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OPEN SESSION

INVESTIGATIONS INTO THE CONTACT STRUCTURE OF THE BRITISH SHEEP POPULATION

C.R. WEBB[1] AND C. SAUTER-LOUIS[2]

SUMMARY

Control policies for farm animal disease are often implemented either at an individual animal level or at a flock level with the hope that such targeted control will result in an overall reduction of prevalence in the national population. However, the effectiveness of control measures may depend on the frequency, distance and type of between-flock contacts and on the level of clustering within the network. Failure to take into account the true contact structure of national populations can result in a poor understanding of how diseases may spread and also in an overestimation of the efficacy of proposed control policies.

Many of the existing models developed to explore disease transmission and control options assume homogeneous mixing of flocks or take on an explicit spatial structure but assume a random contact structure. A clear understanding of the contact structure of the national sheep population would enable models to capture the true heterogeneity in the system and allow more realistic comparison of control strategies than is currently possible. In this paper, we explore the use of network theory to describe the contact structure of populations. In particular, we focus on the contact structure of the British sheep flock. The problems associated with trying to ascertain the true contact structure are discussed and consideration is given as to what the contact structure might elucidate about the rate of disease transmission via different contact routes with specific reference to the prion disease, scrapie.

INTRODUCTION

Networks occur at all levels of biological systems from molecular biology through to population dynamics. Graphs of networks and graph theory have provided a structured approach to the description and understanding of such systems as chemical structures, cellular and metabolic pathways, neural pathways and food webs (Strogatz, 2001). More recently, graph theory has been used to explore the effect of a heterogeneous contact structure between pairs of individuals within a population on the transmission of sexually transmitted diseases such as AIDS and gonorrhoea (Gupta et al., 1989; Ghani et al., 1997). These studies highlight the importance of social ties, which may act as a constraint to the spread of infection; a characteristic which cannot be adequately described using more traditional homogeneous mixing models or reaction diffusion models.

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Relatively little consideration has been given to the contact structure of farm animal populations. The need for a clearer understanding of the frequency and distribution of between-farm contacts was highlighted by the 2001 foot and mouth disease epidemic in Great Britain, where early efforts to use mathematical models to forecast disease spread were hampered by a lack of information on this contact structure. Tracing data from FMD affected farms highlighted the role of long distance movements in the initial stages of the epidemic and provided data with which to parameterise dispersion kernels (Morris et al., 2001; Woolhouse et al., 2001; Keeling et al., 2001; Ferguson et al., 2001). Subsequent movement restrictions simplified the modelling of contact between farms to local and windborne spread only. Movement restrictions require the co-operation of both farmers and the general public and are unlikely to be implemented in the future unless it can be demonstrated that limited restrictions, such as the proposed 21 day standing rule (Anon, 2001a), will cause a significant reduction in disease transmission rates. For the majority of farm animal diseases, contact patterns will continue to play an important role throughout the course of the epidemic.

Historically, the viability of developing a true picture of the contact structure of large populations has been dismissed. However, developments in network theory, improvements in the recording of animal movements and increasing computer power, mean that we can begin to explore the between-farm contact structure of farm animal populations. The aim of this work is not to produce an exact replicate of the national contact structure, but to characterise the network structure in order to improve our understanding of disease spread and improve estimates of the efficacy of control strategies. In this paper, focus is on the national sheep population.

In the first section of this paper, we will summarise some of the key terms used in social-network theory and consider how graph theory might be applied to investigate epidemiological problems relating to scrapie in sheep. We present two independent data sets and demonstrate some of the methods available to investigate the structure of these contact networks.

DEFINITIONS

Graph: a diagram consisting of a set points, called VERTICES or NODES, plus a set of connections, called EDGES, linking some or all of these nodes to each other (Fig. 1).

Directed edge: an edge (or arc) which can be followed only in one direction (usually illustrated on a graph by the addition of an arrow) (Fig. 1).

Loop: an edge which joins a vertex to itself.

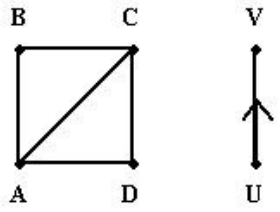

Fig. 1 Example of a graph consisting of 6 vertices {A,B,C,D,U,V}, 5 undirected edges {AB,AC,AD,BC,CD}, one directed edge {UV} and two components {A,B,C,D} and {U,V}.

Connected component: a set of vertices that are linked to each other, although perhaps only through other nodes in the set. Two components of a graph are defined as separate if no vertex is a member of both (Fig. 1).

Adjacency matrix: an n by n matrix representing a graph with n nodes. A one in the ith row and jth column signifies that node i can be reached by node j using just one link. The adjacency matrix for an undirected graph with no loops is symmetrical about the main diagonal, with all elements of the main diagonal equal to zero.

The adjacency matrix for the component, **G**, consisting of the vertices {A, B, C, D} in Fig. 1 is given by $G = \begin{pmatrix} 0 & 1 & 1 & 1 \\ 1 & 0 & 1 & 0 \\ 1 & 1 & 0 & 1 \\ 1 & 0 & 1 & 0 \end{pmatrix}$.

Characteristic path length: let d(i,j) be the shortest path between two vertices i and j, then the characteristic path length, L, is d(i,j) averaged over all vertices.

Risk-potential network: this is defined as a pattern of risk-potential linkages between people (Friedman & Aral, 2001) or, in this case, flocks. A risk-potential linkage is a tie between two flocks that can spread infection, if the infectious agent is present.

Application of graph theory to scrapie

The long incubation period of scrapie (2-3 years on average) and the inability to diagnose scrapie in sheep that are not showing clinical signs, means that there is still much uncertainty about how scrapie spreads, both within and between flocks. The development of a risk-potential network for scrapie can potentially assist at all levels of scrapie epidemiology:

- Aetiology: identify possible sources of infection;
- Transmission routes: help to eliminate/identify routes of transmission between farms and to determine risk factors for transmission;
- Prevention: help to identify high risk farms, hence allowing optimal implementation of preventative measures;
- Control: help to identify how rapidly a disease will spread and where it is likely to spread;
- Economics: help determine the efficacy of prevention programmes.

Potential routes for between-farm transmission can broadly be divided into local and global (long distance) contacts. These groups can be further divided into temporary (short term) and permanent (long term) contacts (Table 1).

In this paper, transmission via local contacts and contact at shows is studied and consideration is given as to whether an edge does exist. The model does not include variation in probability of transmission according to the contact type or the duration of contact.

Table 1: Potential routes of transmission of infectious diseases between farms for sheep flocks in Great Britain

Type of contact	Local	Global
Temporary	Local shows; Shared pasture; Shared lambing sheds; Shared equipment; Poor fencing between neighbouring farms; Borrowed/ Shared rams.	Summer grazing; Shared rams; County, regional and national shows.
Permanent	Direct sale between farms; Local sales; Local ram sales.	Dealers; Breed specific sales; Large markets.

MATERIALS AND METHODS

In the first section of the methods, the data used in this analysis are summarised. In the second section, the risk-potential networks used in this analysis are described, In the final section, the algorithms used to investigate the behaviour of these risk-potential networks are presented.

Data

Data are derived from two distinct datasets relating to the national sheep population. Data relevant to the current paper are summarised below:

Dataset 1 - Show data: A questionnaire consisting of ten short answer questions was sent out in November 2000 to all show society secretaries listed in The Showman's Directory (1999) and in the Farmers Guardian Year Book and Desk Diary (2000). Responses were received from 186 of the addressees (58%) of which 141 responded 'YES' to 'Are sheep shown/ present at the show?'. Information collected relevant to this paper were:

- The location of the show in 2000 (post-code and/or a map);
- Whether the respondent considered the show to be a National, Regional, County or 'Other' (details requested if 'other' response given) show. If 'Other' the show was categorised as Local, County, Regional or National according to the details given;
- Estimated number of breeders who brought sheep to the show in 2000;
- Estimated number of different breeds on display at the show in 2000;
- Names of breeds on display at the show in 2000 (extracted from show booklet where a copy was provided – 137 shows).

Grid references for each show were estimated using the information supplied on the location of the show-ground and/or the post-code of the show-ground.

Dataset 2 - Breed data: A letter was sent out to all breed societies listed in the National Sheep Association handbook (Anon., 1998), requesting information on the location of flocks registered with their society. Data have been supplied by 26 breed societies and a number of

other societies are still being followed up. Data were received either electronically or, in the majority of cases, as flock books. The post-codes of all members of each breed society were entered into a database and these post-codes were used to obtain grid-references for the member flocks.

Risk-potential network

For illustrative purposes, one breed was selected to provide an example of the work carried out within this paper. In order to maintain anonymity, this breed is called, 'Defaid'. The subset of data consists of:

- Grid references, category, number of breeds and number of breeders for all shows at which there was a competition/ display category for breed, Defaid;
- Grid references for all farms with sheep of breed, Defaid.

A unique identifier (ID) was given to each farm and show:
farmID = 1….n; showID = n+1 … n+m; where n = number of farms and m = number of shows.

Three alternative risk-potential networks for breed, Defaid, were compared. Namely, spread of infection through contact at shows, spread of infection through local contacts, and spread of infection through contact at shows and through local contacts. For each scenario, it was assumed that edges are undirected and that there are no loops, that is, a flock can't infect itself. The adjacency matrices for each of these scenarios are calculated using the following assumptions:

A - Edges linking farms to shows: The number of farms of breed, Defaid, attending each show was estimated to be the number of breeders at a show divided by the number of breeds on display at that show. Where either of these data values were missing, the number of entrants was estimated to be the average number of entrants for all shows in the database categorised by show type: Local (3); County (5); Regional (5); National (11). The distance entrants were prepared to travel to compete in each show was assumed to be related to the size of the show as follows:

- Local show ≤ 50 km (≈ 30 miles)
- County show ≤ 100 km
- Regional show ≤ 200 km
- National show ≤ 500 km

The farm ID of the entrants to each show were calculated by randomly drawing from all farms within the catchment area of the show up to the total number of entrants per show (if there were insufficient farms in the locality to meet the estimated number attending the show, then there were fewer entrants).

B - Edges linking farms to farms: An edge is assumed to exist between two farms if they are less than 50km apart. Regular mixing between these farms might occur through shared fencing (immediate neighbours), shared rams, attending local sales, shared veterinary facilities, shared equipment and contact between personnel who visit the farm.

C - Edges linking farms to farms and to shows: This is the summation of the adjacency matrices for the previous two categories, that is, the network has risk-potential linkages both between farms and between farms and shows.

Algorithms

Path length between farms: The path length between farms gives an indication of how closely two farms are connected. The elements of the adjacency matrix, **X**, for a risk-potential network indicates whether two farms, *i* and *j*, have a direct path between them. The matrix, $\mathbf{X}^2$, indicates the number of farms that are two path lengths away from one another (that is, there is another show/ farm which is linked to both of them). The number of nodes that are reachable in *r* links are represented by positive integers in the product matrix, $\mathbf{X}^r$. The summation matrix, P(*r*), is the sum of these product matrices, $P(r)=\sum_{k=1}^{r} X^k$, and represents all the possible paths with *r* links or fewer between two nodes (note $r \leq (n+m-1)$) (Riolo et al., 2001). A general overview of the structure of the summation matrix can be obtained graphically using a 'spy' graph (Anon., 2001b). The spy graph is a picture of the matrix where each point on the graph represents the location of a non-zero element of the matrix. In other words, if there is a path linking node *i* to *j* then there will be a dot on the graph at the intersection of row *i* and column *j*.

Number of disconnected graphs: The number of disconnected graphs in a risk-potential network gives an indication of the likely scale of an epidemic if an infection is introduced to a random flock in the population. In order to determine the number of disconnected graphs in a network, a reachability matrix, **R**, needs to be created as an initial step. The reachability matrix, **R**, is a transformation of the summation matrix **P** such that:

if $\mathbf{P}(i,j) \geq 1$, $\mathbf{R}(i,j) = 1$; if $\mathbf{P}(i,j) = 0$, $\mathbf{R}(i,j) = 0$; if $i = j$, $\mathbf{R}(i,j) = 0$.

That is, the reachability matrix tells us whether two nodes, *i* and *j*, are connected, but not how many paths exist between *i* and *j*. The number of disconnected graphs in a network is calculated using the reachability matrix, $\mathbf{R}^N$, such that:

$\mathbf{R}^N = \mathbf{R}^{N+k}$ for all $k \leq m+n-1-N$.

RESULTS

A relatively small breed society was deliberately chosen for this analysis as the primary interest was to illustrate the techniques available rather than any exact quantitative results of the output. The breed is found throughout England and in parts of Scotland with the largest density of flocks being in North Yorkshire (Fig. 2). The location of shows for which there was a category of sheep of breed, Defaid, correlated well with the location of flocks of breed, Defaid (Fig. 2).

Path length between farms

For a path length of one (that is, a path exists only if there is a direct link between any two nodes), the spy graph of the adjacency matrix for scenario **A** (farm to show) illustrated that there was no direct links between farms (Fig. 3A). The spy graph of the adjacency matrix for scenario **B** (farm to farm) demonstrated that there was no direct links between farms and shows (Fig. 3E). It is clear that there will never be links between farms and shows for scenario **B** no matter how much the allowable maximum path length was increased (Fig. 3E, F, G). For scenario **C** (farm to farm and farm to show), there were direct links both between farms and between farms and shows (Fig. 3I).

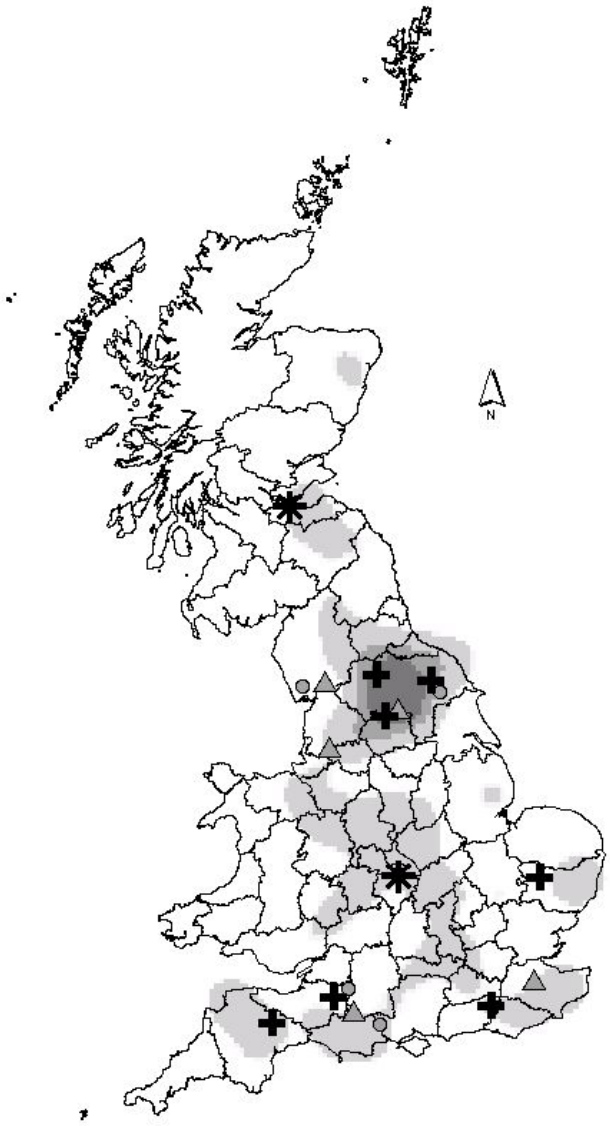

Fig. 2 Distribution of breed, Defaid, and location of shows at which there were classes for this breed. National (star); Regional (cross); County (triangle); Local (circle) shows.

The spy graph of the summation matrix, **P**(2), for scenario **A,** illustrates the number of farms that meet at shows. For example, suppose animals from both farms α and β attend show λ, then it is possible that a highly infectious disease may pass from farm α to farm β via show λ. For each type of risk-potential network, the number of farms that were linked by at least one path increases as the maximum allowable path length increases (*cf* Figs 3A, 3B, 3C; *cf* Figs 3E, 3F, 3G; *cf* Figs 3I, 3J, 3K).

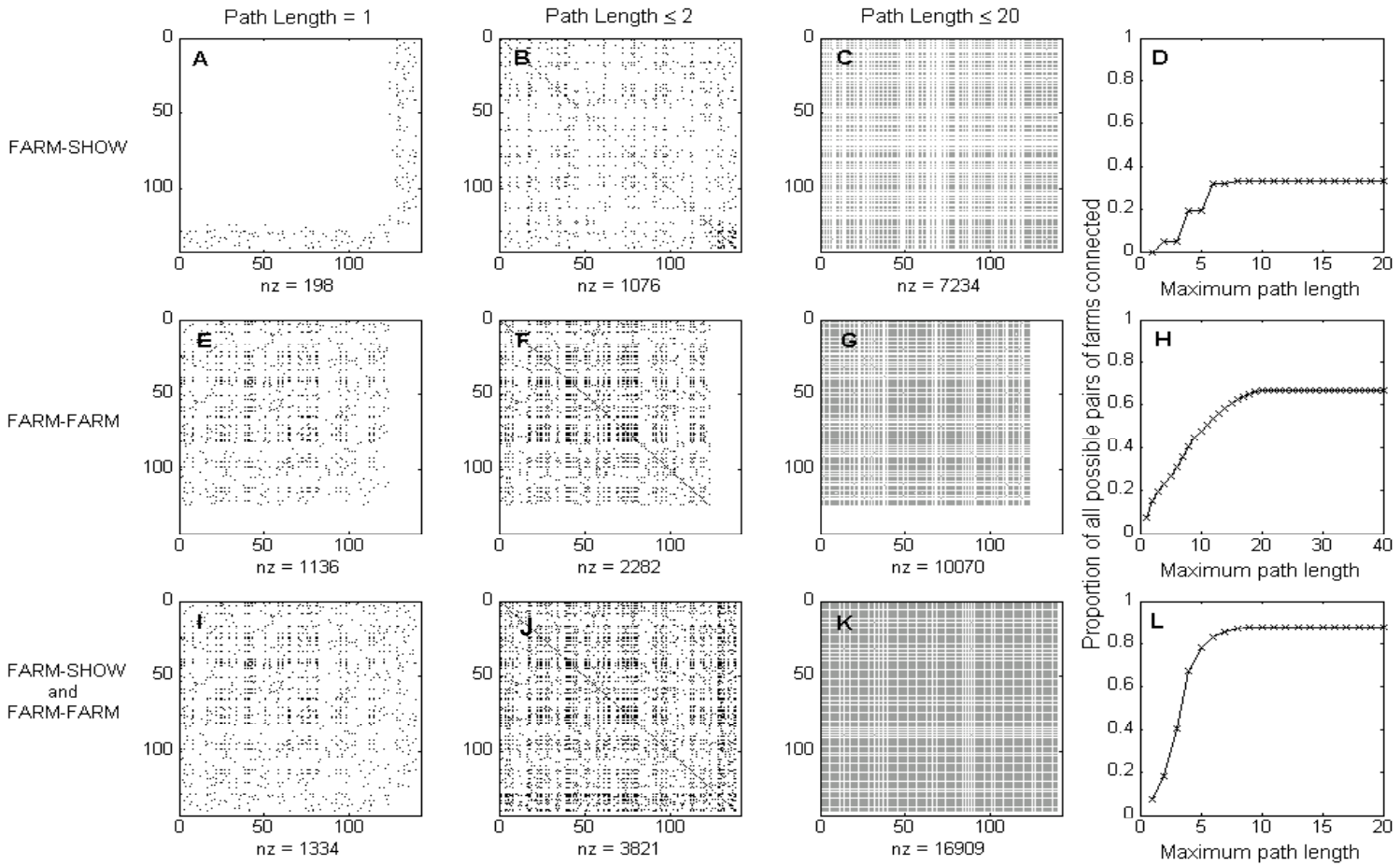

Fig. 3 Summary of path length analysis for sheep breed, Defaid. Graphs A, B, C; E, F, G; and I, J, K are spy graphs of the summation matrices, $\mathbf{P}(r)$, for the farm-to-show, farm-to-farm, and 'farm-to-farm & farm-to-show' risk-potential networks for $r = 1$, 2 and 20. A dot indicates that there is at least one path between nodes i and j. The parameter, nz, is the number of non-zero elements in the summation matrix. The maximum value of nz is 141^2. Graphs D, H and L give the proportion of all possible pairs of nodes that can reach one another by a path shorter than or equal to the maximum path length for the farm-to-show, farm-to-farm, and 'farm-to-farm & farm-to-show' risk-potential networks, respectively.

The relationship between path length and the proportion of all possible pairs of farms connected for each scenario are summarised in figures 3D, H and L, respectively. The asymptotes are reached when all farms that belong to each connected component of the network can reach all other farms that are a member of that component. For the breed, Defaid, approximately 30% of all possible pairs of farms are connected via a path of length 8 or less for scenario **A**. For a farm-to-farm contact structure (scenario **B),** with radius 50km, approximately 65% of all possible pairs of farms are connected via a path of length 20 or less. Allowing both local contact and contact at shows (scenario **C**) results in around 85% of all possible pairs of farms being connected via a path of length 8 or less. Decreasing the farm-to-farm catchment area to 30km, decreases the number of pairs of farms connected to approximately 12% (data not shown) but the addition of the farm-to-show contacts increased the number of pairs of farms connected to around 67%.

Note that since the ID of farms attending each show were drawn randomly, the output of the model varied according to the initial value of the seed for the random number generator. For the data set presented, this variability did not have a significant qualitative effect on the output of the model.

Number of disconnected graphs

Risk-potential network **A** (farm-to-show) consisted of one large component of 80 nodes (including shows) and 61 isolated farms. For scenario **B**, with a farm catchment area of 50km, there is one large component of 100 farms, four smaller components consisting of 7, 4, 3 and 2 farms and 25 isolated nodes (7 farms and 18 shows). Combining scenario **A** and **B** resulted in a network consisting of a component of 128 nodes, a small component of four nodes and 9 isolated nodes.

Reducing the catchment area of farm-to-farm contacts from 50 km to 30 km caused a reduction in the size of the largest connected component to 41 nodes, with 22 smaller networks of between 2 and 7 nodes and 36 isolated nodes (18 farms and 18 shows). Combining scenario **A** with the modified scenario **B,** lead to a component of 118 nodes, one network of 4 nodes, three of 2 nodes and 13 isolated nodes.

DISCUSSION

The relationship between contact structure and disease dynamics has recently been brought into focus by renewed discussion of small-world networks. Small-worlds are characterised by high clustering (a local property) together with small characteristic path length (a global property) (Watts & Strogatz, 1998). Networks for the power grid of the western United-States, the neural network of the worm *Caenorhabditis elegans* and the collaboration graph of film actors have all been shown to be 'small-world' and it is likely that small-world networks are widespread in biological systems (Watts & Strogatz, 1998). In this paper, the preliminary work to characterise the contact structure of the national sheep population has been presented. The work is driven by a need to understand the between-flock dynamics of scrapie. However, at this stage, it is not disease specific. Three alternative risk-potential networks were considered (local farm-to-farm contacts, farm-to-show contacts, and a combination of farm-to-farm and farm-to-show contacts).

Examination of the risk-potential networks presented in this paper highlighted a number of important features of 'real-life' networks. Risk-potential networks do not necessarily consist of

a single large component. Indeed, all of the networks investigated consisted of one large component together with a number of small components and isolated nodes. The basic definition of characteristic path length requires that the network consists of a single component with no isolated nodes since path length between nodes, which are members of different components of a graph, are defined as infinite (Watts, 1999). Much of the theory that has been developed for analysis of contact networks is based on single component graphs (Watts, 1999). However, ignoring disconnected components could result in overestimation of the likely proportion of a population that could be exposed to an infectious disease. If infection is introduced to the largest component of the network then a large proportion of the population is at risk. However, if it is introduced to an isolated node then the disease will not spread to the rest of the population.

For the networks studied, the addition of a risk-potential linkage between farms and shows to local farm-to-farm links increased the size of the largest component in the network and decreased the maximum path length within this component. This is not surprising, as the incorporation of shows changes the structure of the network from something that is likely to be close to regular (i.e. all nodes of similar degree) to a clustered network with a few long distance links (i.e. small-world type network). Therefore, the choice of what constitutes a risk-potential linkage can have a large impact on the predicted between-flock dynamics of diseases.

The networks presented in this paper can be described as sociometric since the set of nodes were defined prior to establishing which nodes were linked. An alternative approach is to collect egocentric network data whereby only the direct links to a particular node ("ego") are recorded (Friedman & Aral, 2001). For human populations, the collection of egocentric data is complicated by differing perceptions of what constitutes an 'acquaintance' and by recall difficulties (Watts, 1999). In contrast, farmers are required to keep records of all movements on and off the farm of sheep for a rolling 5-year period. It is clearly unfeasible to collect movement records from all farmers. However, a sample of egocentric data would improve estimates required for the sociometric approach, such as whether there is frequent contact with neighbouring farms and whether some farmers are more likely to attend shows than others.

There are a wide range of options for future exploration of the data already collected. These include the investigation of the effect of incorporating links between breeds on the network structure, as well as a variety of options for additional data collection. An important feature of epidemiological contact networks is that links are constantly being formed and dissolved and the order of these events can have a large impact on the potential spread of infection. Capturing the dynamics of the national sheep population is not a simple task. However, the rewards for achieving this objective include an improved understanding of how diseases spread and thus a better tool with which to compare control strategies for infectious diseases.

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LET DISEASE TELL THE FULL STORY BEFORE 'SLAUGHTER'

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SUMMARY

Spatio-temporal survey data often form the main decision support mechanism in disease management. Consequently, disease managers rely on adequate description of the data supplied from epidemiological analysis. Practically speaking, such a description comprises of a visualisation, a deduced hypothesis and a statistical evaluation of the hypothesis. Prior to the application of traditional methods of data visualisation and hence data description, an aggregation of the data is required. This paper illustrates the resulting uncertainty in hypotheses generation that can arise from traditional analytical techniques. Additionally, a method of avoiding this typical drawback in traditional data exploration is demonstrated, by the use of data movies.

Data from a ten-year study on the spread of pseudorabies virus in wild boar populations were revised. The visualisation of the data using a data movie instead of using traditional approaches, enabled the immediate observation of the spreading infection in time and space, for the first time. This dynamic perception of the spread drastically changes the understanding of the epidemiology of the virus. The paper demonstrates how data movies can account for the complete information contained within the data set and hence provide a more appropriate starting hypothesis than any a-priori aggregation of the data accompanying traditional approaches. Finally, we recommend the data movie approach as an important gain in the epidemiological toolbox as data movies can easily be integrated into the usual demonstration and publication process.

INTRODUCTION

Disease managers largely rely on the information contained within available data sets to manage disease. However, raw data cannot directly generate useful hypotheses nor can it support the decision making process. Consequently, managers largely depend upon adequate description of the data supplied by epidemiological analysts. In practical terms, such a 'description' resembles a process comprising of a visualisation (e.g. prevalence bar charts, time-series, disease maps), hypothesis generation based on the visualisation (e.g. increasing prevalence in the study period, central epidemic focus) and (statistical) evaluation of the hypothesis. When the resulting epidemiological description is presented, the manager will initially be confronted with a visualisation of the data. The analyst uses the visualisation to

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convince the manager of the hypothesis which was investigated during the process of data exploration. However, the traditional visualisation techniques work in only one, two or three dimensions (cf. Fig. 3). Consequently, whenever disease data comprise of more than two measurements (e.g. disease state, point in time, spatial location), traditional visualisation must suppress some of the attributes of the data. Hence, although managers expect to see the full information contained in the data, they unfortunately cannot while observing spatio-temporal data presented by traditional visualisation methods.

An obvious example to demonstrate this point, is the limitation when the spread of a disease is of interest to management. Here, data records from a disease survey usually consist of disease state (e.g. animal level or sub-population level), the point in time of sampling (e.g. date of shooting or investigation) and the geographic information (e.g. x-y co-ordinates or a spatial unit). Thus, such spatio-temporal data sets need more then three physical dimensions to be visualised (e.g. disease, time, x-coordinate and y-coordinate). Consequently, any exploration method that excludes a-priori one of the sampled information due to technical limitations may suppress a part of the information that could only be observed from the total data set. In addition, the a-priori aggregation may later exclude the managers from a full insight into the spread of the disease, as the manager cannot resolve aggregated data.

The use of disease data movies as a method for the epidemiological toolbox is advocated within this paper. Using the technique allows visualisation of four dimensions. Data movies are particularly powerful when spatio-temporal data are being investigated. This is demonstrated for a data set concerning pseudorabies virus (PrV) infections in wild boar and illustrates how the observation of the full spatio-temporal data set, instead of some aggregated measures, changes the thinking about the disease. The data were explored originally by traditional methods which, for the case of disease mapping, suggested that there was a central epidemic focus (Müller et al., 1998). This traditionally derived hypothesis is compared with the observations from the disease data movie. Furthermore, we demonstrate the feasibility of the data movie method within the standard publication process and hence indicate how it may be used in the future as a general method in epidemiological data exploration.

MATERIALS AND METHODS

Disease data

The study area comprised of a region in the eastern part of Germany (the Federal State of Brandenburg) which is situated between 11.7°-14.7° E and 51.3°-53.4° N, covering an area of 29,530 km^2. Data on PrV consisted of the number of diagnosed positive and negative results from two serological surveys that were directly linked to the spatially defined administrative unit. Survey 1 was conducted between 1985 and 1990 by the three local state veterinary laboratories situated within the study area (Müller, unpublished data). The database, sampling frame and investigation procedure of survey 2 (1991-1994) is described elsewhere (Müller et al., 1998). The majority of data were based on district level for the former German Democratic Republic (1985-1990), whereas more detailed spatial data (municipalities) were available from survey 2. For comparability reasons, all data were summarised to the same administrative level. The Federal State of Brandenburg is divided into 38 administrative units (districts), each with an average area of 748 km^2.

Depending on the survey, the serological data were either based on an enzyme-linked immunosorbent assay (ELISA) and/or a serum neutralisation test (SNT). For this analysis, only data based on the SNT were used because a complete set of ELISA tests was not available (Table 1).

Table 1. Period prevalence data in the region compiled by district level.

	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994
District units sampled	23	36	36	35	36	24	28	27	36	34
Positive SNT	0	0	25	41	22	12	5	26	84	86
Negative SNT	273	749	2,528	3,895	2,320	198	125	323	1,328	1,184
Mean prevalence[a]	0	0	0.01	0.01	0.01	0.06	0.04	0.07	0.06	0.07
Max. prevalence[a]	0	0	0.07	0.04	0.12	0.50	1.00	0.36	0.50	0.40

[a] Prevalence was calculated separately for each spatial unit

Disease mapping

Local seroprevalence values were estimated within district units and plotted geographically on a map of the Federal State of Brandenburg with the software, 'RegioGraph' (Macon Markt und Konzept, Waghäusel, Germany). Chi-square tests were used to detect differences in seroprevalences between local sub-populations.

Data movie

A mathematical smoothing technique was applied to produce a spatio-temporal disease data movie from the data. In particular, the proportion of seropositive wild boars was determined per year and district unit. This raw information was used to create eight three-dimensional grid-surface plots of the seroprevalence estimates, one for each year of the survey (1987-1994; prior to 1987, no cases were recorded and thus the data movie starts with a zero grid). To synthesise one of the grid-surface plots, a regular grid was drawn over the map of the Federal State of Brandenburg (map scale 1:2 million; node spacing of the grid approximately 8 km). Each node of the grid enters a certain district for which the proportion of seropositive animals in the respective year was already known. The seroprevalence value (percentage seropositive) was assigned as a z-value to the node and to all the other nodes falling into the same district. Furthermore, the node spacing was refined on the grid surface to correspond to approximately 1 km, and the spline interpolation (Harder & Desmarais, 1972) implemented in the SAS G3Grid Procedure (SAS 6.1, SAS Institute Inc.) was used to interpolate the intermediary z-values.

The total changes in the seroprevalence value per node and per year were linearly allocated on six new grid-surface plots to smooth the coarse annual scale in the data set. Hence, the eight original annual scatter-plots were replaced by 48 consecutive plots (8 original + 8*5 intermediary plots), interpolating the seroprevalence values in two-monthly time-steps. As a result, two of the eight original grid-surface plots were always connected node by node by a linear interpolation figure (the 1987 plot was virtually connected to the zero grid from 1986). The linear interpolation line then determined the z-values of one node, albeit for each of the five intermediary surface-plots. In other words, we cumulate a sixth of the total annual change per node according to each two-monthly time-step.

The resulting grid-surface plots were converted into coloured contour plots. A non-linear colour scheme is used to highlight low sero-prevalences. Finally, the contour plots were concatenated to produce the spatial-temporal disease data movie. Within the data movie, rivers and highways were fixed in black and grey respectively to highlight natural borders.

RESULTS

Disease mapping

The disease map for the study period indicated a division of the study area (Fig. 1; Müller et al., 1998). The western part had a very low infection prevalence, while the eastern part appeared to be responsible for the majority of pseudorabies-positive animals ($P < 0.05$). Moreover, the regions east of the city of Berlin appeared to have a functional focus (the black region being the central focus). The focal appearance indicated large epidemics of the infection in the eastern part with some offshoots to the remaining areas (Müller et al., 1998).

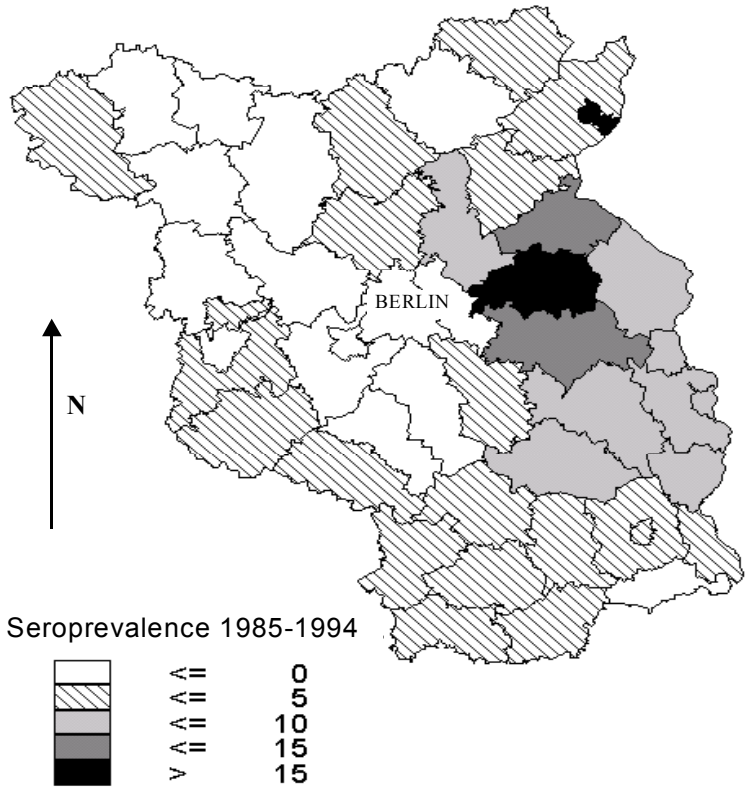

Fig. 1 Disease mapping of the seroprevalence data for the study of PrV infections in wild boar in Brandenburg from 1985-1994 (updated after Müller et al., 1998).

Data movie

In Fig. 2, the slides of the data movie are plotted. The animated, colour data movie is available upon request from the authors (Email: hanst@oesa.ufz.de, selhorst@wus.bfav.de, thomas.mueller@wus.bfav.de).

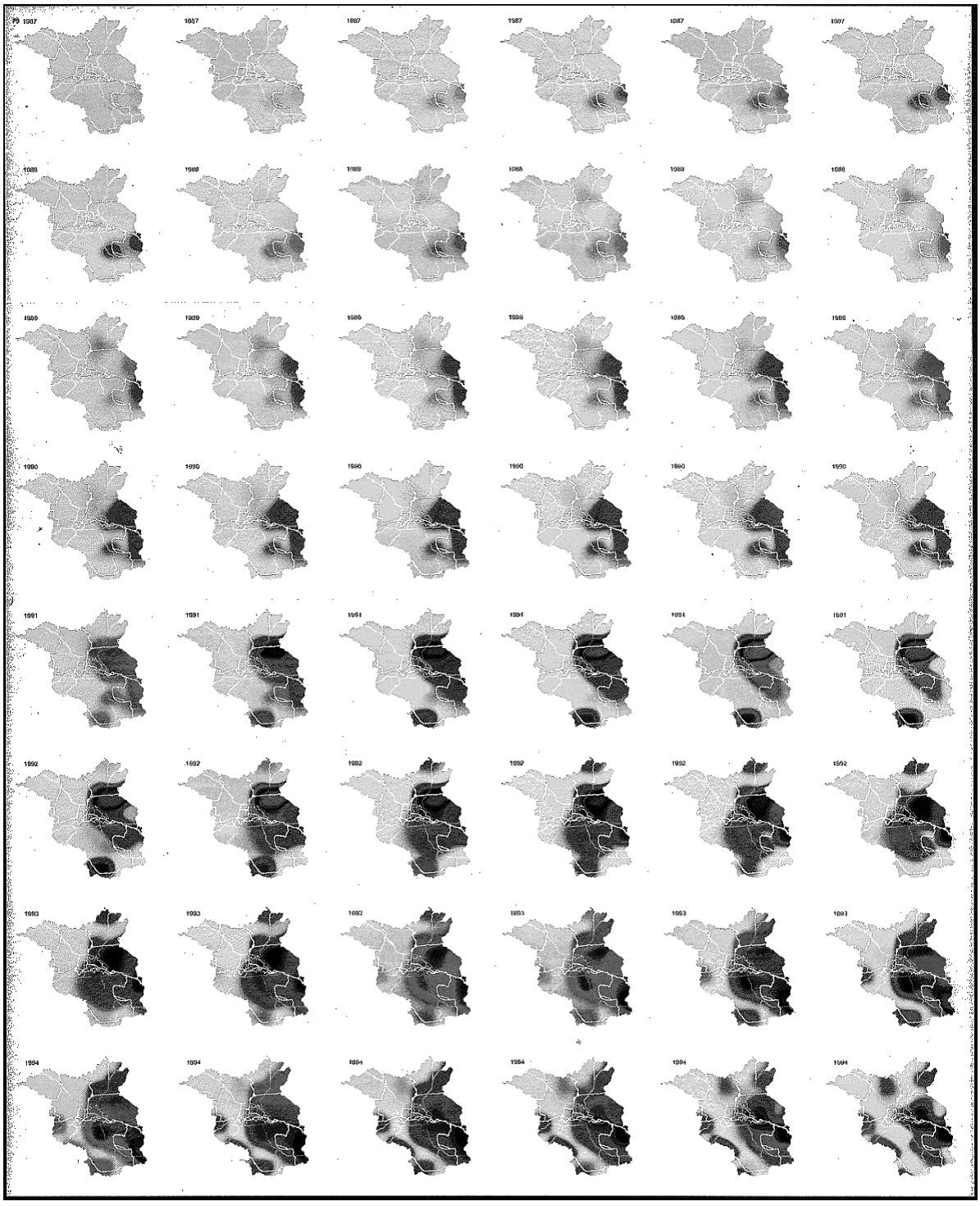

Fig. 2 Plot of the data movie slides. Each row represents one year (1987 – 1994). Increasing seroprevalence is represented by darker shading.

The data movie revealed the apparent endemic focus to be evolving along with other disappearing seats of infection. In particular, two centres with low seroprevalence (locally up to 7%) were observed evolving in the south-eastern part (1987), while one year later (in late 1988), the left centre faded, indicating a decrease in prevalence. At the same time, a new triangular

centre (also with a very low seroprevalence) appeared in the north-eastern part, although this also fades in 1989/1990. In 1991, another centre appeared in the south. The last epidemic centre occurred in the north during 1994.

DISCUSSION

The patterns formed by a disease spreading in space or time are acknowledged as a valuable source of knowledge in epidemiology (Cameron et al., 1997). This is verified by the increasing number of studies in the literature which have recorded spatio-temporal disease data e.g. CSF outbreaks (Dijkhuizen, 1999; Elbers et al., 1999), FMD outbreaks (Sorensen et al., 2000; Mahul & Durand, 2000; Woolhouse et al., 2001; Enserink, 2001), rabies epidemics in European foxes (Serokowa, 1968; Wandeler et al., 1974; Bacon, 1985; Sayers et al., 1985) and rabies in American racoons (Moore, 1999)(Curtis, 1999; Krebs et al., 1999). This research group has added an example from a survey for PrV infections in German wild boars.

However, when starting the exploration of sampled spatio-temporal data with traditional techniques, some information from the spatio-temporal data is lost. Why? Any data exploration starts with a particular visualisation in order to find some marked pattern, which then allows for the generation of a hypothesis. The hypothesis is then investigated using sophisticated epidemiological tools. In the case of spatio-temporal disease data, there are three traditional approaches of visualising the data: measuring disease abundance (prevalence) by bar charts, measuring temporal changes by time-series plots and measuring spatial features by (disease) mapping. Epidemiologists are familiar with all these approaches. This familiarity is probably the major reason why epidemiologists sometimes ignore the fact that, these techniques cannot visualise the spread of a disease simultaneously in time AND space. Although one might explore the data set successively with each of the three traditional methods, this does not yield an exhaustive understanding of the disease, as represented by the original data. This paper has illustrated a method of experiencing time and space effects from a study of PrV.

The original epidemiological exploration of the data (Müller et al., 1998) went through the three standard aspects of disease data: disease prevalence, temporal changes in prevalence and spatial distribution of prevalence. For each of these approaches, one will find the respective visualisation and deduced hypotheses in Müller et al. (1998). For the purpose of this paper, focus has been on the spatial approach of Müller et al. (1998). The respective visualisation was completed by mapping the period prevalence for all districts in the study area (Fig. 1). From Fig. 1, one is apt to conjecture a disease focus left of Berlin radiating to the remainder of the study area. This is the hypothesis that was investigated by Müller et al. (1998). After statistically testing the differences in prevalence within the districts, an endemic area distinct from the surrounding districts was identified.

When one has performed the static visualisation (Fig. 1), the analysis Müller et al. (1998) performed seemed appropriate. This is because of the way the data was visualised, determined the thinking on disease dynamics and thereby the hypotheses that were considered. Indeed, plotting a map makes one think in static terms which are related to static spatial disease patterns (focal, cluster, scattered infections, as well as others). As an illustrative example, the analysis of Müller et al. (1998) was continued by a point source related estimation of the PrV infection risk in the study area (Müller et al., unpublished) which was methodologically correct under the assumption of a radiating point source (Bithell & Stone, 1989; Bithell, 1992). However, the point source assumption was violated. Indeed, the focal hypothesis itself was rejected in the

light of the data movie. To stress the point once more, the reason for the trapping interpretation was the a-priori aggregation of the raw data and not the visualisation by the map.

The data movie approach can also be used to visualise the same data set. Surprisingly, in the data movie, there was no steady spread from initial hot spots of the infection into the eastern part of the study area. In fact, the locations with greatest prevalence vary with time. The moving hot spots contradict the central focus hypothesis. Instead of the static prevalence distribution shown by the map, the data movie discovered local cyclical accumulation of the infection with forwarding into non-infected areas followed by a rapid reduction in seroprevalence. Consequently, the working hypothesis changed from a static 'endemic region' (Müller et al., 1998) into dynamic 'emergence and dissolution of seats of infection'. Consequently, one could ask: 'Do the moving seats of infection indicate independent events?' This seems to be supported by the distances between the temporary foci, which are further apart than known wild boar dispersal distances (Stubbe & et al., 1989).

The evaluation of the dynamic hypothesis suggested by the data movie is in progress. For the current rationale, the two working hypotheses are compared and the fact that the initial visualisation of raw data influences subsequent investigations, is recognised. Finally, the fact that disease managers are initially confronted with data visualisation is recalled. Thus, as the example of PrV infections in wild boars demonstrated, the basic thinking of the disease manager is determined by the visualisation of the data and by the subsequently deduced hypothesis. Although, the static mapping of the disease is not a wrong approach, it does detract from the available information held within the data by aggregating the temporal dimension. Hence, presenting the disease map at the beginning can be misleading because managers are not provided with the full information contained within the raw data about the spread of the disease.

Another solution to the data visualisation problem may be to identify the spatio-temporal dynamics from annual disease maps (Grimm, pers. comm.). In fact, this was our initial approach. However, the result was unsatisfactory because the actual dynamics of disease could not be observed. The spread of the disease is a spatio-temporal process. Consequently, it has to be visualised simultaneously in space AND time, which only can be done with a data movie.

Why do data movies enable the visualisation of real spatio-temporal dynamics and all the other approaches do not? The situation is summarised in Fig. 3. Traditional visualisation techniques can only present data in up to three physical dimensions. Thus, as in the usual case of disease mapping, they easily can be plotted in a two-dimensional representation and can then be printed, projected and disseminated. However, spatio-temporal disease data require four physical dimensions for an adequate visualisation (disease, time and x-y location). Four dimensions cannot be produced in a two-dimensional representation. To overcome this limitation, sometimes less instructive plots, like the right diagram ('4D') in Fig. 3, are used. However, only the data movie can truly visualise the fourth dimension in an acceptable manner. This is possible because the imagination of the observer creates the fourth dimension. Therefore, the data movie allows us to perceive the spread of the disease simultaneously in time AND space. Consequently, we do not need to aggregate any of the information in the raw data but can look at it directly.

However, the demonstrated advantage of perceiving dynamic disease spread by data movies does not mean the other methodological approaches are of less value. The initial investigation of the spread of a disease should use all informal dimensions (disease, time, space) hence inducing an appropriate dynamic notion of the spread. Subsequently, the information can be

aggregated in order to prove a particular hypothesis congruent with our basic dynamic concept. Hence, disease data movies are a powerful method for data exploration and hypothesis generation.

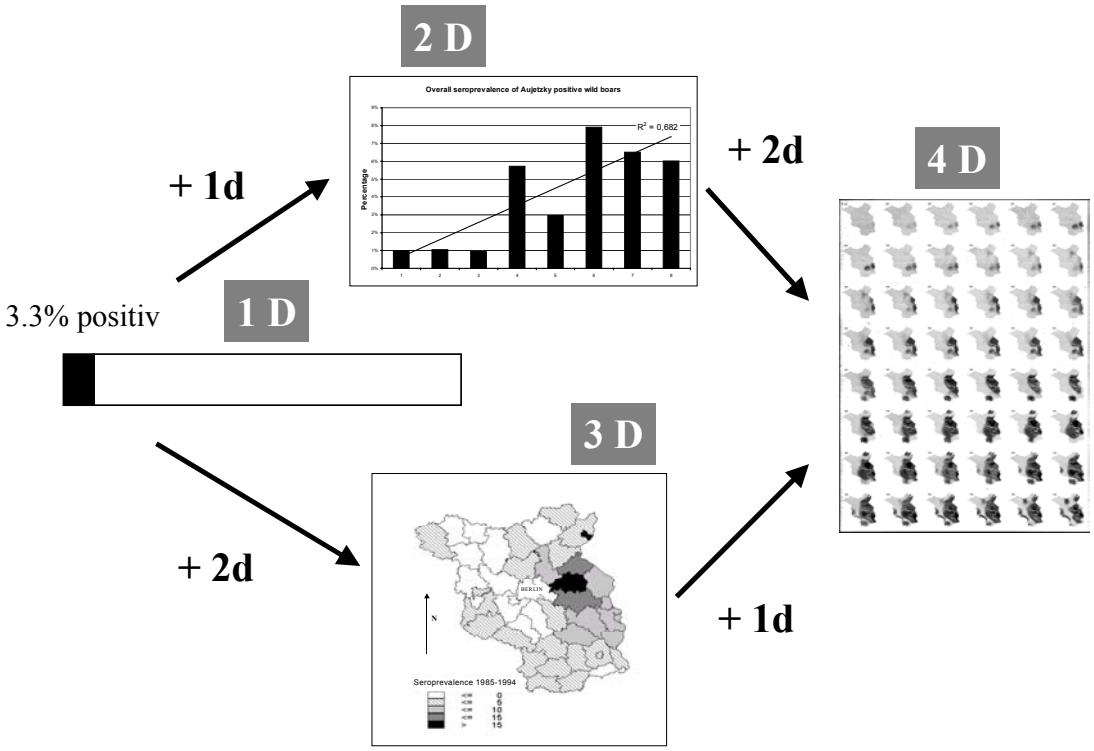

Fig. 3 Schematic representation of the approaches for visualisation of spatio-temporal disease data (left: prevalence bar chart; top: annual time-series of prevalence; bottom: map of district prevalences; right: disease data movie plotted as slides). Black boxes indicate the physical dimensions for each respective diagram. Numbers and arrows indicate the dimensional increase between diagrams.

The perception gained by the data movie is influenced by the methods used to collect the raw data from a population (e.g. sample size and geographical reference unit) and the accuracy of the measures used in data collection (e.g. specificity and sensitivity of the diagnostic test used). However, these limitations are inherent in the actual data and cannot be eliminated by the data movie. Additionally, although the arbitrarily chosen interpolation between the annual seroprevalence records used for construction of the data movie does introduce uncertainties, the interpolation strengthens the dynamic character of our perception.

The spread of disease is a spatio-temporal phenomenon. Consequently, the simultaneous examination of ‘space and time‘ in disease data will produce a considerable insight into epidemiological phenomena. These new insights into spatio-temporal dynamics and patterns pave the way for improved disease management as the latter has to be planned in space as well as time. Additionally, the Internet enables the direct use of these data movies for illustration when they are provided online along with the standard three-dimensional printed figures. Furthermore, recent computer and telecommunication advances enable the efficient transfer of disease data movies. In conclusion, the routine use of disease data movies in all epidemiological research is recommended.

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FIELD INVESTIGATION OF OVINE REPRODUCTIVE LOSS ATTRIBUTABLE TO *Toxoplasma gondii* AND ITS ECONOMIC IMPACT IN 14 FLOCKS IN SOUTHWEST WALES

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SUMMARY

A randomised intervention trial, involving 4,062 breeding ewes from 14 different farms in Southwest Wales, yielded estimates of the effect of vaccination against *Toxoplasma gondii* on the physical reproductive performance of sheep. These estimates were incorporated into partial budget simulation models designed to investigate the economic merits of the two vaccination regimes that are encountered in the field.

Over a five-year period , the models indicated the economic superiority of a regime in which all breeding ewes are vaccinated in the initial year, with the vaccination of replacements in following years over an alternative in which replacements only are vaccinated in all years. Vaccination was predicted to result in a mean of 6.4 additional lambs per 100 ewes tupped per year, with a mean value of £226. This represents an estimate of the costs of primary *T. gondii* infections in the intervention trial population that are preventable by vaccination.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that, since its discovery in 1908, has become recognised as having a worldwide distribution (Buxton 2000). It was first associated with ovine abortion in New Zealand in the 1950s (Towle & Dubey, (1986) and is now believed to be one of the most important causes of ovine abortion in many sheep producing countries, including the United Kingdom (Dubey & Beattie 1988).

Sheep are moderately susceptible intermediate hosts (Innes 1997) and primary infections of non-pregnant sheep are usually mild or asymptomatic (Anderson et al. 1994). Clinical toxoplasmosis can be considered to be primarily a disease of the conceptus (Blewett & Watson

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1983), since the clinical pattern seen in sheep is one of foetal/neonatal disease following localisation of infection in the gravid uterus of pregnant ewes (Anderson et al. 1994; Buxton 2000). The consequence of a primary *Toxoplasma* infection of a susceptible pregnant ewe is primarily dependent on foetal immunocompetence, and thus gestational age at the time of infection (Blewett & Watson 1983). Infection in early pregnancy may result in foetal death and resorption, while infection during mid-pregnancy (approximately 70 to 100 days) may lead to abortion, mummification, stillbirth or congenital infection of the lambs. Congenital infection may also result from infections in late pregnancy. Such lambs may be weak and die or the infection may be subclinical in nature.

Sheep are obligate herbivores and the ingestion of *T. gondii* oocysts with feed or water is almost certainly the primary source of infection (Blewett et al. 1982; Dubey & Beattie 1988). Oocysts are produced by members of the *Felidae* (the definitive hosts) following a primary *Toxoplasma* infection. Many millions of oocysts may be shed in the faeces of a newly infected cat over a period of up to 15 days, until shedding ceases with the development of immunity (Buxton 2000; Kimberling 1988).

The true magnitude of the ovine reproductive losses caused by *T. gondii* in the UK is unknown. Data from the Scottish Agricultural College Disease Surveillance Centres (ANON 2000a) and the Veterinary Laboratories Agency Regional Laboratories (ANON 2000b), consistently show *Toxoplasma* to be one of the two pathogens that dominate the diagnosed causes of ovine foetopathy in submissions to these institutions (the other being *Chlamydophila abortus*). Several authors have estimated the average incidence of *Toxoplasma* abortion in the UK national flock to be 1-2% (Bennett et al. 1999; Blewett & Watson 1984; Stubbings 1995).

In the absence of over-riding implications for human health or animal welfare, most disease control decisions on commercial farms are made on economic grounds. Disease control is rarely an all-or-nothing decision (Dijkhuizen & Morris 1997) and it is generally subject to the law of diminishing returns (McInerney et al. 1992). Application of a basic economic principle, the equimarginal principle, leads to the conclusion that disease control input should be increased to the level where the cost of an additional input equals the return from the additional output (Dijkhuizen et al. 1995).

Partial budgeting has been described as 'simply a quantification of the economic consequences of a specific change in farm procedure' (Dijkhuizen et al. 1995). Such models are particularly suited to herd level analyses of relatively small changes within the production process, of which vaccination against *T. gondii* is an example. Intervention studies produce comparative data from the different intervention cohorts (in this case vaccinated and non-vaccinated sheep) and such data are suited to analysis using the partial budgeting approach (Dijkhuizen et al. 1995; Noordhuizen et al. 1997).

Partial budgets consider only those costs and returns that are changed by the intervention and therefore do not calculate the total income or expenses for the enterprise concerned (Dijkhuizen & Morris 1997; Morris 1999). The method of partial budgeting has been efficiently described by Dijkhuizen and Morris (1997).

Animal production systems are complex environments and many of the elements of which they are composed are subject to uncertainty (Gummow & Mapham 2000). If such elements influence the performance of a given intervention, then successful decisions require that these properties of complexity and uncertainty must be considered. Deterministic models, in which

values are fixed, fail to do this. Fortunately, partial budgeting and simulation models can be combined to generate stochastic models that can take account of the dynamic and risk aspects of livestock production and disease (Gummow & Mapham 2000; Macchi et al. 1999). It is this approach that is used here.

While calculations of total costs can help to provide a better overall understanding of the impact of a disease (Dijkhuizen et al. 1995), it has been argued that they provide little or no indication as to what, if anything, should (or could) be done about the situation (McInerney et al. 1992) and that they incorrectly suggest that these amounts are completely recoverable (Martin et al. 1987). Instead, where decisions are to be guided by economic considerations, the focus should be on the magnitude of avoidable costs (Martin et al. 1987; McInerney et al. 1992) and thus the potential benefits of control (Morris 1999).

MATERIALS AND METHODS

Farms were recruited to the randomised intervention trial through the Farmers' Union of Wales, local veterinary practices and by word-of-mouth. The criteria for recruitment were a minimum of 50 ewes to be put to the tup, indoor lambing, located within a one hour drive of Carmarthen and confidence that the flock owner was willing and able to maintain the levels of surveillance and recording required by the study. A history of reproductive losses caused by *T. gondii* was not a selection criterion.

Enrolment of participating flocks took place prior to tupping in 1999. Only female sheep that would be bred that year were included. Each ewe, which was identified by a unique ear-tag, was aged and condition scored. Systematic (quasi-random) allocation was used to divide ewes into two groups, one of which was vaccinated with a commercially available *Toxoplasma* vaccine (Toxovax®, Intervet UK, Milton Keynes). The cohort of the first animal was decided by the toss of a coin.

Ewes enrolled in the study were followed throughout pregnancy. The results of ultrasound scanning at around 90 to 100 days gestation and the outcome of pregnancy itself were recorded by flock owners on purpose-designed forms provided to them. Participants were asked to record all incidences of the deaths of ewes, barren ewes, abortions, mummifications, stillbirths, live births, and the deaths of live-born lambs between 0 to 7 days of age and 8 to 14 days of age. Recording ceased when lambs reached 2 weeks of age.

The data from this study were recorded in a database created using MS Access 97 (Microsoft Corporation) and statistical analyses were performed using Stata version 6 (Stata Corporation).

Partial budget models were built to investigate the economic benefits of the two vaccination regimes that are encountered in the field. In the standard regime, all breeding ewes in the flock are vaccinated in the first year of vaccination and vaccination in following years is confined to replacements. In the alternative regime, only replacement animals are vaccinated in all years. The only differences between the structures of the two models and their input values were those necessary to represent the different vaccination protocols.

Both models ran for a period of five years, selected because this was the period required for the alternative vaccination regime to achieve a fully vaccinated flock, assuming an annual replacement rate of 20%. A unit of 100 ewes was selected as a convenient unit of simulation. It

is of the same order of magnitude as the majority of flocks and also figures quoted 'per 100 ewes tupped' are easily adapted to different flock sizes.

The models were built in a spreadsheet using MS Excel 2000 (Microsoft Corporation). The simulation analysis software, @Risk version 3.5 (Palisade Corporation), which functions as an 'add-in' to MS Excel, was used to include input variables that were subject to appreciable variation as BetaPERT probability distributions. The BetaPERT distribution was selected because it is less sensitive to extreme values than the triangular distribution and therefore more precisely reflects the distribution of skewed data (Vose 2000). These distributions were defined by their 'most likely', minimum and maximum values. Where the data describing an input variable were derived from the intervention trial, these corresponded to the mean, minimum and maximum 99% confidence limits of the appropriate estimate.

Each simulation was composed of 5,000 iterations. In each iteration, latin hypercube sampling was used to randomly select a value from each probability distribution. These values were then used by the model to calculate output values for that iteration. The models were designed so that the distributions were sampled independently within each year.

The additional returns attributable to vaccination are comprised of the value of the additional lambs produced following vaccination. The models valued all additional lambs on the assumption that they were sold for slaughter. The number of productive ewes per 100 ewes put to the ram was multiplied by the number of lambs per productive ewe to yield the number of lambs produced per 100 ewes tupped in each of the vaccinated and non-vaccinated cohorts. Subtraction of the latter from the former gave the additional number of lambs per 100 ewes tupped expected from the vaccinated cohort.

These data came from the intervention study. In the standard model these distributions remained unchanged across the five years of the model, since the flock was always fully vaccinated. In the alternative model, the number of vaccinated animals in the flock increased by the number of replacements in that year. At the same time, the maximum age of a vaccinated animal increased by one year. The reproductive performance data of the appropriate age cohort in the intervention trial was used for each year of this model. However, the ewes in the intervention trial were aged by their dentition and this method cannot accurately determine the age of a sheep beyond 4 years old. This meant that the trial could not provide the data required to represent the population in the alternative model in year 4. The model therefore considered the flock to become fully vaccinated in year 4 (one year earlier than would actually be the case) and included a replacement rate (and associated costs) of 40% in this year.

The input values of lamb liveweight and price per kilogram were supplied by the Meat and Livestock Commission (personal communication). The price values are the mean, minimum and maximum of the standard quality quotation (SQQ) series for the year 2000. The SQQ series presents a monthly value that summarises the values achieved by lambs in all weight categories during that month. The value of the additional lambs produced in a vaccinated population was calculated as the product of the number of additional lambs, liveweight per lamb and price per kilogram liveweight.

The additional costs included in the models are comprised of the costs of gathering and vaccinating ewes together with the costs of worming and vaccinating additional lambs.

The input values for the time required for gathering and vaccination were selected by the author in the light of experience gained during the intervention study and from discussion with participating farmers. The labour rate of £4.77 per person per hour used in the model is the minimum full-time rate for an individual aged 19 years or more in England or Wales quoted by the SAC (2001).

The model assumed that all female breeding sheep are vaccinated only once in their lifetime. This assumption is in agreement with the datasheet recommendations for Toxovax® which state that 'Toxovax® is known to protect for at least two lambing seasons' and that 'it is likely that within this time natural infection will have boosted the animal's immunity making further revaccination unnecessary' (Intervet UK). While, as the manufacturer points out, this cannot be guaranteed, revaccination of ewes is very rarely practised. A mean reproductive life of 5 years was assumed, giving a mean annual replacement rate in the breeding flock of 20% (Stubbings 1999).

The cost per dose of Toxovax® was entered as £2.15, the recommended retail price (Intervet UK, personal communication). There appears to be very little variation around this figure and it was therefore treated as a fixed value. Farmers who participated in the intervention trial provided a figure of £2.50 per lamb for the costs of worming and vaccination against clostridial diseases.

In each year, the predicted margin of a vaccinated population over a non-vaccinated population was calculated by subtracting the value of additional costs from the value of additional returns. Discounting was applied to margins from the second year onwards according to the formula described by Dijkhuizen and Morris (1997). The discount rate of 2.15% was derived by the subtraction of the UK inflation rate from the base rate (2.6% and 4.75% respectively; BBC Ceefax, 1 October 2001). The mean predicted annual margin of a vaccinated population over a non-vaccinated population was calculated as the sum of the discounted year-specific margins divided by five.

Objective validation of models is difficult (Dijkhuizen & Morris 1997) and there is an absence of appropriate published data against which to evaluate the models that were used. The assumptions, inputs and outputs of the models were discussed with external parties considered to have an expert knowledge of modelling, economic analysis or the system being modelled. This was to ensure the credibility of the model represented the best that could be achieved.

Sensitivity analyses were used to investigate the relative significance of input variables in determining the value of the mean annual margin following vaccination compared with no vaccination. Both multivariate stepwise regression and rank order correlation methods were used. These methods are available in the sensitivity analysis module of the @Risk software.

RESULTS

A total of 4,062 breeding ewes from 14 farms were enrolled in the intervention trial, of which 2,029 (50%) were vaccinated with Toxovax®. The results of the intervention trial that were used in the models are shown in Tables 1 and 2. The data are presented in the form in which they were included in the model and thus 99% confidence limits (C.L.s) are presented

rather than the more usual 95% values. The values used in years 1, 2 and 3 of the alternative model were those of the cohorts aged ≤ 1, ≤ 2 and ≤ 3 years old, respectively. In years 4 and 5 of the alternative model and all years of the standard model, the values derived from the entire trial population (all ages) were used.

Table 1. Age stratified proportions of productive ewes in vaccinated & non-vaccinated cohorts.

Age cohort (Years)	Vaccinated			Non-vaccinated		
	Mean	Lower 99% C.L.	Upper 99% C.L.	Mean	Lower 99% C.L.	Upper 99% C.L.
≤ 1	0.752	0.706	0.797	0.732	0.685	0.779
≤ 2	0.805	0.771	0.840	0.785	0.750	0.821
≤ 3	0.840	0.813	0.867	0.818	0.789	0.846
All ages	0.862	0.841	0.882	0.846	0.825	0.868

Table 2. Number of lambs surviving up to two weeks of age per productive ewe in vaccinated & non-vaccinated cohorts, stratified by age

Age cohort (Years)	Vaccinated			Non-vaccinated		
	Mean	Lower 99% C.L.	Upper 99% C.L.	Mean	Lower 99% C.L.	Upper 99% C.L.
≤ 1	1.297	1.237	1.357	1.289	1.230	1.347
≤ 2	1.403	1.351	1.456	1.390	1.338	1.441
≤ 3	1.486	1.441	1.531	1.449	1.405	1.494
All ages	1.529	1.493	1.566	1.482	1.446	1.517

The standard model predicted that vaccinated ewes would produce a mean annual margin of £117.31 per 100 ewes tupped over unvaccinated ewes during the first five years of vaccination. However, this figure was variable taking a minimum value of £-14.20 and a maximum value of £278.37. This is illustrated by the cumulative probability distribution of the mean annual margin as shown in Fig. 1.

The mean annual margin over a five-year period predicted by the alternative model was £58.62 per 100 ewes tupped. This value was also subject to wide variation with the minimum value of £-31.91 and the maximum value of £166.44. The cumulative probability distribution is shown in Fig. 2.

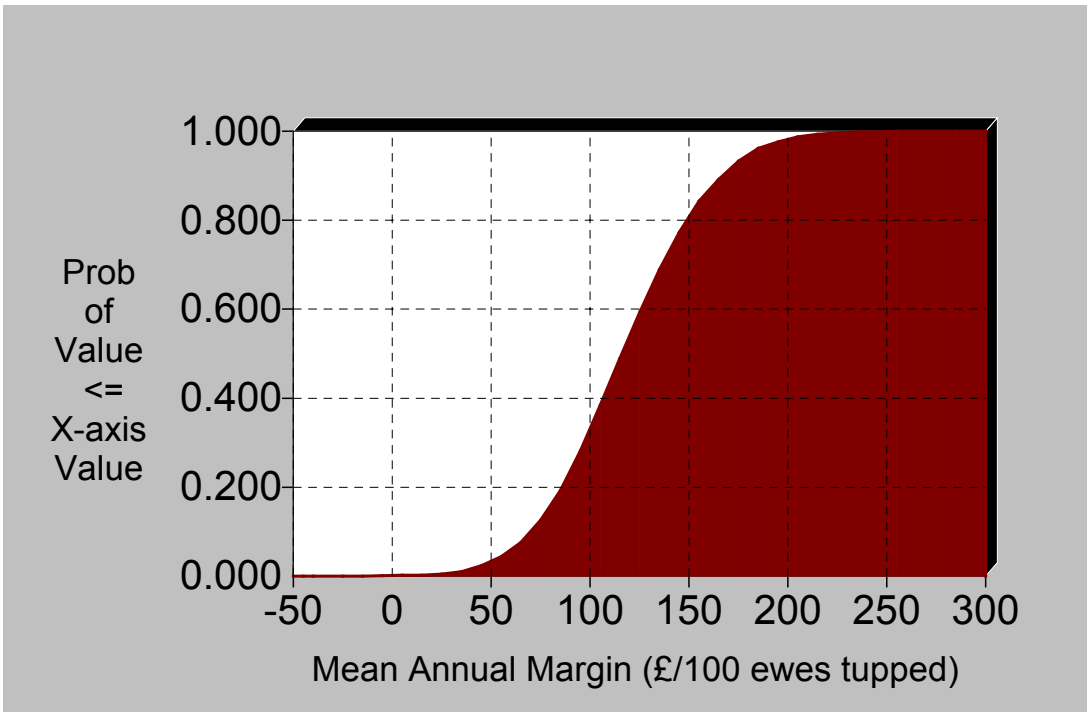

Fig. 1 Cumulative probability distribution of mean annual margin of vaccinated over non-vaccinated ewes in the standard model

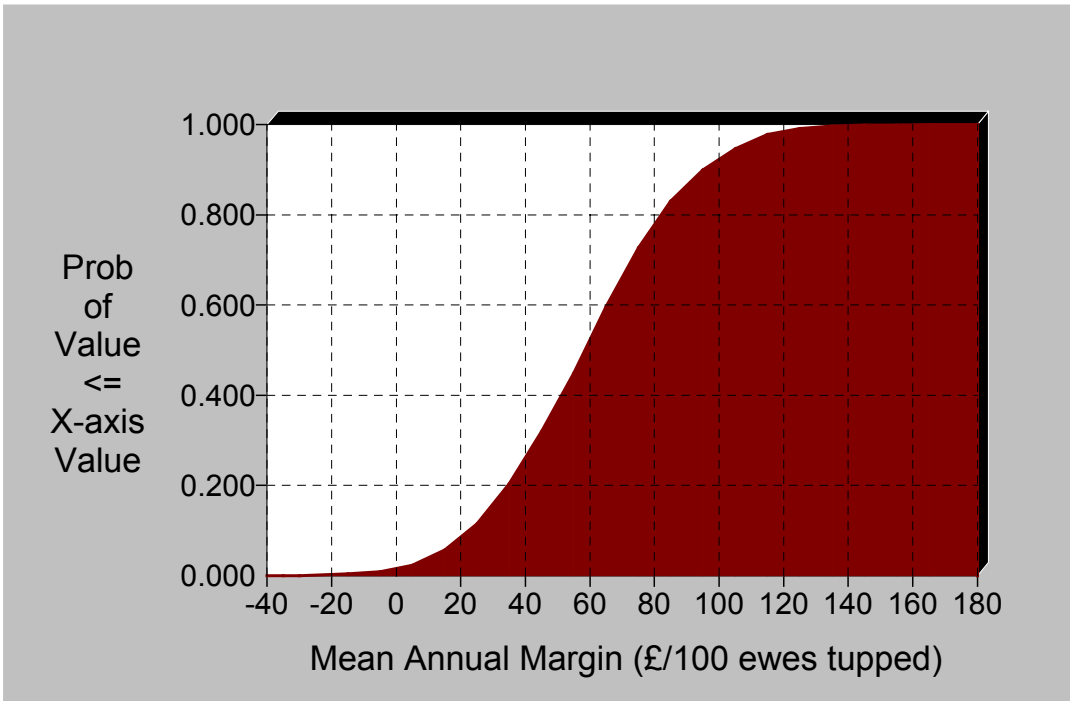

Fig. 2 Cumulative probability distribution of mean annual margin of vaccinated over non-vaccinated ewes in the alternative model

The year-specific margins (before discounting) following vaccination compared with no vaccination in both models showed similar variation as shown in Table 3.

Table 3. Annual margins of vaccinated over non-vaccinated ewes before discounting

Year	Margin (£/100 ewes tupped)					
	Standard model			Alternative model		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
1	-19.53	-264.85	338.51	-28.74	-131.65	85.07
2	159.72	-103.24	570.77	0.83	-159.61	202.38
3	159.64	-96.78	505.93	74.57	-132.47	383.64
4	159.72	-120.62	512.63	111.90	-150.92	406.91
5	159.92	-85.36	501.37	157.43	-101.97	594.36

In the standard model, the number of additional lambs per 100 ewes tupped following vaccination was consistent from year to year. The mean over five years was 6.36, with a minimum of 2.15 and a maximum of 10.14. The monetary value (before discounting) of these additional lambs was similarly consistent, having a mean annual value of £225.79 and minimum and maximum values of £84.55 and £401.48, respectively.

Table 4. Annual number of additional lambs (lambs/100 ewes tupped) and their value (£/100 ewes tupped) produced following vaccination in the alternative model

Year	Variable	Mean	Minimum	Maximum
1	Number of lambs	0.65	-2.19	4.22
	Value (£)	23.03	-76.03	154.77
2	Number of lambs	1.54	-2.61	6.97
	Value (£)	54.84	-104.14	271.82
3	Number of lambs	3.78	-2.64	11.13
	Value (£)	134.19	-89.03	466.27
4	Number of lambs	6.30	-1.95	13.29
	Value (£)	223.19	-56.00	547.55
5	Number of lambs	6.30	-1.77	14.46
	Value (£)	223.33	-67.54	686.88

In the alternative model, the number of additional lambs produced and their values changed significantly over successive years (Table 4). The pattern exhibited a progressive increase towards the values seen in years of full vaccination (years 4 and 5). Mean values calculated over this period are consequently of limited informative value. Such variation is entirely expected since the reproductive performance of the vaccinated cohort within the flock is changing each year, as is the proportion of the flock that is vaccinated. The intervention study

demonstrated a significant variation in reproductive performance with age. One characteristic of the early years of the alternative model is that the age structure of the vaccinated cohort changes from year to year until full vaccination is achieved. There is a consequent variation in the impact of vaccination during this period.

Analysis of the sensitivity of the mean annual margin of vaccinated ewes over non-vaccinated ewes to its input variables using multivariate stepwise regression, yielded an R^2 value of 0.96 in both models. These values indicated that the linear relationship assumed by the models was able to explain the vast majority of the variation in the mean annual margins. Repeats of these analyses using rank order correlation produced very similar results to those utilising multivariate stepwise regression.

These analyses indicated the mean annual margin in both models to be most significantly affected by changes in the values of the number of productive ewes and the number of lambs per productive ewe. Of the two values, the number of productive ewes appeared to be marginally the more influential. In the alternative model, there was a pattern of increasing sensitivity of the mean annual margin to both of these variables as the proportion of the flock that had been vaccinated increased.

DISCUSSION

The models predict a marked difference in the mean annual margins associated with the two different vaccination regimes over the option of no vaccination. The mean annual margin of £117.31 per 100 ewes tupped in the standard model was twice the £58.62 seen in the alternative model over the same period. This indicates that the initial vaccination of all breeding ewes was the most economically desirable option. The option of vaccinating replacements only (the alternative model) theoretically offsets the longer period taken to achieve full vaccination relative to the standard model with lower initial costs. However, the models indicate that the relative reduction in reproductive performance in the years of incomplete vaccination in the alternative model compared to the standard model more than outweigh the benefits of reduced vaccination costs in the former.

Vaccination of all breeding ewes was predicted to result in a mean of an additional 6.4 lambs per 100 ewes tupped per year. Accepting a mean of 38 kg for the liveweight of a lamb at slaughter and a value of 0.93 £/kg liveweight, this equated to a mean monetary value of £226 per 100 ewes tupped per year. This figure represents an estimate of the costs of reproductive losses caused by *Toxoplasma gondii* occurring each year in the intervention trial population that were preventable by vaccination.

In fact, this figure is almost certainly an underestimate of the true preventable costs since the models do not include all the possible economic benefits accrued through vaccination. An example of this is any effect on the costs associated with the premature culling of breeding ewes from the flock. The removal of ewes from the breeding flock before they have successfully completed five breeding cycles is associated with significant economic penalties, the magnitude of which increases with an earlier age of culling (Stubbings 1999). Vaccination resulted in a reduction of approximately 1.5 ewes per 100 tupped in the number of unproductive ewes. Unproductive ewes are more likely to be prematurely culled than productive ewes suggesting that vaccination is likely to result in reduction in the costs associated with such culls. Similarly,

the models do not include the additional costs potentially associated with caring for weak lambs. Adequate data were not available to allow the inclusion of these factors in the models.

The models estimate the results that would be expected by the sample population of the intervention trial. They clearly demonstrate the economic benefit of vaccination in a flock in which vaccination results in the production of an additional 6.4 lambs per 100 ewes tupped per year. The break-even point occurs when the additional returns achieved through vaccination exactly equal the additional costs incurred. The mean annual additional costs incurred by the standard regime of vaccination was £102 per year over the initial five-year period. In the years in which replacements only were vaccinated, additional costs averaged around £66 per year. If a mean lamb value at slaughter of £35.00 is accepted, this indicates that vaccination will break even around the point where an additional 3 lambs per 100 ewes is achieved per year. Assuming a vaccine efficacy of 75%, this indicates that any flock losing 4 or more lambs per 100 ewes tupped each year because of *T. gondii* infections would probably benefit from vaccination.

Whilst the above calculation is approximate, it is important because it addresses a fundamental question that is often ignored, namely how does an individual flock owner assess the economic viability of vaccination in his or her flock? This decision demands two crucial pieces of information. One is an accurate estimate of the annual losses attributable to *Toxoplasma* in the flock, and this will require the combined expertise of the flock owner, their veterinary surgeon and probably some external diagnostic facility. The other is the estimate of the level of reproductive loss caused by *T. gondii* at which vaccination will probably become viable. If this type of work is to genuinely support the decisions faced by stockholders, it must seek to provide this information. It should always be remembered that flocks in which the losses caused by *Toxoplasma* are close to this break-even point have a higher probability of experiencing years in which the costs of vaccination are not recovered than flocks in which losses are greater. The higher the annual losses attributable to *Toxoplasma*, the more certainty there is of a positive margin and the more compelling the justification for vaccination.

ACKNOWLEDGEMENTS

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EQUINE EPIDEMIOLOGY

AN EPIDEMIOLOGICAL INVESTIGATION OF MARE REPRODUCTIVE LOSS SYNDROME: BREAKING GROUND ON A NEW DISEASE

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SUMMARY

In late April 2001, an increased number of early foetal losses (45-60 days gestation) and late term abortions were noted by veterinarians and the state diagnostic laboratory in Lexington, KY, USA. This was the start of an abortion outbreak resulting in more than 1000 foetal losses and 500 late term abortions in central Kentucky. Full necropsies on all late term foetuses and 9200 diagnostic tests revealed no primary infectious aetiology or cause for the outbreak.

Highly unusual weather patterns during this time, multiple breeds involved and acute time related nature of the abortions indicated an environmental aetiology. Additionally, an unusually high emergence of Eastern tent caterpillars (*Malacosome americanum*) occurred in April and May. A field epidemiological investigation involving 133 farms was undertaken. Over 300 factors were evaluated on the farms including management practices, feed sources, pasture composition, etc. Information on more than 200 pastures was available for comparison of risk factors.

Results indicated that the moderate to high exposure of Eastern tent caterpillars by mares, breeding date in February and farms with >50 mares were risk factors for early foetal loss. Protective factors included low or no exposure to caterpillars and feeding hay to mares outside. Only four farms had only late term abortions, so risk factors for that part of the syndrome could not be determined in this study.

INTRODUCTION

Central Kentucky is one of the largest Thoroughbred breeding centres in the world with more than 18,000 mares being bred in 2000. Additionally many other types of horses are bred within the state. Thoroughbred stallion fees for 2001 ranged from $1000-$500,000. The equine industry is one of the top three economic industries for the state of Kentucky. Epidemics of any type, including abortions, are carefully monitored by farm veterinarians and managers, diagnostic laboratory personnel and extension veterinarians. Historically, the majority of aborted foetuses are submitted to the University of Kentucky Livestock Disease Diagnostic Center (LDDC) for a complete diagnostic and pathologic workup, as the work is completed at no charge.

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An acutely increased number of early foetal loss (EFL) and late term abortion (LTA) cases were brought to the attention of extension veterinarians at the Maxwell H. Gluck Equine Center during the last week of April 2001. With no aetiological agent determined by necropsy and other testing, this disease was termed Mare Reproductive Loss Syndrome (MRLS).

On an initial faxed survey to over 280 Thoroughbred farms in central Kentucky on May 7, farm managers whose mares had received an ultrasound examination, reported a 20% early pregnancy loss at approximately 60 days gestation. Pregnancy loss for Thoroughbreds has been studied, and from 40 days to gestation, an approximately 12% loss is normal (K. McDowell, personal communication).

The number of late term abortions necropsied at the LDDC were extremely high with 318 abortions received between April 28 and May 8.[1] Eighteen breeds and multiple counties in Kentucky were represented in the submitted materials.

As events unfolded, team leaders were formally assigned areas of organisation and investigation: pathologic studies, toxicological and experimental studies (including mycotoxins, ergot alkaloids, phytoestrogens, toxins, *etc*.) and field epidemiology survey involving 133 farms. This report reviews the results of an initial study.

MATERIALS AND METHODS

On May 7, 2001, questionnaires were faxed to the 270 members of the Kentucky Thoroughbred Farm Managers' Club. This group represents the most organised of breed associations in Kentucky and also has the most members in the Central Kentucky area which appeared to be the most affected. The purpose of this short questionnaire was to obtain information on the severity of the problem of early foetal loss through this sample population.

Numbers of late term abortions were well documented by the LDDC and were not analysed in this initial questionnaire. The following questions were faxed, and requested return by noon on May 9, 2001 (48 hours). Results of this survey were to be communicated at a public information forum on May 10, 2001 at 5:00 PM.

1. How many mares on the farm have been covered in 2001? _____
2. How many of the mares in question 1 were considered in foal when checked at 42 days of gestation? _____
3. How many of the mares in question 2 were still in foal as of May 7, 2001? _____
4. Location of farm (county) _____
5. Would you be willing to participate in a more detailed questionnaire, if deemed necessary? Yes/No If yes, please provide contact information.

RESULTS

Of the 270 questionnaires which were faxed, 159 (59%) responses were returned in 48 hours. Data were summarised within 24 hours utilising Microsoft Excel 97.

[1] http://www.uky.edu/Ag/VetScience/mrls/lddcdataupdate.htm

The farms providing data were primarily located in Fayette (Lexington, KY) and the surrounding counties of Woodford, Bourbon, Scott and Jessamine. Smaller numbers of responses were from nine other counties in Kentucky, all within the central region of the state, which also represented where most of the Thoroughbred farms were located.

Of the 159 farms, 37 (23%) reported no early foetal losses. Seventeen had over 50% early foetal losses; however these farms had 30 or fewer mares which were pregnant at 42 days and therefore at risk.

Over 8100 mares on the 159 farms had been mated in 2001. The number of mares considered in foal when checked at 42 days of gestation was 3294. Obviously, not all mares bred in 2001 as of May 7 would have reached 42 days of gestation. Of these 3294 mares, 2616 were still in foal on May 7 which represents a 79.4% pregnancy for mares bred in Spring 2001.

DISCUSSION

With an excellent return rate, this initial questionnaire confirmed the significant loss of early foetal deaths in central Kentucky. Normally there is a 12% loss of equine pregnancies between 40 days and term (K. McDowell, PhD, personal communication, June 2001). In this sample, about 20% of early foetuses were being lost between approximately 42 and 60 days of gestation. However, contrary to rumours widespread at the time, there were unaffected farms within central Kentucky, and losses of 50% or more were primarily on farms with low numbers of mares at risk.

Nonetheless, a 20% early foetal loss, coupled with significant late term abortions occurring at the same time was cause for extreme concern. Therefore, a scientifically based study was designed to evaluate factors on the farm, pasture and individual animal levels to attempt to determine risk factors and protective factors for MRLS.

The geographic distribution of the responses indicated that responses mirrored the general distribution of farm managers of the Kentucky Thoroughbred Farm Managers' Club, but information from LDDC showed that late term abortions were occurring throughout central Kentucky in multiple breeds.

Nearly all of the farms were willing to participate in a more detailed epidemiological study, and were the starting point for contacting the 133 volunteer farms.

Recognised causes of abortions in mares include many infectious agents: equine herpesvirus-1, equine arteritis virus, *Ehrlichia risticii*, *Streptococcus* spp, *E. coli*, *Pseudomonas*, *Klebsiella* and other bacteria. Bacteria usually cause an ascending placentitis which interferes with foetal growth (Merck, 1998). *Nocardiaform actinomycetes* and *Leptospira* spp have also been associated with abortions in central Kentucky (Donahue & Williams, 2000). Non-infectious early embryonic loss is not well understood in the mare and is the subject of ongoing research (Zavy & Geisert, 1994).

CONCLUSIONS

With any disease outbreak, communication is paramount to understand the extent of the problem and extinguish erroneous rumours. Because of an existing database and fax capabilities

on 270 Thoroughbred farms, information on the extent of early embryonic loss could be estimated through a faxed questionnaire. Prior knowledge indicated that a 20% foetal loss by 60 days gestation was high and, with the concurrent late term abortions, needed further investigation. Considering that unaffected breeding farms were also in the geographic area of farms with significant losses further indicated that a larger epidemiological study was required. This preliminary study provided basic facts which were essential to the undertaking of the subsequent study.

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MODELLING EQUINE INFLUENZA: EPIDEMIOLOGY, VACCINATION, SPATIAL SPREAD AND STRAIN VARIATION

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SUMMARY

A stochastic model of equine influenza (EI) was constructed to assess the risk of an outbreak in a thoroughbred population at a typical flat racing yard. The model was parameterised from clinical data and also published data on previous epidemics.

Using unique data from the Animal Health Trust (AHT), a functional relationship between pre-challenge antibody and probability of becoming infectious was derived. Changes in antibody level were quantified, again using data from the AHT.

A realistic population was examined over the course of a year and the model used to assess the risk of an outbreak of EI in the yard under the current minimum vaccination policy in the UK. The model was adapted to consider an alternative vaccination strategy and risk of outbreak was again quantified showing that plausible changes offer a significant increase in protection.

Further data were used to examine scenarios where there was a mismatch between the vaccine strain and the circulating strain. Spread of infection between yards was also considered to ascertain the risk of secondary outbreaks.

INTRODUCTION

Equine influenza is a highly contagious infectious disease of equidae, which in fully susceptible animals causes a high temperature, harsh cough, and mucopurulent or serous nasal discharges. Secondary bacterial infections cause significant problems (Sarasola et al., 1992) and broncho-pneumonia occurs in a proportion of cases. In partially immune animals, the signs of disease are moderated and may just consist of a mild cough or mucopurulent nasal discharge (e.g. Newton et al., 2000).

Vaccination against equine influenza has been practised since the 1960s but although vaccines have improved considerably since then, there are continued problems with failure of efficacy under field conditions. Most products available internationally consist of whole killed virus, or sub-unit vaccines. The datasheets for most licensed equine influenza vaccines in

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Europe recommend that an annual booster dose of vaccine be given after an initial course of three doses.

In this paper, we construct and parameterise a stochastic model of equine influenza to assess the risk of an outbreak in a flat racing yard under this recommended dosing schedule (which also represents the minimum vaccination policy under the Jockey Club rules in the UK). A stochastic model is essential when dealing with relatively small populations as chance events (such as failure of the infection to transmit) become important (Grenfell & Dobson, 1995). The model assumes that all horses in a yard are in one of four states: susceptible to infection (*S*), exposed to infection but not yet infectious (*E*), infectious (*I*) and recovered (*R*). The *SEIR* model is parameterised from clinical data and also published data on previous epidemics.

A key component of the model is a functional relationship between pre-challenge antibody and probability of becoming infectious (given exposure) which was derived from quantitative evaluation of data from equine challenge experiments performed at the Animal Health Trust. By using this relationship in conjunction with the model it is possible to simulate epidemic development in a yard provided that antibody levels of all the horses in the yard are known (other complicating factors such as horse age, gender and vaccine history need not be considered).

A realistic yard population structure, which takes account of population dynamics over the course of a year in a flat racing yard (e.g. sale of older horses and purchase of yearlings), is incorporated into the model which is then used to assess the risk of an outbreak of equine influenza in the yard under the current minimum policy in accordance with Jockey Club rules. A key preliminary finding of the models was that small epidemics are far more likely than large epidemics (Glass et al., 2002) and these small outbreaks could be responsible for maintaining equine influenza in the population at large. Consequently, our definition of risk includes small outbreaks and throughout the paper we ask: if equine influenza were introduced to the yard (from an external contact), what are the probabilities of epidemics affecting 3% and 10% of the yard population.

The model is then adapted to consider an alternative vaccination strategy (where the frequency of vaccination of older horses is increased) and the probabilities of both small and large epidemics are again calculated, providing a quantitative comparison between the current minimum policy and a plausible alternative.

A key feature of equine influenza biology is viral evolution, termed antigenic drift, which can, as in man, result in compromised vaccine efficacy when there is a mismatch of vaccine and circulating strains (Mumford, 1998; Newton et al., 1999). In equine influenza, there is also co-circulation of two H3N8 sub-lineages, which have evolved over the last 10 to 15 years. Although viruses from these two sub-lineages cross-react serologically, there is significant reduction in vaccine derived protection against heterologous viruses (Yates & Mumford, 2000). Accordingly, in our model, further data are used to extend the functional relationship between pre-challenge antibody and probability of becoming infectious to consider scenarios when there is a mismatch between the vaccine strain and the circulating strain, thus building a model which can include the effects of virus drift.

Finally, a two-yard model is implemented to address the question of risk of transmission between yards. This can occur locally at shared training areas such as gallops and nationally at

race meetings. This two-yard model is the beginning of a more complex model which can look at large spatial scales, up to the national level.

MATERIALS AND METHODS

Transmission parameters

The stochastic SEIR model uses the three key epidemiological parameters for equine influenza: latent period ($1/a$), infectious period ($1/g$) and transmission rate (β). The first two rates (a and g) have been estimated from clinical observations of vaccinated animals (Table 1). The transmission rate is notoriously difficult to estimate but was successfully done for equine influenza in unvaccinated animals (Glass et al., 2002). It is not entirely clear how vaccination changes the transmission rate. In this paper we consider all values for this rate up to and including that for unvaccinated animals (Table 1). In the presentation of some results (see next section) we consider a plausible intermediate value of the transmission rate.

Table 1. Model parameters

Name	Symbol	Value
Latent period	$1/a$	1.25 days
Infectious period	$1/g$	3.1 days
Transmission rate (unvaccinated)	β_u	1.85 days^{-1}
Transmission rate (vaccinated)	β	$0 \le \beta \le \beta_u$ days^{-1}

The stochastic model uses the rates (a, g and β) to calculate the probabilities of both when and which events occur in the standard Monte Carlo fashion (Bartlett, 1961).

A realistic British Thoroughbred flat race training yard population structure is used. Typically there are around 100 horses in the yard. The age-structure of the yard is noted as yearlings and older horses have different vaccine dosing schedules. In the racing industry all horses have their official birthday on January 1 and so throughout the year no horse changes age class. The yard contains 4 age classes: yearlings, 2-year-olds, 3-year-olds and 4-year-olds.

Some older horses (2-, 3-, and 4-year-olds) are lost from the yard throughout the second half of the year (July to December), for example through injury or a sale to a stud farm. The probability of departure is assumed to follow a normal distribution. Yearlings enter the yard in the latter part of the year. The probability of an admission is assumed to follow a lognormal distribution with the peak in late October to coincide with the peak in yearling sales. The mean number of horses from each age class to leave and enter the yard over the entire year is prescribed in the model as are the initial number of horses in each class (Table 2). The mean number of horses to leave and enter on a given day is simply the product of the prescribed mean and the value of the normalised distribution (normal or lognormal) on that day. This daily mean is then used in a realisation of a Poisson process to determine the actual numbers of horses from each age class departing and entering the yard on a given day.

Because the population dynamics in the yard are stochastic (e.g. it is most likely that a yearling will enter in late October but it could be as late as December) many replicates of the model are used to ensure a true picture of typical flat training yards. A typical replicate is

included which shows a characteristic dip in the population size around September as older horses generally leave the yard before the new yearlings arrive (Fig. 1).

Table 2. Initial number of horses and average number of imports and exports over the year

Age	Initial number	Mean imports per year	Mean exports per year
Yearling	0	52	n/a
2 y.o.	52	n/a	8
3 y.o.	44	n/a	40
4 y.o.	4	n/a	4

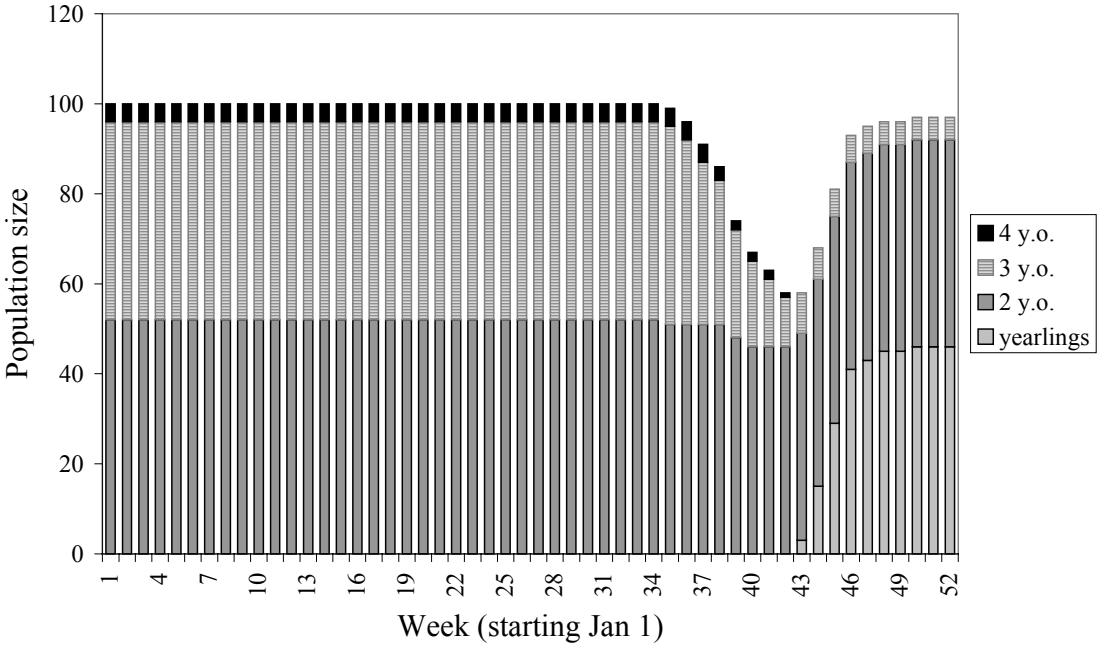

Fig. 1 An example of the population dynamics within a yard over the course of a year.

Vaccine derived antibody levels

A yearling coming into the yard will either be immunologically naïve to influenza or will have been vaccinated previously. (Infection is not common on stud farms). This affects their dosing schedule, as previously vaccinated yearlings often have to wait for a designated time until it is appropriate for further doses to be administered. Here, we assume that in previous and current years 25% of incoming yearlings are naïve and 75% have previously been vaccinated. Consequently, each age class is subdivided into 'naïve' and 'previously vaccinated' categories.

Published and unpublished experimental data were used to estimate the mean antibody level of a horse vaccinated under the current minimum policy at any point during the year. These same data showed that the variability in the antibody levels between horses is approximately constant over time and this is assumed in the model. The distribution of antibody levels at any

time fitted a normal distribution. The mean antibody levels under the current minimum policy are given in Fig. 2.

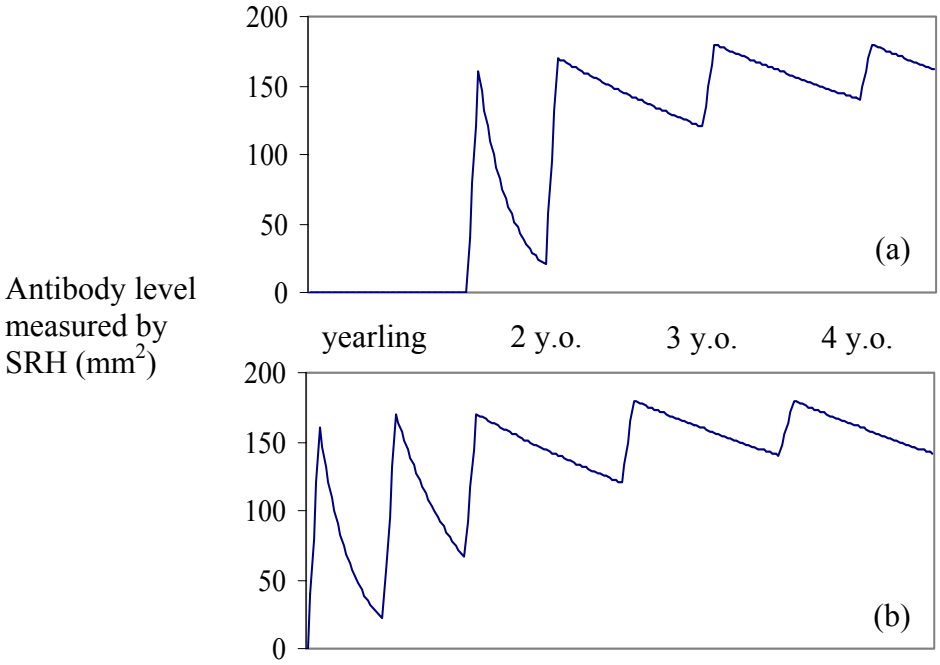

Fig. 2 Mean antibody levels over a four year period (yearling to 4 y.o.) measured by single radial haemolysis (SRH) for (a) horses entering the yard naïve to vaccination; and (b) horses vaccinated prior to entering yard under the current minimum policy.

An alternative strategy in which 2-, 3- and 4-year-old horses are vaccinated every 6 months (as opposed to annually) is also investigated and the mean antibody levels for this scenario are given in Fig. 3.

Relationship between infectivity and antibody levels

We have evaluated data from many of our experiments and have shown a relationship between pre-challenge antibody level (conferred by vaccination) and the probability of becoming infectious upon challenging with a homologous virus strain (Fig. 4). Pre-challenge antibody is measured by single radial haemolysis (SRH) and virus excreted is measured as $\log_{10}$(total titre excreted). All unvaccinated horses excreted virus whereas some of the vaccinated horses excreted no detectable virus. The probability of excreting virus is high if the pre-challenge antibody level is low and *vice versa*. Non-linear regression of these data suggested the best fit to this relationship was sigmoidal (Fig. 5).

Thus, for every horse in the yard in a given week there is an antibody level conferred by vaccination. For each antibody level there is an associated probability of becoming infectious upon challenge. As antibody levels change over time, for each week we use these probabilities (together with the binomial function) for each horse to determine whether the horse should be put into the susceptible class (*S*) or the removed class (*R*). The latter class assumes the horse plays no part in the epidemic process and corresponds to a horse that excretes no virus. Many of these binomial trials are performed in replicates to ensure that a representative picture of the number of protected horses is obtained.

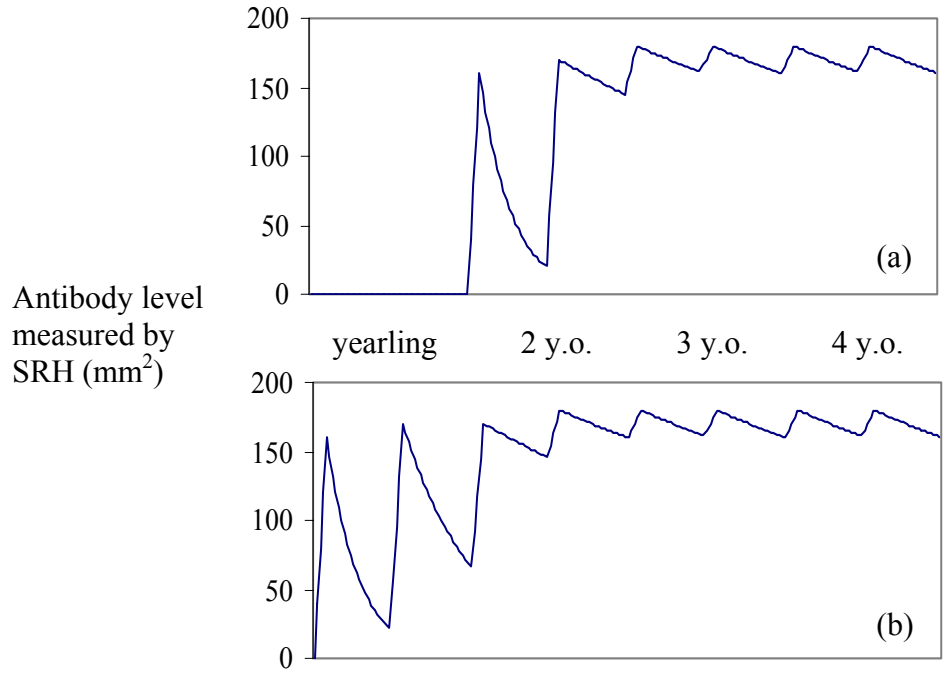

Fig. 3 Mean antibody levels over a four year period (yearling to 4 y.o.) measured by single radial haemolysis (SRH) for (a) horses entering the yard naïve to vaccination; and (b) horses vaccinated prior to entering yard under a strategy where all horses are vaccinated every 6 months.

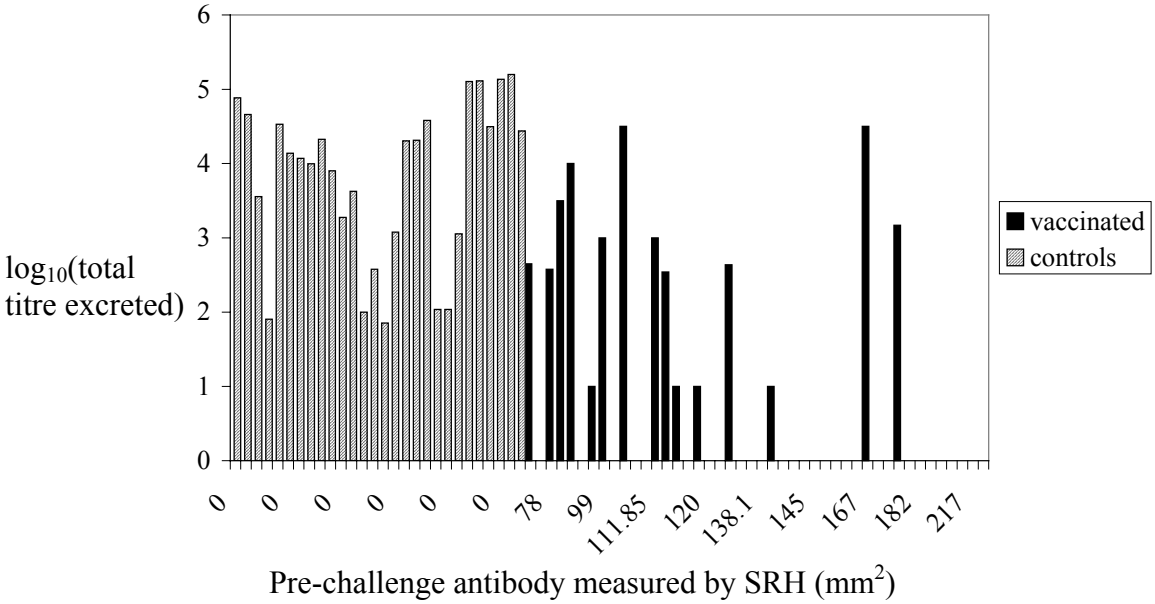

Fig. 4 Total titre excreted by vaccinated and unvaccinated ponies as a function of their pre-challenge antibody level.

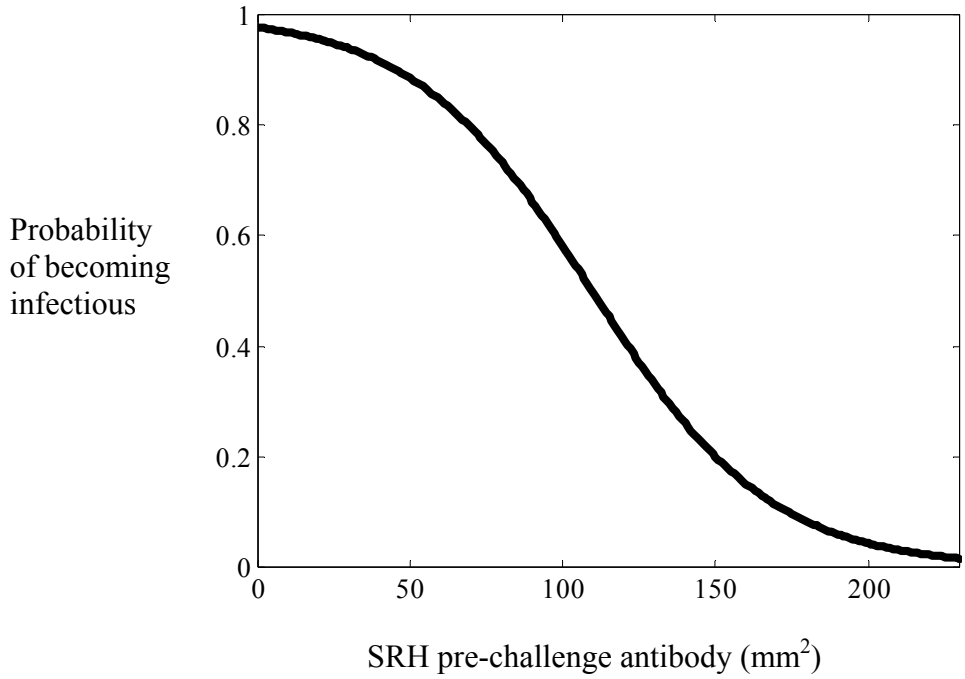

Fig. 5 Non-linear regression of homologous challenge data giving the probability of becoming infectious as a function of antibody level.

Once all of the horses have been put into either the *S* or *R* class, one of the susceptible horses becomes infectious (*I*) to initiate the epidemic. The model is allowed to run until all the infection has died out of the population and the size of the epidemic is recorded. Again, many replicates of the model are used to give a reliable central estimate of risk. After all simulations have been completed, the probabilities of an epidemic affecting at least 3% and at least 10% of the population are calculated for that week.

Antigenic drift

We have used further experimental challenge data to parameterise our model to estimate the effects of challenge of yards with strains of virus not in the vaccine used, as is the case for many vaccines around the world. These experiments were designed to assess, at the individual animal level, the effects on vaccinal immunity of the evolution of circulating H3N8 viruses into two sub-lineages (the 'American like' and the 'European like' branches). We were particularly interested in the effects on herd immunity of challenge with a virus from a different sub-lineage to that in the vaccine

The functional relationship between pre-challenge antibody and probability of becoming infectious upon challenging for cases when the vaccine strain belonged to one sub-lineage and the challenge strain belonged to the other (Fig. 6). The key difference between this heterologous relationship and the homologous relationship shown in Fig. 5 is that when there is a mismatch between the vaccine and circulating strains, the probability of becoming infectious does not approach zero when SRH antibody levels get high (>150mm^2). Rather, the probability of becoming infectious remains high even when the response to vaccine is optimal. This is consistent with field observations, where vaccine breakdown has occurred in the presence of high antibody levels (Newton et al., 1999). Again, the risk of small and large epidemics in a yard

are calculated for this new scenario (where the dosing schedule reverts to the current minimum policy under Jockey Club rules).

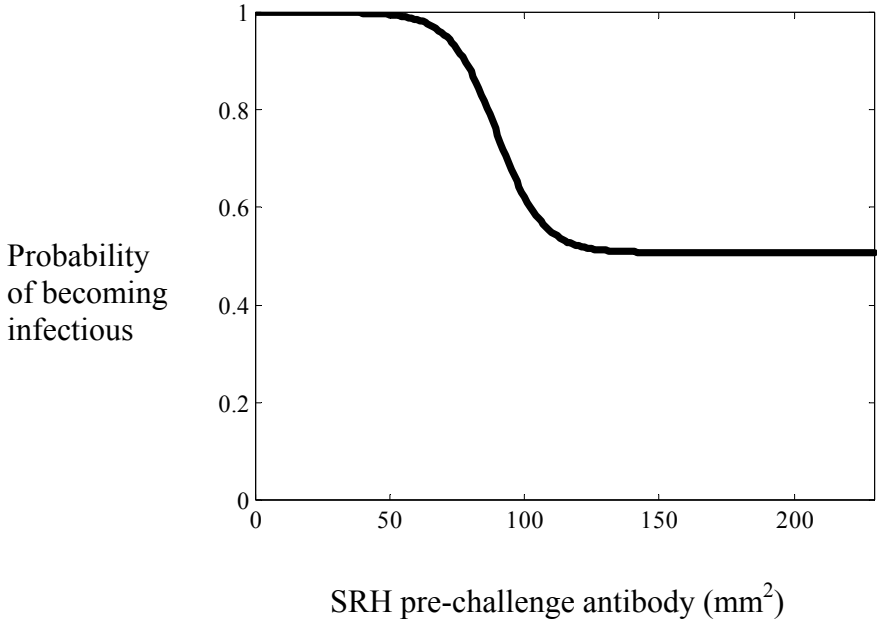

Fig. 6 Non-linear regression of heterologous challenge data giving the probability of becoming infectious as a function of antibody level.

The model is adapted to couple together two similar yards one of which contains one infected horse initially. The coupling is due to mixing at a shared training facility (such as racing gallops). The force of infection experienced by yard A is given by

$$\lambda_A = (1-\mu)\beta I_A / N_A + \mu\beta\left((I_A + I_B)/(N_A + N_B\right),$$

where I_A and I_B are number of infected horses belonging to yards A and B, respectively, N_A and N_B are the total number of horses belonging to yards A and B, respectively and μ is the proportion of time spent at the shared training facility. The force of infection experienced by yard B is derived in a similar manner. The model assumes the transmission rates within a yard and between yards are the same. This model is used to assess the risk of transmission between yards.

RESULTS

The following results address the question: If equine influenza were to enter the yard (from an external contact) in a given week of the year, how likely is it that there will be a small epidemic or a large epidemic. Probabilities of a within-yard outbreak affecting 3% and 10% of the yard population under the current minimum strategy are presented as three dimensional surface plots (Figures 7 and 8, respectively). The x axis represents the transmission rate for equine influenza in vaccinated animals and the y axis represents the week of the year under consideration. The z axis represents the probabilities mentioned above.

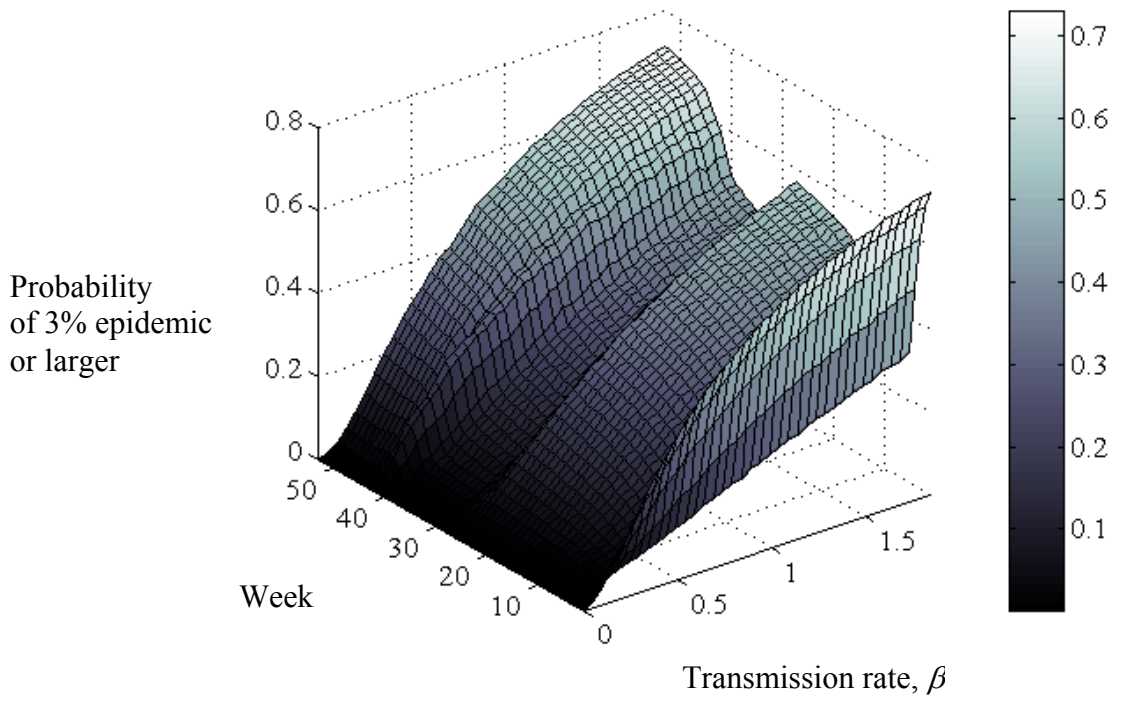

Fig. 7 Surface plot showing the probability of an epidemic affecting at least 3% of the yard population under the current minimum vaccination policy as a function of the transmission rate and the week of the year (week 1 represents the week beginning Jan 1)

Currently, there is uncertainty as to the value of the transmission rate, β. However, in vaccinated animals it is assumed to be less than (or equal to) the transmission rate in unvaccinated animals, which was found to be $\beta_u = 1.85$ (Glass et al., 2002). Once a good bound on the transmission rate is obtained it is possible to quantify the risk of within-yard outbreaks. For a particular value of β, we see that the probability of an epidemic varies throughout the year. Periods of high risk correspond to periods when the antibody status of horses in the yard is generally low. Also, as the transmission rate increases, the probability of an epidemic increases asymptotically towards one. Although this is an intuitive result, the rate at which the asymptote is approached will be a useful guide in assessing risk against the backdrop of virus evolution. For clarity and comparison, subsequent results focus on a plausible intermediate value of $\beta = 1.0$.

The alternative strategy which we put forward here involves all horses being vaccinated every six months after their initial course of three doses. The probabilities of both small and large epidemics are reduced under this vaccination strategy (Fig. 9). At certain times of the year the reduction in risk is highly significant and these times frequently correspond to peaks in racing activity.

It is also possible to observe the effect of a mismatch between the vaccine strain and the circulating strain as outlined in 'Materials and methods' (Antigenic drift). If there is such a mismatch the probability of both small and large epidemics increases (Fig. 10). Further, the risk of an outbreak is approximately constant over time.

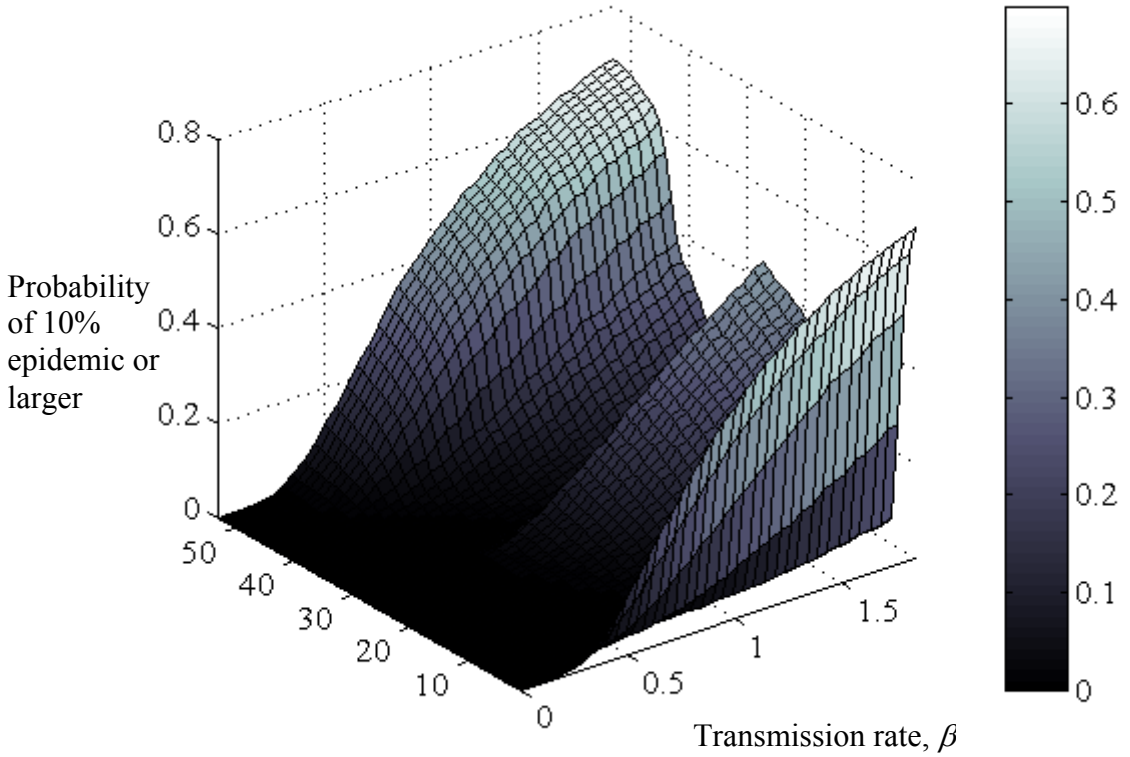

Fig. 8 Surface plot showing the probability of an epidemic affecting at least 10% of the yard population under the current minimum vaccination policy as a function of the transmission rate and the week of the year (week 1 represents the week beginning Jan 1)

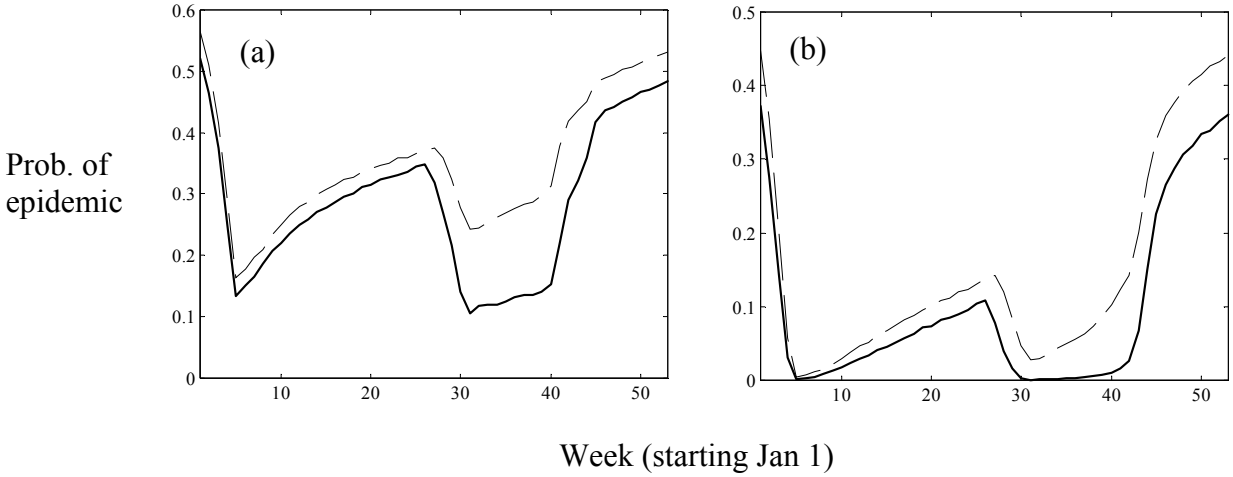

Fig. 9 Comparison of the probability of (a) a 3% outbreak or larger; and (b) a 10% outbreak or larger under the current minimum vaccination strategy (dashed line) and the alternative six monthly vaccination strategy (solid line). The transmission rate is taken to be $\beta = 1.0$.

Finally, we give preliminary results concerning the probability of an epidemic in a yard neighbouring one which has just 'recruited' the infection (Fig. 11). The yards are assumed to be coupled through a shared training facility such as gallops. The amount of time spent at the

shared facility by any horse is assumed to be 10 hours per week. A secondary outbreak is clearly possible and this refinement to the model is the beginning of a larger body of work which will investigate epidemic development in a spatial context.

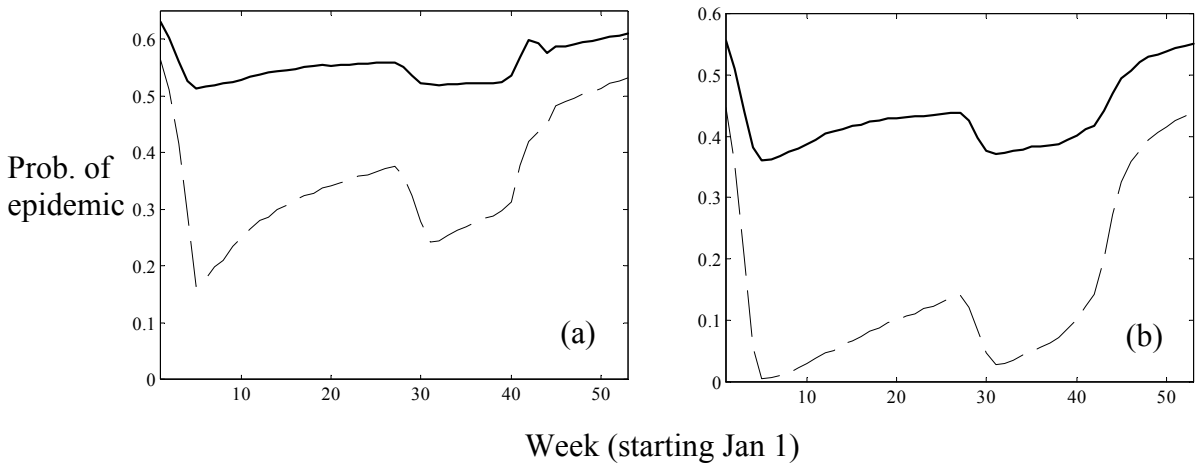

Fig. 10 Comparison of the probability of (a) a 3% outbreak or larger; and (b) a 10% outbreak or larger under the current minimum vaccination strategy with homologous vaccine and circulating strains (dashed line) and heterologous vaccine and circulating strains (solid line). The transmission rate is taken to be $\beta = 1.0$.

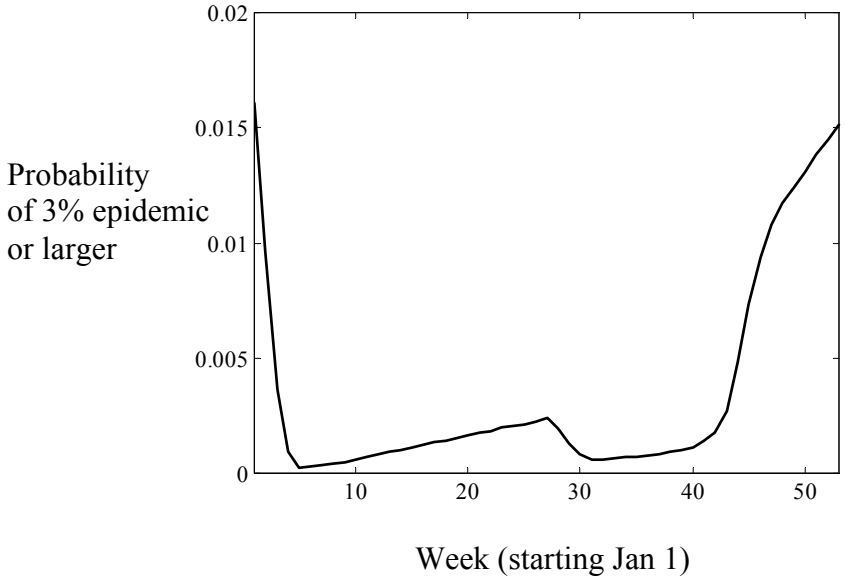

Fig. 11 Probability of an epidemic affecting at least 3% of a neighbouring yard population with shared training facilities with a yard that has just 'recruited' the infection from an external contact. Mixing time at shared facilities is assumed to be 10 hours per week and the transmission rate is taken to be $\beta = 1.0$.

DISCUSSION

We have constructed and parameterised a model that can predict the likelihood of an outbreak of equine influenza in a training yard of Thoroughbred racehorses. The latent period and infectious period for equine influenza in a vaccinated population are known from clinical observations. The transmission rate is more difficult to estimate. However, it is known for an unvaccinated population and this provides an upper bound to the transmission rate. Current on-going research is utilising data from the 1989 epidemic in the UK which will increase the accuracy of this key parameter, giving scope for the model to be a truly quantitative tool in helping to control equine influenza.

Establishing the relationship between antibody level and the probability of becoming infectious is a significant and novel aspect of the model and has been done for both homologous and heterologous vaccine and circulating strains. Its power lies in the fact that only the antibody levels of the horses needs to be known (further information such as horse age, gender and vaccine history is not required). Antibody levels and their dynamics are fairly easy to derive (e.g., from vaccine trial data sheets) and allow an unlimited choice of vaccination scenarios to be considered.

The model predicts that the alternative strategy (which involves increasing the frequency of vaccination in older horses to six months instead of one year) significantly lowers the risk of small and large outbreaks. This strategy is plausible both in terms of logistical operation and also in adhering to ethical practice. It may be possible to refine this strategy so that peaks in the level of protection coincide with periods of high risk (e.g., the high racing season). This is an important consideration which will form the basis for future work. Currently, we assume that the risk of 'recruiting' the infection into the yard is constant over time. In reality, the risk will depend on racing seasons and sales. Obtaining relative risks is fairly straightforward (e.g., by comparing the number of race meetings in certain weeks). However, establishing an absolute risk remains a more difficult challenge.

Risk of both small and large outbreaks increases significantly when the vaccine strain is heterologous to the circulating strain. Also, risk is approximately constant over time so that there is little difference in the probability of an epidemic following vaccination compared with periods when antibody levels are known to be low. The key difference between the homologous vaccine/circulating strain relationship and its heterologous counterpart occurs when antibody levels are high. For a vaccine strain that is homologous to the circulating strain, high antibody levels ensure that the probability of becoming infectious is very low (effectively zero). However, for a vaccine strain that is heterologous to the circulating strain the probability of becoming infectious is significant (approximately 0.5) even when antibody levels are high. Several licensed vaccines only contain one strain which means that if the 'wrong' strain were to come into the yard the outcome could be a large epidemic.

The preliminary spatial model which considers two yards coupled by a shared training facility shows that secondary transmission is a real concern. Currently the transmission rate at the shared training facility (such as gallops) is assumed equal to that within a yard. In reality the transmission rate on the gallops is likely to be much lower but because of the uncertainty over the transmission rate generally, they were assumed to be equal and the model provides a worst case scenario. Future work will build on this two yard model to consider spatial spread up to the national level. It is hoped that model validation will be achieved by examining spatial data for the 1989 epidemic in the UK.

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DIGESTIVE PARASITISM AS A RISK FACTOR FOR COLIC IN HORSES – RESULTS OF A MULTICENTRE CASE-CONTROL STUDY CONDUCTED IN EUROPEAN VETERINARY UNIVERSITIES

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SUMMARY

The colic syndrome is thought to be one of the main causes of death in horses. The aims of our study were first to assess the importance of colic amongst all of the causes of death in horses in veterinary universities, and secondly to evaluate digestive parasitism as a risk factor for death from colic.

The results of all post mortem examinations conducted on horses during 1997 and 1998 were compiled from nine European veterinary universities. Within the population, a sample was chosen in which to conduct a case - control analysis. The association between death from colic and the presence of gastro-intestinal parasitic lesions at necropsy was evaluated.

At the end of the study, a population of 1,265 horses was obtained. In each country, colic was the most common cause of death in horses (33%). Statistical analysis showed a significant association between the presence of parasitic lesions and death from colic (OR = 1.91; $p \leq 0.001$).

INTRODUCTION

In France, knowledge of the causes of horse mortality is still very limited whilst the importance of horses and ponies is rapidly growing at economic, emotional and sporting levels. Two previous studies, one reviewing the causes of death in a population of insured horses in France during 1995 (Leblond et al., 2000), the other a retrospective survey conducted among French speaking equine veterinarians in four countries (Leblond et al., 2001), showed that the main circumstances of death were colic, foaling and locomotor disorders. However, neither of these studies used representative samples of the general equine population, and they showed some conflicting results.

The colic syndrome, defined as abdominal pain of digestive origin, is a major pathological entity in the horse. Numerous risk factors, most frequently digestive parasitism, have been reported in the literature, (Cohen et al., 1995; Kaneene et al., 1997; Reeves, 1997b). Other factors also suggested include age, gender, breed, health history, diet, type of activity and weather conditions.

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In the first part of the study, the causes of mortality were determined from the records of post mortem examinations carried out on equine animals in European universities. Our aim was to evaluate the relative importance of each of these entities as causes of death and to make comparisons between European countries. In the second part of the study, potential links between fatalities associated with colic and parasitic injuries were assessed.

MATERIALS AND METHODS

Study Design

The survey was descriptive and retrospective. The target population consisted of all the equine animals subjected to post mortem examination in the European veterinary universities in 1997 and 1998.

Preliminary study

A preliminary questionnaire was sent to Belgian, French, German, British, Swiss, Italian and Spanish veterinary universities in order to:

- Estimate the number of post mortem examinations carried out;
- Estimate of the number of horses that died from colic each year;
- Obtain access to the data;
- Ascertain how the records were classified and how to gain access to them.

Certain veterinary universities performed few post mortem examinations for horses, and these were excluded from our study. Some universities did not answer our pre-questionnaire in spite of repeated requests and others refused us access to the data. In the end, nine European universities were selected and data were collected over two years, 1997 and 1998.

Data collection and analysis

The same two veterinarians collected the data manually in each university from the veterinary school of Lyon, France. Information was available on the age of the horse, gender, date of death and lesions diagnosed at the post mortem examination.

The causes of death were classified according to the anatomical site of the main lesion by the principal organ system affected: into digestive, locomotor, neurological, cardiovascular or respiratory disorders or into more than one of these categories; and according to aetiology: into tumour, unknown aetiology or other causes. A specific category was created for the horses under one year of age, because causes of death in foals are quite different from adults. The exact location of the lesions was recorded. Further criteria were used to categorise the nature of the disease (acute, chronic) or the aetiology (e.g., infectious). White's classification was used to describe the various categories of colic: non-obstructive, obstructive but non-strangulated, obstructive and strangulated, inflammatory or other (White, 1990).

The study of the association between death from colic and the presence of parasitic lesions at post mortem examination was conducted as a case - control analysis. A case was defined as a horse whose death resulted from colic of digestive origin (with or without euthanasia). The post mortem examination allowed precise definition of the type of colic and the location of the digestive injuries. The control was defined as the following horse in which a post mortem

examination was performed in the same institution and whose death was unrelated to the digestive system. Gastro-intestinal parasitism was defined as the presence of parasitic lesions or parasites at necropsy. Wherever possible, the specific parasites were identified. The other factors studied included the age, gender, season and country of origin.

To facilitate the analysis, the data were split into several groups. Age groups were defined as follows: [0 - <1], [1 - <5], [5 - <10], [10 - <15] years of age and ≥15 years old.

The data were organized and analysed using the EPI - INFO version 6.1 software (Centers for Disease Control / World Health Organization) and Statview (Abacus Concepts). The results were expressed as the exact number or percentage of occurrences for the qualitative variables and the mean (± standard error) for the quantitative variables. Comparisons of qualitative variables were made using bilateral χ^2 tests. A difference was considered significant when $p \leq 0.05$.

The association between the cause of the death (colic versus other) and the risk factors (parasitism, age, sex and season of death) was first evaluated through a univariate logistic regression and the odds ratios and 95% confidence intervals were calculated for each variable. When $p \leq 0.05$, the association was considered significant. A multivariable logistic regression model was constructed, using a backward step by step procedure, including all variables in the initial model for which the association with death from colic showed a significance with $p \leq 0.25$ (Bouyer et al., 1993).

RESULTS

The first objective of the study was to describe the causes of mortality of horses according to their physical characteristics and geographical origin.

Description of the sample

A population of 1,265 equine animals was obtained (Table 1). Cases originated from Belgium (n=275, 21.7%), France (n=81, 6.4%), Germany (n=511, 40.4%), Switzerland (n=178, 14.1%) and the United Kingdom (n=220, 17.4%). The study population consisted of 78% horses, 20% ponies and 2% asses. The proportion of ponies was higher in the United Kingdom (n=60, 27.3% versus 19.5% in the whole population; p=0.0003).

The records for the deceased equine animals provided information about age in 93% cases (n=1182). The mean of the age in the whole population was 8.1 (±7.1) years. In Germany, foals less than one year old comprised a higher proportion of the population than in other countries (n=111, 23.5% versus 18.1% in the whole population; p = 0.015).

The gender was available in 96.6% of the cases. A parity of the genders was observed with 50% females and 50% males (22% stallions and 28% geldings). Seventy three percent of stallions were less than one year old. The proportion of stallions observed in France was higher than expected (n=27, 35.5% in the French sample versus 22.3% in the whole population; p=0.017).

Table 1. Distribution of the causes of death among all equines in the studied population according to their geographical origin (percentages are given in parentheses)

Cause	Belgium	France	Germany	Switzerland	UK	Total
Digestive disorder	130 (47.3)	16 (19.8)	211 (41.2)	63 (35.4)	72 (32.7)	492 (38.9)
Foal (<1year old)	43 (15.6)	10 (12.4)	112 (21.9)	22 (12.4)	24 (10.9)	211 (16.7)
Locomotor disorder	17 (6.2)	12 (14.8)*	33 (6.4)	30 (16.8) *	20 (9.1)	112 (8.9)
Nervous disorder	17 (6.2)	4 (4.9)	32 (6.3)	6 (3.4)	31 (14.1) *	90 (7.1)
Cardiovascular disorder	6 (2.2)	9 (11.1) *	27 (5.3)	7 (3.9)	19 (8.6)	68 (5.4)
Tumour	11 (4.0)	5 (6.1)	18 (3.5)	13 (7.3)	19 (8.6) *	66 (5.2)
Respiratory disorder	13 (4.7)	4 (4.9)	23 (4.5)	13 (7.3)	9 (4.1)	62 (4.9)
Urinary disorder	5 (1.8)	5 (6.2)	10 (2.0)	4 (2.2)	5 (2.3)	29 (2.3)
Others	25 (9.1)	10 (12.4)	34 (6.7)	11 (6.2)	14 (6.4)	94 (7.4)
Unknown	8 (2.9)	6 (7.4)	11 (2.2)	9 (5.1)	7 (3.2)	41 (3.2)
Total	275 (100.0)	81 (100)	511 (100)	178 (100)	220 (100)	1265

* $p<0.001$

Description of the causes of the death

The reported causes of death are summarized in Table 1. In all countries, diseases of the digestive tract represented the most common cause of death. Colic represented 85.6% of these digestive disorders (n=421), diarrhoea was the second most frequent problem encountered (n=54, 11% among digestive disorders). The frequency of other causes varied depending on the country.

Specific causes of death in foals were described for equine animals less than one year old. Ninety-eight had infectious diseases (50.3%), 21 (10.8%) had congenital malformations and 14 (6.5%) died following a digestive disorder. *Escherichia coli* was the most frequently isolated infectious agent. In this population, the proportion of stallions was higher than expected ($p<<0.001$), and the proportion of ponies lower than expected ($p=0.01$).

The aetiology of locomotor disorder included fractures in 37.5% cases (n=42), followed by joint diseases in 22.3% (n=25) and laminitis in 10.7% (n=12). Case fatalities due to locomotor disease were comprised a higher proportion of fatalities in Switzerland than in other countries ($p<0.001$). Among horses that died following a fracture, the age group ≥15 years old comprised a smaller proportion than expected ($p=0.012$).

Nervous diseases and tumours were more frequently diagnosed in the United Kingdom ($p<0.001$). Grass sickness was the most frequently diagnosed nervous disease (n=20 cases 21.3% of nervous diseases) and led to the death of 8 horses in the United Kingdom. In Germany, 10 deaths were attributed to the Borna virus disease. This infection was never identified in the other countries.

Among the tumours, lymphosarcoma was diagnosed in 25.8% cases (n=17), followed by carcinoma (16.7%, n=11), melanoma (13.6%, n=9) and adenoma of the pituitary gland (9.1%, n=6). These animals had a mean age of 14.8 (±6.9) years and the category of horses older than 15 years of age was over-represented ($p<0.001$). The percentage of tumours having led to death was higher in the United Kingdom than in other countries ($p<0.001$).

Case-Control analysis

The study of the relationship between death from colic and the presence of parasitic lesions was conducted in 421 equine animals that died from colic and 421 controls.

Description of the cases of colic: The most frequently represented types of colic were colic due to inflammation (29.4%), obstructive and strangulated colic (28.7%) and non obstructive colic (26.6%). The obstructive but non strangulated category of colic represented 10.8% of all cases and was more frequent in ponies ($p=0.045$). The distribution of lesions among colic cases is described in Table 2.

Colics can also be described according to the affected segment of digestive tract. The results are presented in Fig. 1. Thirty nine percent of colic of large intestinal origin were diagnosed in the [5 - <10] years age group, significantly more than in any other age group (n=19, $p=0.024$).

Table 2. Aetiological diagnosis in 421 horses that died from colic (number of cases in brackets)

Category of colic[a]	Type of lesion	Anatomical location
Inflammatory (122)	Peritonitis (49)	
	Parasitic lesions (24)	Stomach (10)
		Caecum (8)
		Small intestine (5)
		Large intestine (1)
	Enteritis (24)	
	Enterotoxaemia (14)	
	Colitis (7)	
	Typhlitis (4)	
Obstructive and strangulated (119)	Torsion (69)	Small intestine (33)
		Large intestine (33)
		Caecum (2)
		Small colon (1)
	Hernias (21)	Small intestine (20)
		Large intestine (1)
	Intussusceptions (15)	Small intestine (7)
		Caecum (6)
		Large intestine (2)
	Pedunculated lipoma (14)	
Non obstructive (111)	Rupture (72)	Stomach (29)
		Small intestine (20)
		Caecum (11)
		Large intestine (9)
		Rectum (9)
	Infarction (15)	Small intestine (9)
		Large intestine (6)
	Distention (9)	Small intestine (5)
		Stomach (3)
		Caecum (1)
	Ulcers (9)	Stomach (8)
		Large intestine (1)
Obstructive non strangulated (45)	Stasis, impaction (6)	Stomach (6)
	Obstruction (31)	Large intestine (17)
		Small intestine (5)
		Caecum (5)
		Rectum (2)
		Oesophagus (2)
	Displacement (8)	Large intestine (4)
		Small intestine (2)
		Caecum (2)
	Enteroliths (1)	Large intestine (1)
Others (19)		

[a] some cases may have more than one corresponding finding

Description of the parasitic lesions: 17.7 % of the deceased horses in our selected sample had parasitic lesions (n=149). The main species of parasites observed at necropsy were *Strongylus* spp. (11.9%, n=53), *Gasterophilus* spp. (11.2%, n=50), *Anoplocephala* spp. (8.9%, n=39), *Cyathostomum* spp. (6.5%, n=28) and *Ascarides* spp. (1.7%, n=7).

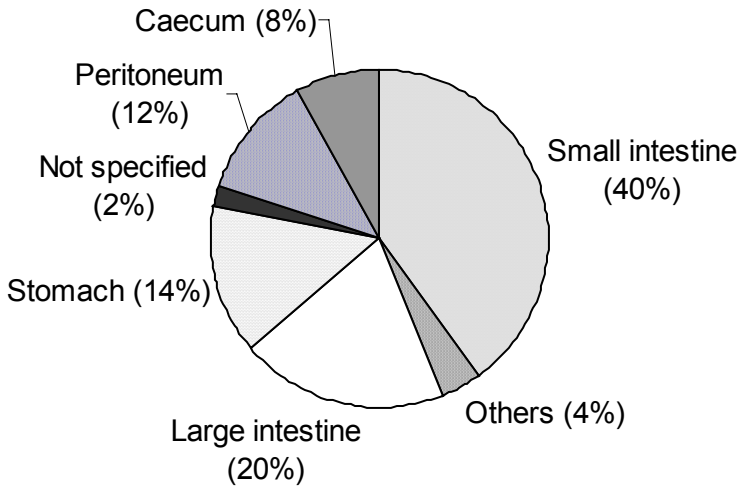

Fig. 1 Anatomical location of lesions in the 421 colic cases

There was no significant relationship between parasitism and gender (p=0.21). Ponies and asses were more frequently infested by *Strongylus sp.*, *Gasterophilus sp.*, *Cyathostomum* spp. and *Anoplocephala* spp. than horses (p<0.001, p<0.001, p=0.003 and p=0.02 respectively). Thoroughbreds were less frequently infested than other breeds (p=0.036). In the age group [1 - <5] the number of horses presenting parasitic lesions due to *Strongylus* spp. was higher than the expected value (29.9%, n=43 versus of 18.4% in the whole population, p<0.001). Seventy three percent of horses with *Cyathostomum* spp. infestation were less than 5 years old (n=19, p<0.001). The number of horses infested by *Strongylus* spp. or *Gasterophilus* spp. was significantly higher in the autumn (p=0.018 and p<0.001 respectively). This was consistent with our knowledge of the parasitic cycles and an infestation occurring mainly in summer.

The study of the association between the type and location of colic and parasitism showed a higher percentage of inflammatory colic among horses having *Gasterophilus* spp. or *Cyathostomum* spp. infestations (p=0.01 and p<0.001 respectively). Obstructive but non-strangulated colic was more frequently associated with *Strongylus* spp. infestation (p=0.03). Horses infested with *Cyathostoma* spp. presented caecal lesions more frequently than other horses (p=0.018).

Association between colic and other factors: The descriptive statistics and odds ratios obtained for the risk of colic for the selected sample are presented in Table 3.

For the multivariable analysis, 764 observations were used. Age and gender remained significant confounding factors. The adjusted OR for the association of colic and parasitism in the final model was $OR_{adjusted} = 2.39$ ($1.55 < OR_a < 3.68$; $p = 0.0006$).

Table 3. Descriptive statistics and odds ratio obtained for the risk of colic in 421 cases and 421 controls

Factor	Cases	Controls	Odds Ratio	Confidence interval (95%)		P
Age (years)						
[0-1[	24	73	1			
[1-5[	73	90	2.47	1.42	4.30	0.01
[5-10[	99	68	4.43	2.54	7.71	<0.001
[10-15[	102	66	4.70	2.70	8.19	<0.001
>15	102	65	4.77	2.74	8.33	<0.001
Gender						
Stallion	47	119	1			
Gelding	100	138	1.83	1.20	2.81	0.01
Female	188	215	2.21	1.50	3.27	<0.001
Breed						
Heavy breeds	5	11	1			
French Warmblood	9	7	2.83	0.67	12.02	0.20
Thoroughbred	26	14	4.09	1.18	14.13	0.04
Arabian	5	17	0.65	0.15	2.77	0.74
French Standardbred	3	6	1.10	0.19	6.29	0.91
Quarter Horse	9	11	1.80	0.45	7.13	0.45
Pony	73	70	2.29	0.76	6.94	0.16
Ass	5	12	0.92	0.21	4.05	0.98
Parasitism						
No	327	366	1			
Yes	94	55	1.91	1.33	2.75	<0.001

DISCUSSION

Our purpose was to determine the causes of death in equine animals and their relative importance in Europe. The quality of a retrospective study depends directly on the quality of the information collected at the time of death, the mode and the duration of conservation of the data and their accessibility (Bouyer, 1993). By choosing the veterinary universities as sources of data, we answered these requirements. The choice was motivated by the large number of equine animals subjected to post mortem examinations in these institutions and by the fact that these examinations are systematic and performed under the supervision of an authoritative pathologist. The data were found to be reliable, relatively homogeneous and properly stored. Thus, errors arising from recall bias should have been minimised in this study. Nevertheless, some necropsy reports did not contain sufficient information on either the physical characteristics or activity of equine animals or on the history of the disease.

Not all of the universities asked to participate were willing to do so. This constituted a bias of selection with regards to the target population. Moreover, the study population came from a hospital environment and included referred animals, which limits the general applicability of the

results. The likelihood of coming to the hospital can be different for the cases and for the controls. Horses affected by colic are generally referred to specialised centres. Knowledge of the general populations of equidae in different countries is limited, so this bias of selection is difficult to evaluate and cannot be corrected during the analysis.

A bias also exists in the techniques of measurement. This bias is associated with the methods used to take measurements, obtain records or perform analyses. The study concerned several centres in different countries. The management of cases may differ between centres (methods of diagnosis, type of operation, surgeon,…). The methodology of the autopsies is potentially different from one centre to the next. For example, in Germany, a bacteriological examination is systematically performed for all digestive, nervous or systemic disorders. This was not the case for the other countries. Furthermore, parasitic infestation may be underestimated in control horses when compared in colic cases in post mortem examinations. Indeed, when the cause of death is of digestive origin, the digestive system is examined more meticulously and the presence of parasites or parasitic lesions is systematically noted on the reports. However, in educational institutions, a complete systematic examination is usually performed.

For the purpose of our study, we established a unique classification of the causes of mortality. The reports of post mortem examination provided a precise diagnosis that was not always easy to integrate into our classification. Several major injuries were sometimes described and it was not always simple to determine which was the cause of death of the animal. By limiting the data processing phase to only two persons, we reduced the bias of interpretation and classification. Furthermore, in every centre, help was available to interpret the records.

There is little information available on the relative importance of health problems in horses, especially in France (Powell, 1989; Traub-Dargatz et al., 1991; Lindner & Offeney, 1992; Reeves, 1997a). In a population of 480 dead horses studied in Liverpool by Baker & Ellis (1981), 3.1% of the post mortem examinations did not reveal the cause of death. In our population, 3.6% of deaths remained unexplained.

We followed the same classification of the causes of death as Baker & Ellis (1981), Barkley (1985) and Leblond (2000 & 2001). A questionnaire survey was performed in 1996 among French-speaking veterinarians to assess the relative importance of the causes of death in horses and compare their geographical distribution. The results of this study showed that colic represented the major cause of death, followed by musculoskeletal diseases (Leblond, 2001). In our study, locomotor disorders remained a major cause of death in all the countries. However, the presence of particular diseases such as Borna virus disease in Germany, grass sickness in the United Kingdom or the over-representation of foals in Germany, involved a different classification of the causes of death in these countries.

Regarding the study of colic cases, disorders associated with a poor prognosis occurred frequently in our study. Although intestinal rupture is not usually a frequent disorder, we recorded 20 cases (6.9% gastric rupture, 2.6% caecal rupture). The percentage of torsions was 7.8 % for the small intestine and 8.8 % for the large intestine, higher than the usual values quoted in the literature. Indeed, White (1990) reported 4.6% of torsions of the small intestine and Baker & Ellis (1981) recorded 5.4%. The proportion of colonic torsions has been reported to be 6% (White, 1990; Mair & Hillyer, 1997). The frequency of herniation of the small intestine in the bibliography has been reported to be 1 % (White, 1990), while in our study, a percentage of 4.75% was observed.

Conversely, the rate of obstruction was low, 1.2% of colic related cases for the small intestine and 4% for the large intestine. Baker & Ellis (1981) reported that 8.4 % of horses died following an obstruction of the small intestine, while White (1990) reported 19.5% of large intestine obstruction, although their study concerned live horses. The mortality rate of this disorder amounts only to 22 % (White, 1990). The low percentage of these cases observed in our study is probably explained by the recent progress in equine veterinary medicine, and therefore a better diagnosis and treatment of digestive obstructions in horses.

Parasitism is frequently incriminated as a risk factor of colic (Barclay et al., 1982; Edwards, 1986; Proudman & Edwards, 1993; Cohen, 1997; Proudman et al., 1998). According to our study, a significant link exists between parasitism and colic. These results confirm the findings of other prospective or case-control studies, where worming was implicated as a risk factor for colic (Cohen et al., 1995; Tinker et al., 1997). The fact that our study was performed with data concerning deceased equine animals, however did not allow us to consider all the potential risk factors identified by these authors.

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IS ISOFLURANE SAFER THAN HALOTHANE IN EQUINE ANAESTHESIA? RESULTS FROM A MULTICENTRE RANDOMISED CONTROLLED TRIAL

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SUMMARY

Perioperative complications, including mortality, are an important problem in equine surgical practice. One to two horses per hundred die unexpectedly. There is great interest in trying to lower the incidence, and we have focused on the choice of agent used to maintain anaesthesia. We performed a multicentre randomised controlled trial to compare the effects of isoflurane and halothane, both fully licensed products. We hypothesised that the death rate would be reduced in the isoflurane group as many benefits were measured in experimental studies. We analysed the study using mixed effects regression models and found no overall benefits of either drug, although the death rate was significantly reduced in horses aged 2 to 5 years that received isoflurane. We present our models in full and discuss our analytical strategy.

INTRODUCTION

It has been recognised for many years that equine anaesthesia carries a high risk of unexpected death – around one in 100 horses die from unforeseen perioperative death within seven days of an anaesthetic (Johnston, 1995). This figure compares with 1/10,000 in man (Lunn & Mushin, 1982) and around 1/600-1,000 in dogs or cats (Clarke & Hall, 1990; Dyson, 1998).

A large-scale observational study of equine anaesthetic fatalities conducted over six years identified several important risk factors for operations other than emergency abdominal surgery (Johnston, 2000). These included age, operation type, day of the week and time of surgery as well as drugs used for anaesthesia (premedicant, induction and maintenance agents). In particular, inhalational anaesthetic induction and maintenance, night-time and weekend operations and fracture repairs were associated with increased risk. After the first six months, risk increased with age of horse. Risk was significantly reduced in animals which received "total intravenous anaesthesia."

During the observational study, the most common approach to anaesthetic maintenance was to use halothane, but towards the end of it, isoflurane was licensed in the UK as an alternative inhalational maintenance agent in horses.

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Experimental evidence had suggested possible cardiovascular benefit of isoflurane over halothane (e.g. Steffey & Howland, 1978; 1980). However, no significant difference was found in the observational study between the mortality rate in animals maintained on isoflurane compared to those on halothane (Odds ratio (OR)=1.66, 95% confidence interval (CI) 0.7-3.6). There was some indication that, probably because of experimental evidence, horses perceived as being at higher risk were more likely to be given isoflurane.

As isoflurane was a newly licensed drug in the UK, with perceived safety benefits over halothane, it was important to know whether the drug was actually safer in clinical practice. Due to the problems of confounding in observational studies and the differences between experimental and general practice, it was clear that the only way to make a comparison of the drugs' relative safety was to perform a randomised controlled trial in general practice.

This paper reports the overall results of a randomised controlled trial comparing isoflurane and halothane in clinical anaesthesia in horses. Multivariable models, incorporating the effects of possible covariates or effect modifying factors were developed to explore the data, to compare the effects of the two drugs and to identify factors associated with perioperative death and complication. We also present models that evaluate the factors associated with specific causes of death. The general analytical and modelling aspects are described in some detail.

MATERIALS AND METHODS

Objectives

A randomised controlled comparison of isoflurane and halothane as anaesthetic maintenance agents in veterinary practice was performed, adequate to detect a 50% reduction in the perioperative death rate in horses receiving isoflurane compared to those receiving halothane. Secondary objectives were to consider specific causes of death as well as perioperative complications, also occurring within seven days.

Design

The study was a prospective, multicentre, randomised controlled trial conducted in a convenience sample of 35 equine surgical clinics in seven countries. Clinics agreed to enter into the trial all *equidae* due to undergo any operation under general anaesthesia and receive inhalational maintenance. Data were collected between May 1997 and September 1999.

Randomisation

Patients were randomised immediately prior to surgery to receive either halothane or isoflurane as maintenance agents using a schedule designated by the study managers. Premedicant and induction agents were chosen and recorded by the responsible clinician unless anaesthesia was induced with an inhalational agent, in which case the allocated maintenance agent was used for induction.

Data

Clinics completed one line diaries for each anaesthetic episode. Information recorded included date, time and type of operation, body position during surgery, and whether this was

changed, and anonymised codes for the surgeon and anaesthetist responsible. The horse's breed, age (in years or months), gender and stage of pregnancy, if appropriate, were recorded together with an assessment of anaesthetic 'risk' (low, medium or high) made by the clinician responsible for each case. All agents used for premedication, induction and maintenance of anaesthesia were recorded using standard abbreviations. Duration of anaesthesia from induction until the vaporiser was switched off was recorded in minutes. Additional treatments recorded were use of nitrous oxide (N_2O), intermittent positive pressure ventilation (IPPV), whether arterial blood pressure (BP) was monitored and whether a sedative was given during the recovery period. Quality of recovery was also assessed. Date, time and type or cause of perioperative death or non-fatal complication were recorded for each case.

Case definition

The primary outcome measure ("death") was whether or not the horse had died, including being euthanased, as a result of a perioperative complication, within seven days of induction of anaesthesia. Horses which died naturally or which were euthanased because of the severity of the lesion, or due to inoperable problems, were recorded as "put to sleep" (PTS) and excluded from analyses, as were multiple anaesthetic episodes within the seven day period. All cases classified as "deaths" were individually checked by one author (GMJ) and were assigned a specific cause of death on the basis of information provided by the submitting clinicians.

Study size and trial conduct

Initial sample size estimates were based on the expectation of a death rate of 1% in the halothane group and a 5% significance level for rejection of the hypothesis of no difference between the two agents. These suggested that around 12,000 randomised events were required to give the study 90% power to detect a 50% reduction of death in the isoflurane group. In fact, the death rate was much higher (1.6% overall) and when funds for the study were running out, a decision was made to re-evaluate the study size on the basis of this increased rate. Accordingly, data collection was ended when around 8,500 randomised episodes were available for analysis. At this stage 134 deaths had occurred; initially it was considered that 90 would be adequate. Wherever possible, we have followed the recommendations in the Consort statement when reporting the results from this study (Moher et al., 2001).

Statistical analyses

Data were analysed on an intention to treat basis. Statistical analysis was carried out using Egret and SAS. Following careful univariable and stratified contingency table exploration, detailed ordinary (OLR) and mixed effects logistic regression (MELR) modelling was performed. Models were developed using a manual, forward build-up approach. All variables that were associated with outcome ($P<0.4$) in univariable analyses were tested for inclusion in multivariable models. Terms were retained in models if they were significantly associated with outcome (Wald $p\leq0.05$) or if they significantly reduced model residual deviance (likelihood ratio statistic $p\leq0.05$). Initial modelling was performed in Egret and confirmed using PROC LOGISTIC and PROC GENMOD in SAS (v6.12). Mixed effects models were fitted in SAS (v8.2) using PROC NLMIXED. Mixed effects models were used to evaluate and adjust for the possible lack of independence of data from within each clinic. The fit of the models was evaluated using the Hosmer-Lemeshow statistic (Hosmer and Lemeshow, 1989) and evaluation of receiver operating characteristic (Greiner et al., 2000).

RESULTS

More than 11,000 anaesthetic episodes were recorded during the course of the study (Fig. 1). However, some clinics contributed a large number of non-randomised events which were excluded (some individual clinicians refused to participate and some case types were electively not included).

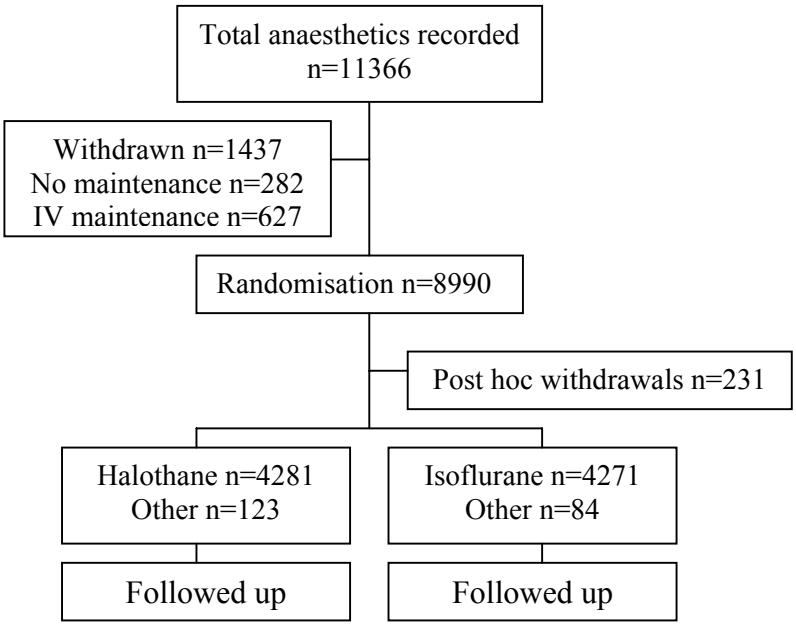

Fig. 1 Trial profile, as recommended in the Consort statement

The effectiveness of the randomisation in ensuring balance in respect of the various factors between the two treatment groups was evaluated by comparing the distribution of all covariates between the two randomised groups. With no exceptions, there was an equal distribution between them (differences<2%).

Overall results

A total of 8,242 randomised episodes were available for analysis, following the exclusion of 517 horses who were PTS. There were 134 deaths in total giving an overall perioperative mortality within seven days of 1.6% (95% CI 1.4-1.9%). If colic or other emergency abdominal surgeries were excluded, perioperative mortality within seven days was 0.9% (95% CI 0.8-1.3%).

Overall, there was no difference in all cause mortality between the two treatment groups (Table 1: $\chi^2=0.07_{1df}$, p=0.8), nor in the occurrence of non-fatal perioperative complications (Table 2: $\chi^2=1.8_{1df}$, p=0.2).

Table 1. Association between maintenance agent and all cause mortality

	Halothane	Isoflurane
Survived	4,080 (98.3%)	4,028 (98.4%)
Died (cases)	69 (1.7%)	65 (1.6%)
Total	4,149	4,093

Table 2. Association between maintenance agent and non-fatal perioperative complications

	Halothane	Isoflurane
No complication	3,961 (97.1%)	3,930 (97.6%)
Non-fatal complication (cases)	119 (2.9%)	98 (2.4%)
Total	4,080	4,028

Stratified analysis suggested that there was significant heterogeneity between different age groups in the risk associated with isoflurane. The overall risk of death was similar between isoflurane and halothane in horses of all age groups other than those aged between two and five years inclusive, where isoflurane was associated with a significant reduction in death rate (Table 3: $\chi^2_{1df}=11.3$, $p<0.001$). A similar significant reduction in non-fatal perioperative complications associated with isoflurane was also seen in this age group (data not shown).

Table 3. Association between maintenance agent and all cause mortality in horses aged 2-5 years

	Halothane	Isoflurane
Survived	1,346 (98.5%)	1,334 (99.7%)
Died (cases)	21 (1.5%)	4 (0.3%)
Total	1,366	1,338

Risk factors for all cause "deaths"

Mixed effects logistic regression models were developed as described. Using standard statistical significance rules for variable retention in models, convergence was achieved for very "over-fit" models, with far too many terms given the number of cases. Accordingly, only variables that were reasonably prevalent amongst the study population were retained in the model (Table 4). These variables included age, anaesthetic 'risk', operation type and an interaction between age of horse and maintenance agent. Although significant, it was difficult to justify inclusion of a term describing the effects of premedicant and induction agent as some combinations were only used in a few clinics. In the "drug" term, the combined use of ACP as a premedicant with benzodiazepines and dissociative anaesthetics as induction agents was associated with increased risk, although this was an uncommon combination (n=51/8242). The fit of models containing the terms described, with and without a drug term, was therefore compared. The HL statistic for the OLR model containing the drug variable suggested an unusually good fit (p=0.90), perhaps consistent with an overfit model, although that for the equivalent MELR model was lower (p=0.44). When drug was excluded, the HL statistic for the OLR model was similar to that from the MELR model (p=0.48 and p=0.55, respectively). The ROC curves for the two models were also compared and there was little difference apparent between the ability of the two models to discriminate cases and controls. (Further evaluation of

the positive predictive values of the models at different predicted value cut offs suggested that the drug variable only really increased discrimination of very high risk animals with predicted probabilities >0.1). We felt that, of the different models, the MELR model with no drug term was preferred and this is shown in Table 4. The coefficient values in this model were in fact the same (to the 1st decimal place) as those in the model including drug.

The model suggested that, even though there was no overall effect of treatment (halothane versus isoflurane), isoflurane was significantly safer in one age group (2-5 years inclusive), having adjusted for confounding variables. Risk was increased in both treatment groups in fracture operations and emergency abdominal surgery and lowest when the operation type was ENT or urogenital surgery. Comparing age groups, risk was lowest in yearlings.

When the model was fitted in SAS using PROC NLMIXED, addition of the random effects term did not result in a significant reduction in residual model deviance (LRS =2.46, p=0.12).

Other variables that were significant in the overfit model, but that were excluded from this final model included the monitoring of blood pressure (associated with reduced risk) and the use of intermittent positive pressure ventilation (increased risk).

Table 4. MELR model of the variables associated with the risk of perioperative "death"

	Alive	Dead	β	S.E. β	P	OR	95% CI
Intercept			-5.77	0.73			
RISK					<0.007		
Low risk	5,392	42				1.00	
Medium risk	765	54	0.83	0.25	0.001	2.28	1.4-3.7
High risk	1,920	38	1.84	0.32	<0.001	6.34	3.4-11.9
TREATMENT							
Halothane	4,080	69				1.00	
Isoflurane	4,028	65	0.01	1.00	0.9	1.01	0.1-7.3
OPERATION TYPE					<0.001		
ENT/Urogenital/Misc	3,624	27				1.00	
Emergency Abdominal	1,112	60	0.90	0.3	0.002	2.48	1.4-4.5
Fracture	208	9	1.57	0.4	<0.001	4.80	2.2-10.6
Other Orthopaedic	3,164	38	0.57	0.3	0.03	1.77	1.06-2.9
AGE GROUP					0.7		
Foal	589	13	0.89	0.8	0.3	2.40	0.5-12.4
Yearling	758	4				1.00	
2 – 5 yrs	2,680	25	0.63	0.7	0.4	1.87	0.4-8.1
6 yrs upwards	4,032	90	0.53	0.7	0.5	1.69	0.4-7.1
TREATMENT × AGE					0.009		
Treatment × Age=Foal	311	7	-0.12	1.2	0.9	0.89	0.08-8.6
Treatment × Age=Yrling	273	2					
Treatment × Age=2-5	1,330	4	-1.75	1.2	0.1	0.17	0.02-1.7
Treatment × Age=6+	1,984	51	0.30	1.0	0.8	1.34	0.2-10.0
Random effects term			0.09*	0.09	0.29		

*variance term from PROC NLMIXED

Different causes of death

The main cause of death was cardiac arrest (43/134) with fractures in recovery accounting for a further 31/134 (Table 5). In addition, when non-fatal complications were also considered, it was clear that myopathy was a very important complication type (n=67), even though many cases were not fatal (c.15% only). Evaluation of factors associated with cardiac mortality and the occurrence of myopathy are shown below.

Table 5. Specific causes of death

System	Halothane	Isoflurane	Total	%
Cardiac	30	13	43	32%
Fracture	16	15	31	23%
Myopathy	6	4	10	7%
Respiratory	2	4	6	4%
Abdominal	7	10	17	13%
CNS/SCM	-	5	5	4%
Other	8	14	22	16%
Total	69	65	134	100%

Cardiac related mortality

Cardiac arrest was the most common specific cause of death. With the same modelling approach as above, we found, consistent with univariable analysis (Table 6), a significantly lower cardiac fatality rate in horses maintained with isoflurane. In addition, the clinical 'risk' status of the horse prior to surgery (positively) and routine blood pressure monitoring (negatively) were significantly associated with the risk of cardiac related mortality (Table 7).

Table 6. The association between cardiac related mortality and treatment

Status	Halothane	Isoflurane
Survived	4,080 (99.3%)	4,029 (99.7%)
Cardiac death	30 90.7%)	13 (0.3%)*
Total	4,110	4,042

*Isoflurane was associated with significantly reduced risk, $\chi^2=6.47_{1df}$, p =0.01

In the model for cardiac deaths there was no significant clinic effect (Wald χ^2 p=0.15) and the fit of the MELR model was satisfactory (HL p=0.37), although somewhat less good than that of the OLR model p=0.82), despite the fact that there was a significant reduction in model deviance associated with addition of the random effect term (LRS = 7.5, p=0.006).

Table 7. MELR model of factors associated with cardiac anaesthetic death

	Alive	Dead	β	S.E. β	P	OR	95% CI
Intercept			-5.18	0.46			
					<0.001		
RISK							
Low risk	1,392	9				1.0	
Medium risk	1,920	9	1.43	0.50	0.004	4.2	1.6-11.2
High risk	766	25	3.76	0.46	<0.001	42.9	17.6-105
TREATMENT							
Halothane	4,080	30				1.0	
Isoflurane	4,029	13	-0.82	0.34	0.02	0.4	0.2-0.8
BP MONITORING							
BP not monitored	1,275	13				1.0	
BP monitored	6,804	29	-1.92	0.41	<0.001	0.1	0.06-0.3
Random effects term			0.79*	0.53	0.15		

*variance term from PROC NLMIXED

Table 8. MELR model of factors associated with the risk of myopathy

	Alive	Dead	β	S.E. β	P	OR	95% CI
Intercept			-6.7	0.7			
					<0.001		
DURATION							
<= 45 minutes	1,350	4				1.00	
45-<=90 minutes	3,326	6	-0.23	0.7	0.7	0.8	0.2-3.2
>90 minutes	2,677	54	2.27	0.6	<0.001	9.7	2.9-31.7
					<0.001		
BODY POSITION							
Dorsal – non-abdom.	2,743	13				1.00	
Dorsal – abdominal	1,027	11	0.25	0.4	0.6	1.3	0.6-3.0
Left and right lateral	3,378	40	0.84	0.3	0.01	2.3	1.2-4.4
Other – moved	665	3	-0.08	0.7	0.9	0.9	0.3-3.4
Random effects term*			0.31	0.3	0.31		

* variance term from PROC NLMIXED

Myopathy

All cases of myopathy, in contrast to many other causes of death or complication (e.g. cardiac related mortality), had to complete surgery and so it was possible to consider duration of operation for this outcome. In the myopathy model (Table 8), both duration of operation and body position during surgery were strongly associated with risk. There were no treatment (isoflurane *v.* halothane) effects. The fit of the MELR model was satisfactory (HL p=0.80),

being perhaps marginally better than that of the OLR (HL p=0.59). The clinic level random effects term had a non-significant Wald chi squared value (p=0.3) and its addition resulted in a non-significant reduction in residual model deviance (LRS = 2.57, p=0.1).

Non-fatal complications

Assessed anaesthetic 'risk', age, treatment and duration of operation were all significant when considering all non-fatal complications. Again, there was a significant interaction between age and treatment and a reduction in adjusted risk associated with isoflurane use in the 2-5 year age group (results not shown).

DISCUSSION

The results raise a number of important issues. They demonstrate that it is perfectly possible to conduct multicentre randomised controlled studies in veterinary practice to evaluate the usefulness of different treatments and other interventions, as is commonly undertaken in human medicine and surgery (e.g. Moher et al., 2001). This supports the conclusions of Morley et al. (1999) who conducted a blinded randomised study of the efficacy of an equine influenza vaccine in North America. There is a need for far more rigorous scrutiny of the treatments used in veterinary medicine than is currently undertaken.

This study was reasonably free from misclassification errors and other biases. Great care was taken in our classification of the deaths as either cases or "put to sleep" (outcome = missing) and checked with clinicians involved wherever there was any question. We are confident that all deaths were identified.

It was rather more difficult, in the absence of post-mortem examination reports, to be sure of the precise reason for death, particularly in the case of cardiac deaths. However, when considering specific causes in these analyses, a classification system broad enough to ensure reasonable accuracy was used. There was likely to have been greater misclassification of the non-fatal problems.

The clinics in this study were not selected at random, but were a carefully selected convenience sample. The majority were selected on the basis of having been highly reliable at recording data in the previous observational studies (Johnston, 2000). We felt that this method of selection was important as throughout the study, great emphasis was placed on data quality.

The randomisation protocol itself was not closely monitored and the co-operation and honesty of the clinics was relied on to a great extent. However, compliance was carefully checked, in particular considering the sequence of operation and the randomisation code, and we are confident that it was adhered to. This is supported by the almost totally uniform distribution of covariates between the two treatment groups.

Data collection was ended in this study somewhat earlier, in terms of numbers of anaesthetic events, than had originally been intended. However, this was only after having noted a higher overall death rate than originally estimated and having taken considerable care to ensure that we had greater study power than originally envisaged.

The primary hypothesis of this study was rejected – that is, the use of isoflurane was not significantly associated with overall reduced risk of perioperative mortality in horses. This is the

main conclusion from the study. However, there was some heterogeneity of effects between different age groups, and although we did not initially hypothesise this, isoflurane use was associated with significantly reduced risk in horses aged 2 to 5 years inclusive. Although this result could just reflect chance variation, the likelihood that it did not, is reduced by the finding that non-fatal complications were also significantly reduced in this age group (effectively an independent group of animal as horses could not suffer both fatal and non-fatal complications).

In this study, horses were randomised to receive either halothane or isoflurane as maintenance agents during anaesthesia. It was impossible to blind the anaesthetists to the treatment and it was not feasible to control the other drugs used for premedication and induction during the study. However, we were able to assess the effects of these other treatments during the course of our analyses and found them to have no effects on our conclusions. Also, and as expected, the surgical interventions varied widely between horses, but again we were able to make some adjustment for this during analysis.

Although the primary purpose of the study was to determine whether or not isoflurane was associated with reduced risk of death, several other results from the regression modelling are also of interest, both when considering overall mortality and type specific complications.

Despite significant interest from ourselves and anaesthetists in modifiable risk factors, such as drugs used as premedicant and induction agents, we were uncomfortable about the interpretation of the drug variable, as well as about the likelihood of confounding by other measured or unmeasured effects. There was a relationship between category of anaesthetic risk, as determined by the clinician, and choice of premedicant within clinic and an association with anaesthetist choice of premedicant drug within clinic. In addition, a few of the larger clinics inevitably dominated these results. Even though we did adjust for clinic effects in our mixed effects models, the interpretation of the results relating to drug were not clear.

We included the term for clinic as a random effect in all our models, whether or not it was significant, in order to adjust covariate estimates and their standard errors. Models in which surgeon or anaesthetist was the random term were also fitted and there was little or no difference between the effects in the models of the different terms.

In the main and cardiac models, the assessed anaesthetic 'risk' status of the horses was highly significant. We are not confident that there was equal classification between clinics of the horses into each category, but the breadth of the terms made it likely that most cases were classified similarly in different clinics. That this was a highly significant variable, with the most 'at risk' horses being over six times more likely to die (and around forty times more likely to die due to cardiac arrest) was not surprising and consistent with previous knowledge (e.g. see Johnston, 2000).

Operation type was also highly significant, even after having adjusted for the animals' risk status, with fracture repairs being inherently the most risky operation. It should be noted that 'fracture in recovery', responsible for nearly a quarter of all deaths, was not restricted to horses following fracture recovery, but was distributed across operation types.

It was not possible to follow simple statistical rules when building our main model without it becoming grossly 'overfit' and it was somewhat arbitrary which variables were included in the model. However, care should be taken in drawing firm conclusions from epidemiological studies, unless they have been confirmed in more than one study. We did find the use of ROC

evaluation of model fit useful in informing our choice of variables and also, although not formally assessed here, in demonstrating the ability of the model to predict. However, other than informing clinicians, most of the terms in the model would not be easy for clinicians to modify prior to surgery. A variable omitted from our main model, but that was highly protective against cardiac arrest, was routine monitoring of blood pressure. This was done routinely in some clinics but not in others, perhaps only being done for high risk patients.

A variable suspected for some time of being highly significant in causing perioperative complications is the duration of the surgery. This is clearly evident in the myopathy model where operations greater than 90 minutes long were around ten times more likely to result in myopathy (Table 8). Despite its significance, it was not possible to use the term in the main model, as duration of operation was dramatically and artificially reduced in horses dying from cardiac arrest. Many of these horses died within 30 minutes of induction (e.g. see Johnston et al., 1995) which made it falsely appear that short operations (< 30 minutes) were particularly risky. The other variable associated with risk of myopathy was body position, with horses kept in lateral recumbency being at highest risk. This variable was closely correlated with operation type, although in this model it was more associated with risk than with operation type.

Even though isoflurane was not associated with an overall reduction in mortality, it was not surprising that its use was associated with a significant reduction in cardiac mortality, as many of the beneficial effect reported in experimental studies related to the cardiovascular system (e.g. Steffey et al., 1978; 1980). Even though cardiac problems were the most important cause of death, the benefits of isoflurane were evened out by small, non-significant increases in risk with all other causes of death, particularly those associated with disease of the CNS, resulting in an equal overall death rate between the isoflurane and halothane groups.

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HORSE FALLS IN NATIONAL HUNT RACING IN THE UK: RISK FACTORS AND SOURCES OF VARIATION

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SUMMARY

The fatality rate for horses in jump racing in the UK is significantly higher than for those racing on the flat, with falling over fences representing a major cause of the difference in death rate between these two types of racing. This study examines the determinants of falling in both steeplechasing and hurdling, and evaluates sources of variation in the risk of falling for targeting future research.

This analysis utilised retrospective data from all starts on UK National Hunt racecourses during 1999. The data consisted of 28,092 starts with 1014 horse falls. The falling rates per 100 starts were 5.6 in steeplechasing and 2.1 in hurdling. The death rate among fallers was 4.9% and 38% of all deaths were associated with a fall.

The final models showed that the risk factors for falling were different for hurdling compared to steeplechasing. For example, the age of the horse, field size, distance and racing experience were all significantly related to the risk of falling in steeplechasing. In hurdling, the sex of the horse, the jockeys experience and the age at which the horse started hurdling were all significantly related to the risk of falling. The highest proportion of variation resided at the horse and race level in steeplechasing, with very little clustering found at any level in hurdling.

INTRODUCTION

Injuries to horses whilst racing has a significant effect on animal welfare and the economics of racing. Furthermore, fatal or severe injuries can have a substantial negative impact on the public perception of racing. Falling during National Hunt racing in the UK contributes significantly to fatality in horses and injury in both horses and jockeys and often occurs in full view of the public.

The fatality and injury rate for racing over fences is higher than for flat racing (McKee, 1995; Bailey et al., 1998; Wood et al., 2000; Williams et al., 2001) with around 74% of all fatalities on racecourses in the UK occurring in hurdle and steeplechase racing despite these races accounting for only 39% of all starts in the UK (Wood et al., 2000). In the UK, overall fatality rates of 0.1 per 100 starts for flat racing, 0.52 per 100 starts for hurdling and 0.71 per

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100 starts for steeplechasing have been reported (Wood et al., 2001). A study by Bailey et al. (1998), conducted at four Australian racetracks, found incidence rates for fatal musculoskeletal injury of 0.06% for flat racing, 0.63% for hurdling and 1.43% for steeplechasing. This study identified harder track surfaces, older horses and one racecourse as significant risk factors although it did not differentiate between risks for flat and jump racing. In two descriptive studies of fatalities on UK racecourses falls were associated with between 50% (McKee, 1995) and 55% (Vaughan & Mason, 1975) of all fatalities in jump racing.

Previous studies on racetrack injuries have used different case definitions such as fatality, fatal musculoskeletal injury and non-fatal serious musculoskeletal injury (Bailey et al., 1998; Cohen et al., 1999; Wood et al., 2000). To our knowledge, there have not been any previous studies that specifically address risk factors for falling in National Hunt racing and risk factors may be very different for different causes of death. For example, 78% of fatal distal limb fractures in hurdle racing in the UK were not associated with a jumping episode whereas fatal fractures of the vertebrae most often occur due to falling (Parkin, personal communication; Vaughan & Mason, 1975).

Analysis of these data sets had two main aims. First, to identify risk factors associated with falling, second, to estimate the contribution of various levels of clustering to the variation in falling so that levels that account for an important amount of the variability can be targeted for further research. Potential areas of clustering in jump racing include starts by the same horse, horses with the same sire, starts within the same race and on one particular track, and starts by horses trained by the same trainer or ridden by the same jockey. Allowing for these sources of variation, using multilevel mixed effects models, improves the estimation of regression coefficients.

MATERIALS AND METHODS

Data

The study utilised retrospective data from Computer Raceform, a commercially available electronic database, and data from the Jockey Club on fatalities recorded on UK racecourses. Weatherbys Ltd provided data on the previous racing history of the horses. Data from 1st January 1999 to 31st December 1999 were analysed providing information on every start in jump races on all racecourses in the UK during this period. Only data from the hurdle starts are presented in this paper and the results are compared to the findings of a previous study of steeplechase starts (submitted for publication). The variables available for analysis are shown in Table 1. The data were checked for validity against an independent data source (Racing Post online – www.racingpost.co.uk).

Statistical analysis

Fixed Effects: Multivariable generalised additive models (GAMs) (Hastie & Tibshirani, 1990) were used to explore the functional form of the relationship between the response (falling) and continuous variables (number of runners, age, distance of race, number of runners in last 12 months, weight carried, age started hurdling). When combined with other techniques, such as the fitting of equal number and equal interval categorised variables, these methods inform the choice of transformations that may be required to represent this relationship in generalised linear models (GLMs). The GAM model fits nonparametric functions to estimate the relationship

between the response and the predictor variables. The advantage of GAMs is that individual variables are not assumed to have a linear relationship with the outcome. The response is modelled as a sum of smooth functions in the predictors. Two functions used for estimating the smooth relationships between the response and the predictors are the smoothing spline fit and the locally weighted least squares regression smooth (loess) (Hastie & Tibshirani, 1990). Loess smoothers were used for the models presented in this paper. The loess smoother takes a proportion or "window" of the overall data and then fits a local regression model with nearest points given greater weight. The proportion in each window is set by the span. In these models the default span of 0.5 was used. In this way for each individual component fit, 50% of the overall data values are taken within a window set on the x-axis. The GAMs were fitted in S-Plus (S-plus 2000, Mathsoft Inc).

Table 1. Description of the variables available for analysis from computer Raceform and Weatherbys Ltd

Variable	Description
Horse identity	Name
Age of horse	Age at time of each start in years
Age at first race	Calendar age at first hurdle race under rules
Sex of horse	Gelding, colt or filly
Headgear	Blinkers or visors
Racing history	i) First race type
	iii) Number runs in the last 12 months
	iv) Number runs in the last 3 months
	v) Date of last run
Official rating	Official rating at each start
Weight carried	Weight carried by horse in pounds
Jockey identity	Name and whether professional or conditional
Trainer identity	Name of trainer
Dam identity	Name of dam
Sire identity	Name of sire
Race identity	Date and race form number
Racecourse	Name of course
Going	Hard-Heavy as recorded by clerk of the course
Distance	In furlongs
Number of runners	Number of horses starting in the race
Race class	Official class of race A-H

Variables considered for inclusion in multivariable logistic regression models included terms that had been shown to be important in previous studies, such as going and age of the horse, categorical variables with a p-value <0.25 on univariable analysis and polynomial terms identified using GAMs . A final model was built using backward elimination procedures where variables with a term-wise Wald test p-value <0.15 or variables that improved the fit (likelihood ratio chi squared statistic P<0.05) were left in the model. Biologically plausible interaction

terms were tested. The logistic regression model was fitted initially using EGRET (Egret Application 2.0, Cytel Software Corporation).

Random Effects models: Initially, intercept only, 2 level models were fitted to assess individually the contribution at each level of clustering. The levels included horse, sire, racetrack, race, trainer and jockey. The models were fitted using a residual generalised iterative least squares (RIGLS) algorithm. Modelling was attempted initially using second order penalised quasi-likelihood (PQL) but in two cases (horse and race) these would not converge so first order PQL was used. Comparative estimates using Markov Chain Monte Carlo (MCMC) simulations were attempted, however prohibitively long chain lengths (up to $9x10^6$) were required to give reliable estimates of both the mean and tails (2.5 and 97.5 centiles) of the posterior distributions of regression coefficients. Comparative estimates were derived from full maximum likelihood models using EGRET. To estimate the proportion of variation attributable to each level of clustering, intra-class correlation coefficients were calculated using four different methods as described by Goldstein et al. (2000). Mixed effects two level models were then fitted including fixed effects from the final logistic regression model to assess the change in variance in these models. Subsequently the 3 level model, which accounted for the most variation, was extended to include significant fixed effects (from the logistic regression model). The random effects models were fitted using MlwiN (MlwiN 1.10,0006, IOE, London). The final fit of the model was assessed by calculating the sensitivity and specificity of the model for varying predicted-value cut off points.

RESULTS

Descriptive Statistics

There were 14,595 hurdle starts available for analysis with 367 horse falls. Runners that did not complete the race for reasons other than falling were not included in the analysis. The falling rate was 2.1 per 100 starts and 7.1% (26) of the fallers died. Of all deaths recorded on the racecourse from these starts 35% were associated with a fall. Table 2 shows the numbers at each hierarchical level and the maximum starts at each hierarchical level.

Table 2. Hierarchical structure of the data.

Hierarchical level	Numbers at each level	Maximum starts per level
Horse	4887	25
Race	1666	30
Trainer	656	656
Jockey	439	439
Track	42	637
Sire	1046	234

Generalised Additive Models

Results from the GAMs are shown in the plots in Fig. 1. Outliers of age (>13 years) and number of runs in the last 12 months (>30) were omitted from this analysis to allow better interpretation of the curves at the points where most of the data lie. None of the variables

demonstrated a significant non-linear ($p<0.05$) relationship with the log odds of falling and so only linear terms were considered in subsequent logistic regression model.

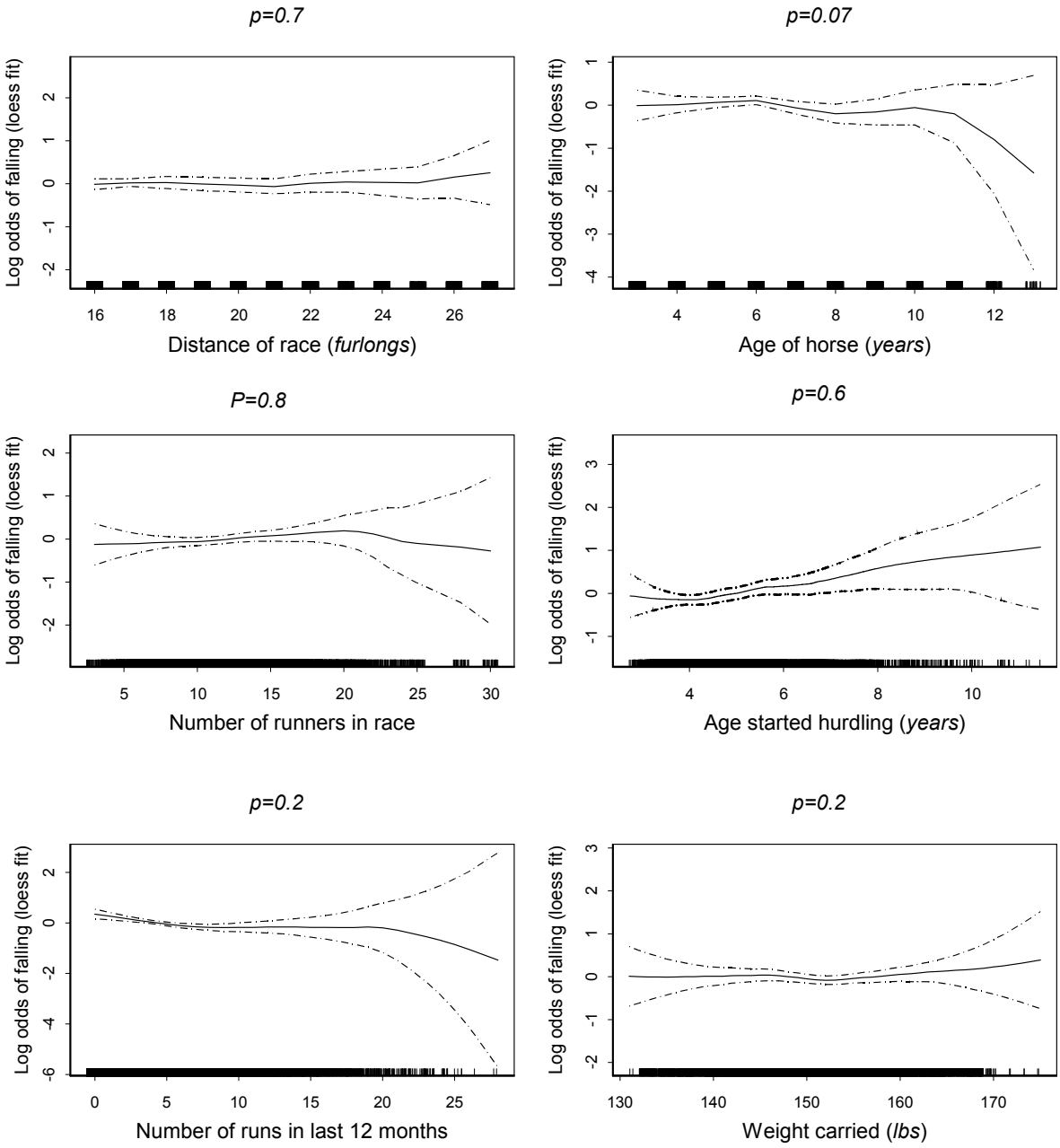

Fig. 1 Graphical representation of the functional form of the continuous variables modelled in a multivariable GAM. The plots show the smoothed, fitted means and the 95% confidence intervals, with rug plots to represent the number of data points along the x-axis.

Random Effects

Table 3 compares the intra-class correlation coefficients (ICC's) for horse, race, track, jockey, trainer and sire, using the four different methods described by Goldstein et al. (2000). The results show that there was very little variation at any level of the hierarchy and only jockey showed 'significant' variation. In all cases the latent variable approach provided the highest estimate for all level 2 variables.

Table 3. Comparison of intra-class correlation coefficients using four different methods. The variance estimates used are from 2-level intercept only models using 1st or 2nd order PQL models.

Level 2	Latent variable approach	Model linearisation	Simulation	Binary linear model
Horse*	4.4%	0.4%	0.4%	0.6%
Race*	2.9%	0.2%	0.3%	0.3%
Trainer	2.6%	0.2%	0.2%	0.2%
Track	0.2%	0.03%	0.03%	0.01%
Jockey	3.2%	0.3%	0.3%	0.3%
Sire	2.4%	0.2%	0.2%	0.1%

* 1st order PQL

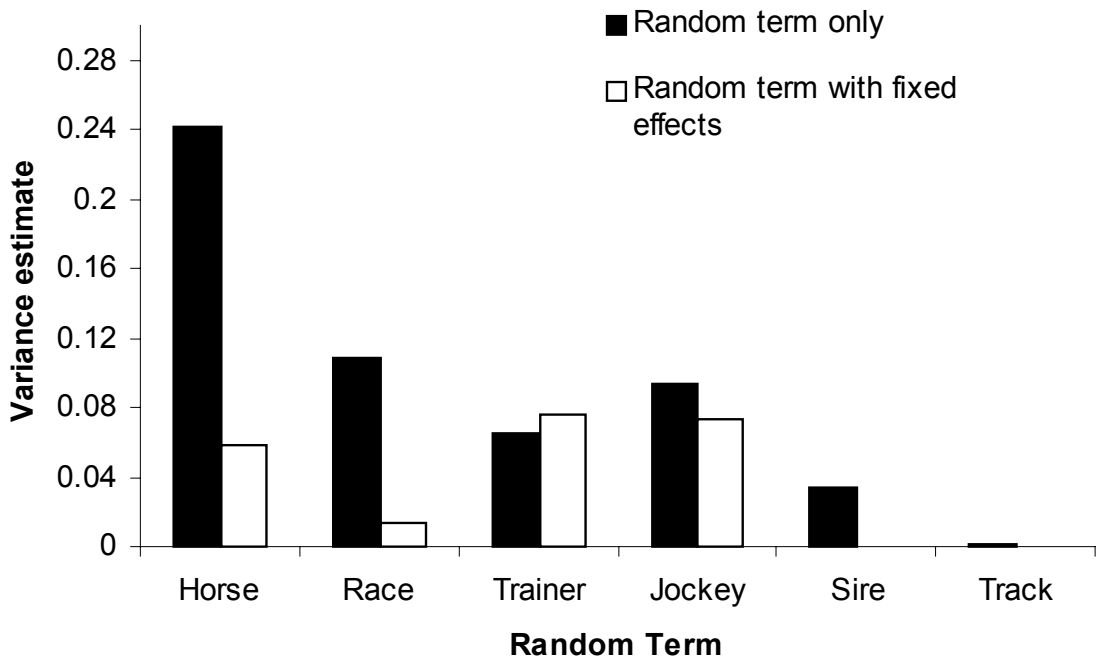

Fig. 2 Variance estimates for each level with and without fixed effects. The latter were obtained using estimates from the intercept only PQL models (for horse and race, 2nd order PQL models would not converge so 1st order PQL models were used so estimates may be biased).

Changes in the variance estimates for each hierarchical level after inclusion of fixed effects from the final multivariable model are shown in Fig. 2. The variance estimate for trainer increased after inclusion of the fixed effects whilst the variance estimates for all other levels decreased.

Multi-level multivariable logistic regression model

The results from the final multilevel mixed effects model (using a 1st order PQL model) are shown in Table 4. The three levels used were jockey, horses and the start (level 1). The nested model of jockey-horse was used as this accounted for the largest proportion of variance (although horse within jockey will not be entirely nested). Two-level models with horse, trainer or jockey as the 2nd level and the three level model trainer-horse-start were also considered. When the three level models were fitted the horse variance was reduced to zero in both cases. The coefficient estimates for the fixed effects did not vary substantially between these three level and two level models. Most coefficients were identical to 2 decimal places, except the estimates for jockeys claiming weight, which decreased in the models including the level jockey by between 0.01 and 0.04.

Table 4. Multi-level multivariable logistic regression model of risk factors associated with falling in hurdle racing fitted using 1st order PQL

Regression terms	Estimates	S.E.	Odds Ratio	95% CI	P-value
Random effect	*Variance estimate*				
Jockey	0.072	0.055			
Horse	0.000	0.000			
Fixed effects	*Coefficients*				
Age (cent)	0.005	0.036			0.9
Age started Hurdling (cent)	0.116	0.052			0.02
No. runs last 12 months (cent)	-0.028	0.014			0.05
Weight carried (cent)	0.019	0.008			0.02
Sex					
-female			1.00[a]		
-male	-0.390	0.124	0.68	0.53 – 0.86	<0.01
Going					
-firm/good to firm	-0.126	0.140	0.88	0.67 – 1.16	0.4
-good			1.00[a]		
-good to soft	-0.177	0.145	0.84	0.63 – 1.11	0.2
-soft/heavy	-0.459	0.153	0.63	0.47 – 0.85	<0.01
Hurdled before					
-No			1.00[a]		
-Yes UK	-0.534	0.152	0.59	0.44 – 0.79	<0.01
-Yes Outside UK	0.120	0.371	1.13	0.54 – 2.33	0.7
Jockey					
-Professional			1.00[a]		
-Claiming 3lbs	0.375	0.182	1.45	1.02 – 2.08	0.04
-Claiming 5lbs	0.291	0.190	1.34	0.92 – 1.94	0.1
-Claiming 7 lbs	0.389	0.180	1.48	1.04 – 2.10	0.03

[a]Indicates reference category; 'Cent' indicates variables were centred.

To reduce collinearity, the continuous variables were centred by subtraction of the sample mean from all observations (age mean=6.97, weight mean =152, number runs in the last 12 months mean=5.96, age started hurdling mean=5.6). From the model it can be seen that the continuous variables, weight carried, the age the horse started hurdling and the number of runs the horse had in the last 12 months were all significantly related to falling, and their relationship with the log odds of falling were best described by single linear terms. The older the horse began its hurdling carrier the more likely it was to fall and greater the weight carried was associated with an increased odds of falling. A greater number of runs by the horse in the last 12 months was associated with a decreased odds of falling. Horses that hurdled before in the UK were also at decreased odds of falling when compared with horses that had never hurdled before. Horses ridden by a professional jockey had a lower odds of falling compared to horses ridden by conditional jockeys (entitled to a weight a allowance) and starts on soft or heavy going were associated with a lower odds of falling. Male horses (geldings and entire males) were also at decreased odds of falling.

Assessing the fit of the model

The fitted probability values calculated from this final model ranged from 0.006 to 0.13. The specificity and sensitivity at various cut-off points are shown in Table 5. For example when a cut off of 0.025 is selected (i.e. if the predicted probability of falling is above 0.025, the horse is predicted to fall) the specificity is 62% (i.e. 62% of non—fallers were correctly classified) and the sensitivity was 54% (i.e. 54% of fallers were correctly classified)

Table 5. Sensitivity and specificity of the model presented in Table 4.

Cut-off	Specificity (proportion of non-fallers predicted)	Sensitivity (proportion of fallers predicted)
0.02	38%	76%
0.025	62%	54%
0.03	77%	38%
0.05	96%	12%
0.1	99%	5%

DISCUSSION

This study, combined with a similar study of steeplechasing (submitted for publication), has contributed to our understanding of the aetiology of equine falls during racing on National Hunt racecourses in the UK. The two studies have identified markedly different risk factors and sources of variation. The only significant level of clustering detected in the hurdling model was at the jockey level, and this only explained a very small amount of variation. This suggests that modification or intervention of start (level 1) variables would be likely to have the most impact on reducing the risk of falling in hurdling. However, in the steeplechase model significant clustering was found at the horse, race, sire and racetrack level and this helped to focus further research in these areas. Few previous studies on racehorse injuries have taken into account levels of hierarchy and clearly race starts may not be independent. A study by Wood et al. (2001) looking at deaths on UK racecourses from 1990 to 1999 detected small but significant clustering at the trainer and race level in hurdling. The use of multilevel models also provides more robust

estimates regression coefficients for fixed effects and assessment of whether or not the effects of these change within different levels of clustering.

Although only two-level-intercept-only models are presented above, 3 level nested models (e.g. start, horse and sire) were also fitted but, possibly because of small variance estimates, only marginal quasi-likelihood models (MQL) would converge and these did not reveal any contribution from higher levels. This is in contrast to the steeplechase study (submitted for publication) where, for example, the sire remained a 'significant' source of variation, having accounted for horse and start. Inclusion of the fixed effects decreased the variance estimates at all levels except trainer where the estimate increased. This may be due to some trainers having more horses with less experience or using claiming jockeys more often than other trainers, so when evaluation of the variation in the risk of falling is assessed over all trainers it is lower than when experience of the horse and/or jockey is taken into account.

All random effects models were fitted using 2^{nd} order PQL models if possible as the second- order Taylor series expansion provides the most unbiased estimates of regression terms and variance estimates. However, 1^{st} order PQL models were used when 2^{nd} order would not converge. All iterative least squares algorithms may have inherent bias (Rodriguez & Golmad, 1995; Dohoo, 2001) and confirmation of estimates was attempted using MCMC techniques; however, very long chain lengths of between 1 and 9 million, according to the Brooks-Draper statistic, were needed which were too computationally and time intensive. The need for long chain lengths may be because of the small variance estimates at all levels and was not the case in the study of steeplechase starts. The final model shown in Table 4 (2 levels only-jockey and start) was also fitted using MCMC simulation with a Metropolis-Hastings sampling, diffuse priors, a burn in of 50,000 and a run of 10^6 iterations, to provide comparative estimates of the regression coefficients. The coefficients of the fixed effects were the same to 2 decimal places. However the estimate for jockey decreased to 0.058. A comparison of four different methods of estimating variance (marginal quasi-likelihood, MCMC with metropolis Hastings sampling, MCMC using Gibbs sampling and maximum likelihood estimates) was performed when analysing the steeplechasing data and all except the MQL models gave similar estimates.

The ICC's showed that the contribution to the variation at any level other than level 1 (the start) was very low. The simulation method and the model linearisation methods both provided similar estimates. Calculations using a latent variable approach provided higher estimates and this pattern was also seen in the steeplechase data analyses. This approach assumes that the binary response is derived from an underlying continuous variable and is possibly a less justified approach for a truly discrete response such as falling or not falling.

The generalised additive models provided a means of exploring the functional form of the relationship between continuous variables and the risk of falling and also graphically assessing the contribution by each variable. In the hurdle model none of the variables had a significantly non-linear relationship with the outcome of falling and for some variables single linear terms provided the best fit in the final multivariable logistic regression model. The graphs of weight carried and number of runs in the last 12 months appear almost flat and close to zero because of the small odds ratios associated with these variables (1.02 and 0.97 respectively), which were detectable due to the high power of the study. Age was not significant, but was forced into the model, and was considered both in a quadratic form and as a piece-wise fit, neither of which improved the fit of the model. In the steeplechase data three variables had a significantly non-linear relationship with the risk of falling. Distance and age of the horse were best described by a cubic relationship and the number of runs the horse had in the last 12 months by a quadratic

relationship with the risk of falling. When combined with the alternatives such as variable categorisation, GAMs provide a powerful way of representing, potentially complex, functional relationships parsimoniously in regression models.

Although age at the time of start was not significant in hurdling, the age at which a horse began its hurdling career was significantly related to the odds of falling. This was best described by a linear term giving an OR of 1.12 per yearly increase, so for example, a horse that started its hurdling career at 8 years old would be estimated to have an increased odds of falling of 1.8 times compared to a horse that started its hurdling career at the age of 3 years old (3 years is the minimum allowed age for hurdle racing in the UK). The age at which horses began their hurdling career ranged from 3 years to 11 years old. This may be due to enhanced learning responses in younger horses, or it may be that horses starting their hurdling career at a later age have had more runs on the flat. Such horses are likely to have been specifically bred for flat racing, and therefore less suitable for jumping in hurdle racing. There was no information in this data set on the number of previous flat races a horse had over its career. There was information on the number of runs (of any type) that a horse had in the last 12 months and this had a small but significant relationship with the odds of falling with a greater number of runs associated with lower odds of falling. This relationship was different to that observed in steeplechasing where the odds of falling started to increase with greater than 17 to 18 runs in the previous 12 months. This may be due to gaining jumping and racing experience and increasing fitness levels. This is backed up by the results of comparing horses that had hurdled before with those having their first ever hurdle race. Horses that had hurdled before in the UK had decreased odds of falling. Interestingly this effect was not seen in horses that had hurdled only outside of the UK and this is in contrast to steeplechasing where a previous steeplechase race anywhere decreased the odds of falling. This may be due to different fences used overseas or may reflect the population of horses sent to race in the UK.

In hurdling, male horses were less likely to fall than females. Males may have a better natural athletic ability for jumping compared to females, or may be of an average greater height than females although this effect was not seen in steeplechasing where there was no significant difference between the sexes.

In contrast to steeplechasing, the jockey was associated with the risk of falling in hurdling and this was seen both in the variance estimates and in the fixed effects. When professional jockeys were compared with conditional jockeys claiming three different weights the odds of falling were almost two fold for horses ridden by a conditional jockey. A conditional jockey is an inexperienced jockey under 26 years of age and the weight they are allowed to claim is dependent upon the number of races they have won (e.g. 7lb until won 15 races, 5lb up to 30 races and then 3 lb). Once a jockey has won 65 races he is classed as professional. Increasing weight carried by the horse also had a small but significant increase on the odds of falling. It may be that extra weight does affect the horses jumping ability or may contribute to fatigue but this really needs to be assessed taking into account whether the race is a novice or a handicap and what the magnitude of handicap weights or horses rating.

The effect of the going in hurdling was opposite to that seen in steeplechasing with softer and heavy going significantly decreasing the odds of falling in hurdling. In steeplechasing these types of going appeared to be associated with an increase risk of falling. This does suggest different mechanisms for falling in the two different types of racing, and speed, which will be affected by going. may have also have an effect.

The results of the sensitivity and specificity of the model suggest that there is a significant proportion of unexplained variation within the model. This may be due to unmeasured, including un-measurable, covariates.

To summarise, this study has identified a number of variables associated with the risk of horse falls in steeplechasing and hurdling in the UK and will help to identify high-risk horses and races. The random effects models have suggested in which areas further research and intervention studies would be likely to have the most impact.

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PORCINE EPIDEMIOLOGY

SIMULATING SALMONELLA PREVALENCE FROM THE GROWING PIG TO THE SLAUGHTERED CARCASS: WHERE SHOULD THE EFFORT BE PUT TO INCREASE FOOD SAFETY?

L. ALBAN[*] AND K.D.C. STÄRK[**]

SUMMARY

Infection with Salmonella is seldom associated with clinical disease in pigs. However, control is important as the public is concerned about the human health impact. The producers, the slaughterhouses and the authorities are interested in implementing procedures to mitigate this risk. To evaluate the effect of different procedures, a stochastic risk model was developed to simulate the prevalence of Salmonella infection during the production process from the live pig on the farm, to the final carcass. This paper describes the model and findings of simulating different control scenarios. The variables with maximum effect on the Salmonella prevalence on the final carcass were 1) number of herds with a high prevalence of Salmonella, 2) singeing efficiency, 3) contamination and cross-contamination at degutting and 4) cross-contamination during handling. However, improvement of any single factor in isolation had a limited impact upon the level of contamination. The largest reduction was observed when several factors were improved concurrently.

INTRODUCTION

In recent years, food safety has gained increasing attention in Europe. Reports describing food poisoning, such as salmonellosis from pork, cause public anxiety. Consumers expect completely 'safe' food, even though this cannot be achieved unless a measure such as radiation is used (which is unpopular for other reasons). On the other hand, a farmer may find it difficult to understand that there is a Salmonella problem in their herd because infection is usually subclinical. Additionally, the bacteria can often not be isolated from environmental faecal samples even though serology results indicate that Salmonella is present in the herd.

In Denmark during 1993, the approximate number of human salmonellosis cases caused by consumption of pork was 1,100. In the same year, a human epidemic was traced back to an infection with *Salmonella* Infantis in certain pig herds. This initiated the establishment of a nation-wide *Salmonella enterica* surveillance and control programme. In 1995, the programme was extended to include serological surveillance of slaughtered pig herds. The primary aim of the control programme was to reduce the prevalence of Salmonella in both pig herds and pork. Approximately £9 million have been invested in the annual control programme; 1/3 of which

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was paid by the Danish authorities and 2/3 by the pig industry. From 2002, the industry will pay an even higher proportion of the costs. Since 1993, the annual number of human cases related to pork has declined and in 2000, only 166 out of a total of 2,308 human salmonellosis cases were attributed to pork (Anon., 2001). To improve pork safety further, the Danish authorities have asked the Danish Bacon & Meat Council to ensure a 25% reduction in the prevalence of test-positive carcasses over a 5-year period. The primary question is, where should the effort be put to achieve this efficiently: is it at farm level, during transportation or at the slaughterhouse? To answer this question, a computer simulation model was developed. This paper describes the model, the results of simulations using various intervention strategies and the limitations of the model.

MATERIALS AND METHODS

The unit of interest in the model is the individual pig (Stärk et al., 1999). Each pig originates from a herd with a given within-herd Salmonella prevalence (Level 1: <1%, Level 2: 1-10%, Level 3: >10%) and of a given size (Table 2). The pig is followed in the herd, during transportation to the lairage and at the slaughterhouse. Furthermore, the model simulates the passage of the carcass through the slaughter process (including singeing, polishing, degutting and handling) until the carcass is split. The outcome of the model is the probability of the final carcass being test-positive for Salmonella using bacteriological testing. This probability can be interpreted as the prevalence of Salmonella contamination in pork after chilling.

Information for the model was collected from publications by the Danish Bacon & Meat Council, scientific journals, information from various Danish slaughterhouses and from expert opinion (Stärk et al., 2002). The model structure was developed using intensive analysis of all hazards in the pig production system from primary production until after slaughter. A hazard was defined as any process, practice or activity that could result in the infection or contamination of a pig. Based on the hazard analysis, a risk model was drafted and then translated into a simulation model. The software program, '@Risk', and Monte Carlo simulations were used to obtain the model output.

Table 1. Transition probabilities[a] during growing for a pig from a Level 1, 2 or 3 herd

Infection status at start of growing		End of growing		
		Free	Infected	Carrier
Level 1	Free	0.99	0.005	0.005
	Infected	0.97	0.020	0.010
	Carrier	0.75	0.010	0.240
Level 2	Free	0.95	0.025	0.025
	Infected	0.50	0.250	0.250
	Carrier	0.60	0.200	0.250
Level 3	Free	0.50	0.250	0.250
	Infected	0.10	0.50	0.400
	Carrier	0.20	0.40	0.400

[a] Each probability was described by a triangular distribution (the listed probability ±10%) based upon the authors' best guess.

Table 2. Description of the variables used in the modelling of the Salmonella status from the live pig to the final carcass

Variable	Distribution	Values	Source of information
True within-herd prevalence	Discrete	Level 1: <1% Level 2: 1-10% Level 3: >10%	Anon. (1998)
Herd prevalence	Beta	$Prev_{L1}$= 1-($prev_{L2}$+$prev_{L3}$) r_{L2}= 113, r_{L3}= 139, =1,962	Anon. (1998)
Herd size	Discrete	0-99: 1.7% - class 1 100-499: 4.6% - class 2 500-999: 6.2% - class 3 1000-1999: 17.9% - class 4 ≥2000: 69.7% - class 5	Status August 2000. Danish Salmonella surveillance and control programme
Probability of common transportation given herd size class	Binomial	Class 1: 97.7% Class 2: 90.8% Class 3: 90.4% Class 4: 79.4% Class 5: 41.7%	Danish Crown Slaughterhouse
Transportation	Triangular	$OR_{shedding\ among\ infected,\ if\ transport\ >3h}$ = 1.7±10% $OR_{increase\ in\ proportion\ of\ carriers,\ if\ mixing}$ = 2.0±10%	Expert opinion
Lairage	Triangular	$OR_{shedding\ among\ infected,\ if\ lairage\ >3h}$ = 1.6±10% $OR_{increase\ in\ proportion\ of\ carriers,\ if\ mixing}$ = 1.6±10%	Expert opinion Authors' opinion
Singeing efficiency	Triangular	Min: 0.90 Mode: 0.95 Max : 0.99	Discussion with staff at the Danish Meat Research Institute
Recontamination after Polishing	Triangular	Min: 0.00 Mode: 0.05 Max : 0.06	Rasmussen (19960
Contamination at degutting	Triangular	Min: 0.10 Mode: 0.80 Max : 0.95	Synthesis of observations made at the Danish Meat Research Institute
Cross-contamination after degutting	Triangular	Min: 0.009 Mode: 0.010 Max : 0.011	Authors' best guess
Cross-contamination during handling	Triangular	Min: 0.009 Mode: 0.010 Max : 0.011	Authors' best guess
Test sensitivity	Triangular	Min: 0.35 Mode: 0.40 Max : 0.75	Expert opinion among Danes
Test specificity	Triangular	Min: 0.98 Mode: 0.99 Max : 1.00	Expert opinion among Danes

The model consists of 5 sub-models. In each sub-model, a pig can change Salmonella infection status (representing combinations of carrier, shedding and surface contamination states) according to a set of predefined transition probabilities for each event. The transition probabilities vary according to the infection level of the herd of origin of the pig (Level 1, 2 or 3 herd) (Table 1). For example, a pig from a Level 2 herd, which is free at the beginning of the growing period, has a 95% probability of still being free at the end of the growing period. Due to the lack of data, many of the transition probabilities were based on the authors' best guess (unless otherwise stated in Table 2).

Influential input variables in the simulation model were identified using sensitivity analysis (data not shown). The effect of changing parameters for selected influential variables was assessed using the following simulation scenarios:

1) The baseline parameters describing the current situation. Different test sensitivities were applied for testing the final carcass;
2) Reducing the number of Level 2 and 3 herds;
3) Separate transportation and lairage facilities at the slaughterhouse of pigs from Level 1, 2, and 3 herds;
4) Increased efficacy at singeing;
5) Reduced probability of recontamination at polishing;
6) Reduced probability of contamination and cross-contamination at degutting;
7) Reduced probability of cross-contamination during handling;
8) Increased effort both in primary production and at the slaughterhouse.

RESULTS

The baseline parameters describing the current situation

When the baseline parameter estimates were used in the model, the Salmonella prevalence increased from loading to the time of kill, where it reached a maximum of 18%. However, singeing induced a substantial decrease, as only 1% of the carcasses were Salmonella positive. Thereafter, the prevalence increased due to evisceration, polishing and handling, but then decreased due to bacteriological testing of the chilled carcass (3.9%: 90%C.I. = 2.6-5.2%) (Fig. 1). If the aim is a 25% reduction, a target prevalence can be defined as 2.9%.

According to the model, the prevalence of test-positive carcasses varied depending on the sensitivity of the test; the higher the sensitivity, the higher the prevalence and vice versa (Table 3).

Reducing the number of Level 2 and 3 herds

The infected pig itself is the primary reason for Salmonella on the carcasses at the slaughterhouse. According to the model, the proportion of Level 2 and 3 herds would need to be reduced substantially before this was reflected in a decreased Salmonella prevalence of pork carcasses (Table 3). If no Level 3 pigs were delivered to the slaughterhouses, then the prevalence of Salmonella in pork would still be 3.2% (18% decrease).

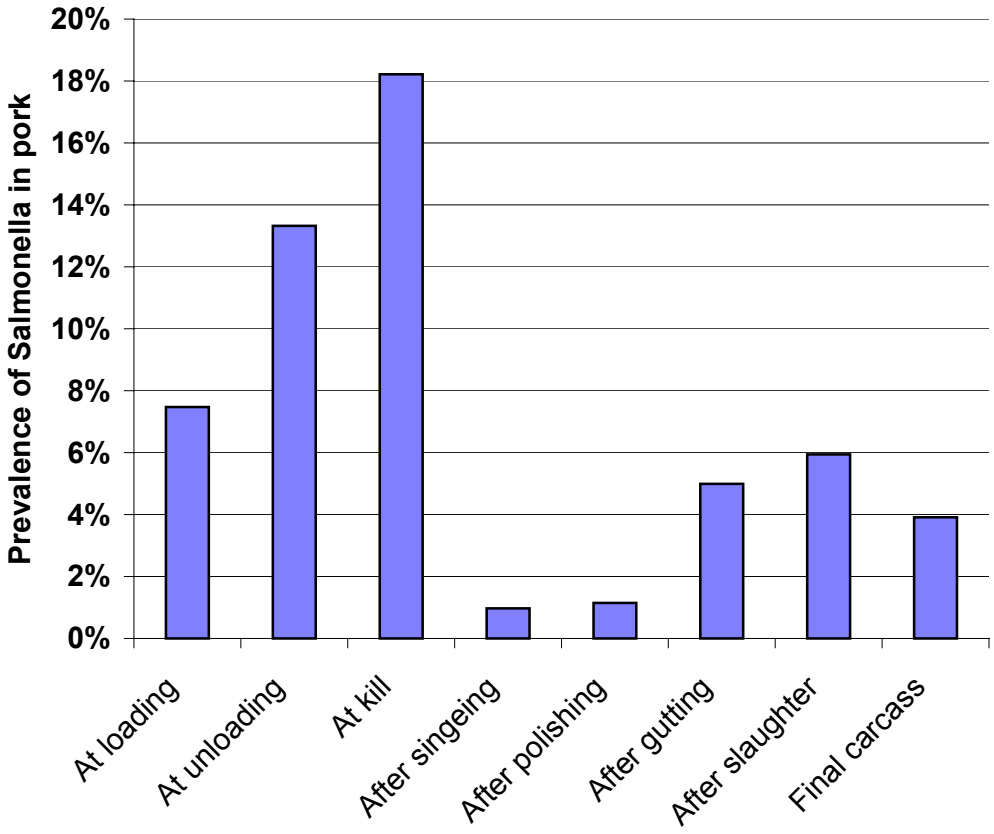

Fig. 1 Estimated prevalence (%) of Salmonella contamination in pork from loading to the tested final carcass (pork after chilling) when baseline parameters were used

Table 3. Effect on Salmonella prevalence on chilled carcasses (P) at different levels of bacteriological test sensitivity or differing proportions of Level 3 herds

Bacteriological test sensitivity[a]				Proportion of Level 3 herds	
Min	Mode	Max	P (%)	Proportion (%)	P (%)
0.60	0.65	0.95	5.3	**100**	**3.9**
0.55	0.60	0.95	5.1	90	3.8
0.50	0.55	0.90	4.8	80	3.8
0.45	0.50	0.85	4.5	70	3.7
0.40	0.45	0.80	4.2	60	3.6
0.35	**0.40**	**0.75**	**3.9**	50	3.5
0.30	0.35	0.70	3.6	40	3.5
0.25	0.30	0.65	3.3	30	3.4
0.20	0.25	0.60	3.0	20	3.3
0.15	0.20	0.55	2.7	10	3.3
0.10	0.15	0.50	2.4	0	3.2

Figures in bold represent the standard parameters used in the model.
[a] The distributions describing sensitivity and specificity were derived from Danish expert opinion.

Separate transportation and lairage facilities for pigs from Level 1, 2 and 3 herds

Mixing during transportation and at the lairage may increase the proportion of contaminated pigs and carriers. Additionally, prolonged transportation and detention in the lairage may induce shedding among carriers, as reflected by an increased proportion of pigs excreting the organism. However, according to the model, there was no effect of separate transportation for herds of different levels. Additionally, it was only if there was no mixing and only short a stay in the lairage (<3 hours) that a minor decrease (from 3.9% to 3.8%) in Salmonella prevalence could be achieved.

Increased efficacy at singeing

Shortly after a pig has been bled, it is scalded and dehaired. Next, the carcass is passed through a singeing oven. The singeing process significantly reduces the level of surface contamination, even though bacteria might survive in deeper skin folds (the base and orifices of the ears) or in the hair follicles (Berends et al., 1997). The model estimated, that the prevalence only fell to 3.6% when the singeing efficacy was increased to 0.99 (Table 4). This implied, that substantial improvements in singeing efficacy would be needed before an effect on the prevalence of salmonella-positive carcasses can be expected. However, if singeing efficacy was reduced, the prevalence increased (Table 4).

Table 4. Efficacy[a] of singeing, degutting and handling on the Salmonella prevalence of chilled carcasses (P)

Singeing efficacy				Probability of contamination at degutting				Probability of handling cross-contamination			
Min	Mode	Max	P (%)	Min	Mode	Max	P (%)	Min	Mode	Max	P (%)
0.80	0.85	0.89	4.6	**0.1**	**0.8**	**0.95**	**3.9**	**0.0090**	**0.0100**	**0.0110**	**3.9**
0.82	0.87	0.91	4.5	0.1	0.7	0.90	3.8	0.0081	0.0090	0.0099	3.8
0.84	0.89	0.93	4.3	0.1	0.6	0.85	3.7	0.0072	0.0080	0.0088	3.8
0.86	0.91	0.95	4.2	0.1	0.5	0.80	3.5	0.0063	0.0070	0.0077	3.7
0.88	0.93	0.97	4.1	0.1	0.4	0.75	3.4	0.0054	0.0060	0.0066	3.7
0.90	**0.95**	**0.99**	**3.9**	0.1	0.3	0.70	3.3	0.0045	0.0050	0.0055	3.6
0.92	0.96	0.99	3.8	0.1	0.2	0.65	3.2	0.0036	0.0040	0.0044	3.6
0.94	0.97	0.99	3.8	0.1	0.1	0.60	3.2	0.0027	0.0030	0.0033	3.6
0.96	0.98	0.99	3.7	0.1	0.1	0.55	3.0	0.0018	0.0020	0.0022	3.5
0.98	0.99	0.99	3.6	0.1	0.1	0.50	3.0	0.0009	0.0010	0.0011	3.5

Figures in bold represent the standard parameters used in the model.
[a] The effects were described by triangular distributions.

Reduced probability of recontamination at polishing

At polishing, the burnt hairs and skin are removed. There is only a low probability of faecal contamination at polishing. In the model, it was assumed that cross-contamination between carcasses did not occur. According to the model, there was almost no impact on the prevalence

of Salmonella positive carcasses when the parameter describing the probability of recontamination at polishing was changed. If cross-contamination was assumed to happen with the same probability as recontamination (mode: 0.05), the prevalence in pork increased from 3.9 to 5.6%.

Reduced probability of contamination and cross-contamination at degutting

Degutting consists of the evisceration process including bung dropping and removal of the pluck (tongue, oesophagus, larynx, trachea, lungs, heart and liver). Faecal contamination is difficult to avoid during this process. However, this only results in Salmonella contamination if the carcass originated from an infected pig (as non-infected or carrier pigs do not shed bacteria). According to the model, the Salmonella prevalence in pork will decrease, if contamination at degutting can be reduced. For example, when the (mode) probability was halved from 80% to 40%, the prevalence in pork decreased from 3.9% to 3.4% (Table 4). Cross-contamination between carcasses at degutting also occurs, but with a lower probability. If the probability of cross-contamination was halved or totally removed, the model estimated that the prevalence in pork would decrease to 3.7 or 3.4%, respectively (data not shown).

Reduced probability of cross-contamination during handling

Handling consists of carcass splitting, meat inspection and trimming. Table 4 shows the predicted effect of lowering the probability of cross-contamination between carcasses during handling. Again, a substantial reduction was needed before a reduced prevalence of Salmonella in pork was observed. For example, if the probability was halved, the prevalence in pork decreased from 3.9 to 3.6%.

Increased effort both in primary production and at the slaughterhouse

To estimate the effect of simultaneous improvements both in the primary production and at the slaughterhouse, the parameters were changed concurrently (5-50% reduction, unless otherwise stated) for the following variables:

1) Proportion of Level 2 and 3 herds;
2) Singeing efficiency (triangular distribution 0.95; 0.975; 0.99 was used);
3) Probability of contamination and cross-contamination at degutting;
4) Probability of contamination during handling.

The larger the concurrent improvements, the higher the estimated impact on the Salmonella prevalence on the final carcass (Fig. 2). According to the model, the targeted Salmonella prevalence of 2.9% was obtained, if the four selected variables were improved by 25% each.

DISCUSSION

Limitations of the model

All models are reduced explanations of the real world. The more sophisticated the model, the more precisely the real world may be explained. However, this is at the expense of the overview, which may make the model too complicated to be of any use. The present model aims at describing the entire pathway from a pig being born into a herd, through growing, transportation, lairage and slaughter, and up to the point where the carcass is split. This is an

ambitious model, which is very data demanding. Due to the lack of data, assumptions had to be made. These assumptions (e.g. the transition probabilities) are open to discussion and are gradually being replaced as relevant results from other studies emerge. The model also requires further validation (Stärk et al., 2000). Using the simulations presented within this paper, can be seen as part of this process with more insight being gained regarding the behaviour of the model. Additionally, the results obtained indicate where efforts should be targeted (primary production, transportation or slaughterhouse). The skeleton of the model was based on a hazard analysis of the general pig production chain. The probabilities describing the single events were adjusted for the Danish context (e.g. the distribution for occurrence of long transportation). As this differs between countries, adjustments are needed before the model can be used for another country. Finally, residual environmental infections were not considered.

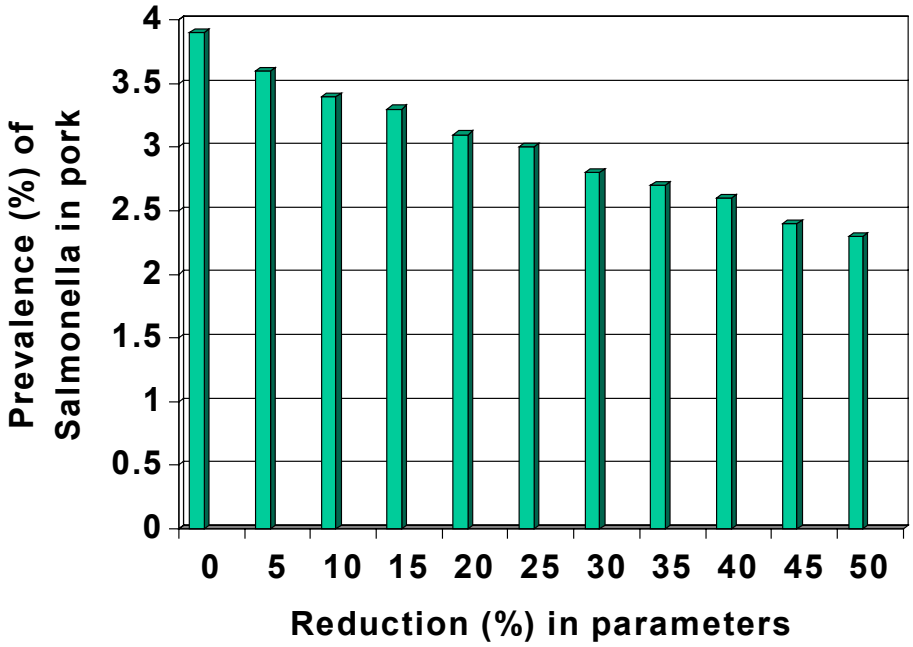

Fig. 2 Impact of increasing improvements in both primary production and the slaughterhouse on the prevalence of Salmonella of chilled carcasses.

When the baseline parameters were used, the prevalence of Salmonella contamination of final carcasses was estimated to be 3.9%. The Danish national surveillance of pork cuts found that that 1.1% were positive in 1998, 1.0% in 1999, and 0.8% in 2000 (Anon., 1999, 2000, 2001). The lower prevalence found in the national surveillance was probably due to the fact that only cuts of pork (where the skin has been removed) were sampled, whereas the model focused on the prevalence on split carcasses. Since January 2001, the surveillance of pork has been based on the swabbing of 300 cm^2 of carcass surface (Anon., 1996). Preliminary results demonstrated a prevalence of 1.6% (January-August 2001). However, if a larger area were swabbed, a higher prevalence would be expected. Part of the difference between the Danish surveillance results and the prevalence estimated by the model might be attributed to differences in test sensitivity. Table 3 showed very clearly the large impact that the test sensitivity had on

the observed prevalence. A Dutch literature review showed prevalences ranging from 5 to 30% (Berends et al., 1997). Therefore, the focus should be on the relative differences in prevalence from farm to fork and between the different scenarios, within the same study.

The effect of selected scenarios was also modelled. During modelling, the parameters for each variable were changed one by one, and the subsequent change in the expected prevalence of carcass contamination was noted. Scenarios with a low prevalence are the ones to consider for the future, as they will provide the highest level of food safety. However, the question is, how likely are they to occur, and at what cost. In the following sections, this is discussed for each scenario.

Reducing the number of Level 2 and 3 herds

There was a relative small effect from lowering the proportion of Level 2 and 3 herds, probably because these two groups only constitute a minor proportion (6% and 7% in this model, respectively) of the pig herds in the baseline scenario. Even though the Salmonella prevalence is <1% in the remaining herds, this is still sufficient to fuel the entire system with salmonella (e.g. when buying in new pigs, mixing during transportation or lairage, and cross-contamination during slaughter). However, if all Level 3 pigs were slaughtered at specific slaughterhouses, which ensured that the salmonella bacteria were dealt with effectively, the prevalence at the other slaughterhouses would be reduced by approximately 20%, from 3.9% to 3.1%. Another approach could be to slaughter Level 3 pigs on specific days only. Reducing the number of Level 2 herds on top of reducing the number of Level 3 herds had limited effect. In conclusion, even if all Level 2 and Level 3 herds were changed to Level 1, the prevalence in pork would not be decreased to the target of 2.9%. The Salmonella prevalence in a herd may be reduced by changing feeding practices, adequate rodent control, minimising mingling and improving hygiene. It has been estimated that a reduction from Level 3 to Level 1 would cost (per pig) £1.40-1.60 for a sow herd, £3.20-3.80 for a slaughter pig herd, and £4.30-4.60 for an integrated herd (Udesen, personal commun. 2002).

Separate transportation and lairage facilities for pigs from Level 1, 2 and 3 herds

There was no effect from separate transportation. This was probably because the degree of cross-contamination occurring at the slaughterhouse is so large in comparison, that it makes no difference whether Level 1 pigs are transported separately. In Denmark, Level 3 pigs are transported and slaughtered separately. However, Danish Level 2 pigs may be transported and slaughtered with Level 1 pigs. Currently, separate transportation and lairage facilities are available at only one Danish slaughterhouse. All pigs from Level 3 herds are slaughtered on one specific day and likewise for Level 1 and 2 herds. However, for other slaughterhouses this approach probably would result in additional costs because of longer transportation and changes in the logistics.

Apparently, there was a limited effect of long lairage (>3 hours). This was because long lairage occurs infrequently (mode: 5%) in Denmark. If long lairage was a common occurrence, for example with a probability of 50%, the estimated prevalence of test-positive carcasses increased to 4.3%. This means, that those days where everything breaks down, and lairage is long for the majority of the pigs, the salmonella prevalence increases. Mixing in lairage had no effect either, even though non-infected pigs become carriers if mixed with pigs from herds with a higher Salmonella prevalence. This is in accordance with Dalsgaard and Rasmussen (2000), who found that Salmonella negative pigs could become infected with Salmonella during lairage.

However, they primarily isolated Salmonella from the throat (17%), less commonly from the stomach content (3.8%) and infrequently from the carcass (0.8%). However, Hurd et al. (2001) found that 18 hours of lairage in clean pens did not increase shedding. Likewise, Boes et al. (2001) found that mixing of finishers from Salmonella-negative herds with finishers from Salmonella-positive herds in the lairage did not increase Salmonella shedding in the pens, nor did it increase Salmonella occurrence on carcasses of these animals. Although, pens should be kept clean, lairage pens are never completely clean (Boes et al., 2001; Swanenburg et al., 2001a).

Increased efficiency at singeing

Singeing is the only step in the production process, where Salmonella can actually be removed. There was a limited effect of increased singeing efficiency, probably because it is already carried out effectively in Denmark. Furthermore, the subsequent evisceration leads to contamination of carcasses (Berends et al., 1997). According to the model, the prevalence of Salmonella-positive carcasses will increase if singeing efficacy is reduced. During recent years, some Danish slaughterhouses have reduced singeing in order to save energy (even though it is still higher than in other European countries). It has been estimated that it would cost £0.02 per pig to increase singeing efficacy again (J. Larsen, personal comm. 2001).

Reduced probability of recontamination at polishing

There was limited impact of polishing on the Salmonella prevalence of pork. In the model, only recontamination was assumed to occur (no cross-contamination between carcasses) and, as only a small proportion of the pigs are infected, a low impact was expected. This is contrary to Berends et al. (1997) who (based on a literature review) estimated that 5-15% of all carcass contamination occurred during polishing. The Danish slaughterhouses have a tradition of intense singeing. Thus, the probability of both contamination and cross-contamination at polishing is probably negligible, but may increase if singeing efficacy were reduced.

Reduced probability of contamination and cross-contamination at degutting

It has been estimated that 55%-90% of all carcass contamination occurs during degutting (Berends et al., 1997). Several ways to reduce the probability of contamination have been proposed by the Danish Meat Research Institute and the Danish Bacon & Meat Council. These include automating procedures for taking out the intestines, removal of glands and blood from the neck area, loosening the bung using lactic acid sterilisation, application of a mechanised bung cutter in connection with enclosing the anus and rectum in a plastic bag. Likewise, improved disinfection of tools will reduce the probability of cross-contamination at degutting. Finally, slaughter with the tongue left in place is currently under consideration, as this has been shown to be associated with a lower prevalence on the carcass (Olsen et al., 2000). However, this is currently not allowed because of meat inspection rules (Fresh meat directive 64/433, revised 91/497). It has been estimated that it would cost £0.25-0.50 per pig to obtain a reduction of 25-50% in the prevalence of Salmonella positive carcasses (H. Christensen, personal comm. 2001).

Reduced probability of cross-contamination during handling

The simulations showed that improved hygiene during handling had an effect on reducing the Salmonella prevalence. The improvement could consist of scalding the knife and washing hands, visual meat inspection as well as proper disinfection and improved precision at carcass

splitting. In support of this finding, Swanenburg et al. (2001b) found that the carcass splitter was a critical control point in one Dutch slaughterhouse. Decontamination of the final carcass with acetic or lactic acid is used in the USA (Hurd et al., 2001). However, this is not currently accepted in the EU where use of acids will probably only be accepted for disinfection of tools. Currently, in Denmark, decontamination is carried out using hot water for carcasses originating from herds with *Salmonella* Typhimurium DT104. This has been shown to reduce the numbers of *E. coli* on carcasses by 2 log-units; regarding *Salmonella* it is assumed, that decontamination will reduce the numbers to below the detection limit on over 90% of the contaminated carcasses (Jensen & Christensen, 2000). Extending hot water decontamination to a larger proportion of the pigs (still focused on pigs from herds with high seroprevalence) would reduce the probability of contamination of the final carcass. It has been estimated that hot water decontamination would cost between £0.13 and £0.30 per pig (Jensen, 2000).

Increased effort both in primary production and at the slaughterhouse

The simulation of improvements in both primary production and at the slaughterhouse demonstrated a substantial decrease in the Salmonella prevalence on carcasses. This is probably related to the cross-contamination occurring in all parts of the production chain, particularly at the slaughterhouse. This is comparable with results of a similar simulation model developed by van der Gaag et al. (1999). However, van der Gaag et al. (1999) identified farms as the most important stage to achieve a reduction in the Salmonella prevalence. This study casts doubt that focusing solely on primary production would be the most efficient as the price per pig for reducing a Level 3 herd to a Level 1 herd is very high. Additionally, the Danish farmers have already put a lot of effort into reducing Salmonella for many years. The current system puts a constant pressure on the herds with highest Salmonella prevalence by use of penalty fees (Nielsen et al., 2001). Contrary to van der Gaag et al. (1999), Swanenburg et al. (2001c) found that the slaughterhouse was the most important source of Salmonella contamination for carcasses. Our results suggest that improvements to all parts (from stable to table) need to be considered, and the most economical optimal solutions should be chosen. A combination of interventions is even more critical if the current level of contamination is already low. Moreover, any further reduction can be expected to be hard to achieve.

CONCLUSION

Computer simulations showed that an improvement of individual factors either related to primary production, transportation, lairage or the slaughterhouse had a limited effect on the Salmonella prevalence of the final carcass. An effect of separate transportation and lairage can probably only be expected if the separation is maintained all the way through the slaughter process so as to avoid cross-contamination. To decrease the Salmonella prevalence in pork efficiently, improvements all the way from stable to table should be implemented. According to the model, even smaller concurrent improvements on the following areas would be beneficial:

1) Lowered proportion of Level 3 herds;
2) Increased singeing efficacy;
3) Reduced probability of contamination and cross-contamination at degutting;
4) Reduced probability of contamination during handling.

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STUDYING AN EMERGING PIG DISEASE, ENCEPHALOMYOCARDITIS, BY MEANS OF A CASE-CONTROL STUDY; MISSION IMPOSSIBLE?

H. MAURICE[1], M. NIELEN, K. FRANKENA, P. VYT AND F. KOENEN

SUMMARY

Over the last few years, Encephalomyocarditis virus (EMCV) has presented as an emerging infection amongst pigs in Belgium. In pigs, disease due to EMCV may take one of two main forms: an acute myocarditis which usually affects young piglets, or reproductive failure in sows. A case-control study was set up in West Flanders, to study potential risk factors associated with the introduction and spread of EMCV on pig farms. In total, 60 farms participated and control farms were initially selected from farms in which clinical signs were absent. Pig-to-pig contact, transplacental infection and rodents were seen as potential mechanisms for virus spread, whilst rodents were also thought to introduce EMCV onto a farm. However, during the analysis, unravelling the epidemiology of EMCV by means of a case-control study turned out not to be a straightforward process. Besides difficulties in recognising clinical signs, the case definition was also complicated by the existence of the two clinical pictures. In addition, a considerable number of potential control farms turned out to be seropositive, suggesting sub-clinical EMCV infections. Although rodents were often suggested as a reservoir host for EMCV, it is difficult to assess whether the seronegative control farms were really at risk, since no details were available on the serological status of the rodents on any farms. Despite the various difficulties that were encountered, it can be concluded that the case-control design is a suitable method for the study of an emerging disease. However, special attention has to be paid to the definition of cases and controls, particularly with regard to the hypothesis being tested. The preliminary results from the univariate analysis indicated clusters of factors that were either positively or negatively associated with clinical EMCV. Namely, rodents, the presence of other enterprise on the farm and general hygiene.

INTRODUCTION

Emerging infections can be defined as those infections that have recently appeared in a population or, that have rapidly increased their incidence or that have expanded their geographic range (Morse and Hughes, 1996). How should an epidemiologist, a veterinarian or a policy maker proceed to control such a disease, especially when little is known about its origin and behaviour at the time of the initial outbreaks? If the pathogen is known, one way to gain additional knowledge about the disease is the analysis of field data. Epidemiology can be defined as the study of the patterns of disease that exist under field conditions and more precisely as the study of the frequency, distribution and determinants of health and disease in

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populations (Martin et al., 1987). Information about what animals are affected, where and when a disease occurs (its distribution) is often suggestive of the cause of disease. Furthermore, determinants (causes) of disease should be formally identified, usually by means of observational studies. These epidemiological studies follow a general scientific method, in which hypotheses are derived from clinical observations and from descriptive studies (descriptive epidemiology and case studies) in combination with existing knowledge about the disease (Martin et al., 1987). These theories/hypotheses are then tested by a formal study, the results of which can either validate or modify current knowledge. Observational studies, often using samples from the study population, are specifically used to investigate the association between a potential risk factor and disease in order to identify and quantify the contribution of those factors to disease occurrence (Noordhuizen et al., 1997). In the case of an emerging disease, information is available on disease status but not on exposure to potential risk factors, hence the case-control approach is usually a suitable method of epidemiological analysis.

EMCV as an example of an emerging disease

Over recent years, Encephalomyocarditis virus (EMCV) has presented itself as an emerging infection amongst pigs in Belgium. In pigs, disease due to EMCV may take one of two main forms: an acute myocarditis which usually affects young piglets (Gainer, 1967) or reproductive failure in sows (Joo et al., 1988). Both forms of EMCV are seen in Belgium (Koenen et al., 1999), while outbreaks of acute myocarditis have also been reported in Italy, Greece and Cyprus (Sidoli et al., 1989; Paschaleri-Papadopoulou et al., 1990; Loukaidis et al., 1996). Since clinical cases occur regularly in Belgium, it was decided to set up a prospective case-control study in West Flanders. The goal was to study potential risk factors associated with the introduction and spread of EMCV on pig farms. Pig-to-pig contact, transplacental infection and rodents were seen as potential mechanisms for virus spread, whilst rodents were also thought to introduce EMCV onto a farm. Based on these hypotheses and prior knowledge from case reports and transmission experiments, a questionnaire with potential risk factors for EMCV was developed which was to be completed on all case and control farms involved in the study.

In this paper, the use of a case-control study design in the analysis of an emerging disease is discussed, using EMCV as an example. The practical implications of the case-control study will be presented and the various pitfalls that were encountered in this process, will be discussed. Based on the initial analysis of the data from the EMCV case-control study, the influence of the results that were obtained upon the initial hypotheses about the epidemiology of this disease will also be discussed.

MATERIALS AND METHODS

EMCV study design

The goal of the current case-control study on EMCV was to obtain more insight into factors associated with the introduction and spread of the EMCV on pig farms in West Flanders, Belgium. Since March 2000, all suspected clinical EMCV outbreaks, as recognised by sudden deaths amongst piglets or reproductive failure amongst sows, that were reported to the Regional Veterinary Investigation Centre (RVIC), Torhout were checked for their true disease status. After the first selection of potential clinical cases, the initial diagnosis was confirmed at the Veterinary and Agrochemical Research Centre (CODA-CERVA), Ukkel, Belgium (Koenen et al., 1999). This provided 29 clinically confirmed EMCV case farms. As potential controls,

farms without clinical signs of EMCV were selected by the RVIC within the same neighbourhood as the case farms. Whenever possible, the EMCV status of the control farms was checked by serological testing of available samples from the Aujeszky's disease control programme. All selected farms were located in the North-western part of Belgium (West Flanders).

Based on the prior findings from the field, expert knowledge from partners in an EU-project and deduced hypotheses about EMCV, a questionnaire was developed for completion on both case and control farms. The questionnaire contained questions on farm layout (type, number of animals, number of locations), secondary enterprises, stable management (manure handling, dead animal handling, feeding practice), disease status, presence of rodents and technical results. With respect to potential risk factors for EMCV, questions were included about rodent control, animal purchase, hygienic measures and contact patterns between pens and animals (Maurice et al., 2000). For case farms, an extra section was included concerning the disease outbreak, referring to the affected age category, observed mortality, duration of the outbreak and the spread of the virus throughout the farm. A full copy of the questionnaire (in English) is available from the author. During the interview, the questions were read out to the farmer by the interviewer and answers were written down or chosen from a multiple-choice selection. The questionnaire produced approximately 220 variables, which were incorporated into the univariate analysis. The case farms were visited by a veterinarian from the RVIC as soon as the clinical signs were detected. At the farm, the veterinarian completed the questionnaire and collected material in an attempt to confirm the initial diagnosis of EMCV. The control farms were visited for questionnaire completion by the first author during one of three time periods (18-20 December 2000, 12-13 February 2001 and 11-12 June 2001).

Statistical analysis

As a standard approach for analysing data from a case-control study, a first screening for statistical associations between the potential risk factors and the response variable was performed. All factors were handled as categorical variables and analysed by means of the Chi-square test and Odds Ratio (OR) (Hosmer and Lemeshow, 1989). In the second part of the analysis, logistic regression will be used to study the multivariate relationships between risk factors and EMCV status, including checks on confounding, interaction and correlation between potential risk factors.

RESULTS AND DISCUSSION

Selection of cases

The term emerging disease can apply to different situations, with different follow up strategies available for those in charge of the control of the disease. In the worst case scenario, an emerging disease is probably recognised by its clinical signs in the field without any knowledge of the causative pathogen. On the other hand, a 'known' pathogen can act as emerging disease by appearing in a new population such as in another species or a different age group, or by expanding its geographical range (Morse and Hughes, 1996). If a pathogen is known, basic questions that have to be answered include, why has the outbreak occurred or why has the incidence suddenly increased? For the current study, the initial goal of the epidemiological research was to obtain more insight into factors associated with the introduction and spread of EMCV on pig farms. It was initially assumed that a randomly chosen pig farm

without clinical signs in West Flanders would be free of EMCV. This seemed to be a valid assumption based on the rather low EMCV prevalence at the regional level (Koenen et al., 1999). Therefore, selection of case farms based on the presence of clinical signs seemed a logical option.

In total, 29 case farms were brought to the attention of the researchers because of suspected clinical signs of EMCV and subsequent laboratory confirmation. EMCV resulted in clinical signs amongst fattening pigs on 7 farms. In 14 farms, EMCV was found amongst suckling piglets while only one farm showed clinical signs amongst weaned piglets. Two farms revealed deaths among more than one age category. Reproductive problems (deaths amongst sows, infected foetuses, early births) were found on 3 farms, while 2 farms also showed clinical signs among suckling piglets.

The observed clinical signs on case farms, illustrated one of the complicating factors in this observational study. In the literature, two clinical pictures due to EMCV are mentioned, acute myocarditis and reproductive failure (Gainer, 1967; Joo et al., 1988). If two different types of EMCV exist (Paschaleri-Papadopoulou et al., 1990; Zimmermann, 1994; Koenen et al., 1999), the question arises whether risk factors for introduction and spread of both types of EMCV can be measured within a single study. This would at least require a sufficient number of cases of each subtype within the study and sound, distinguishing criteria between the two clinical pictures. On the other hand, it was experimentally shown that a myocardial EMCV strain from Greece was also able to induce comparable reproductive problems as observed from a reproductive Belgian EMCV strain (Koenen et al., 1997). These findings may support the hypothesis that the clinical picture shown not only depends on the virus strain involved but also on the age at which the animal becomes infected (Koenen et al., 1994). Because distinction between both clinical pictures is difficult without strain subtyping, initially all cases were handled as one group in the analysis. However, the limited number of specific reproductive cases (n = 3) may easily be left out of the analysis so that the myocardial presentation can be specifically analysed.

Selection of controls

To study the potential risk factors for introduction and spread of EMCV on pig farms, 30 clinically disease-free control farms were selected. To ensure that those selected farms were truly free of EMCV, serological tests were performed on samples available through the Aujeszky's disease screening programme. The serological examinations on non-clinical control farms unexpectedly showed that sub-clinical EMCV infection was widespread. Of the 17 control farms tested, 13 farms turned out to be serologically positive for EMCV. Four farms were serologically negative, while the status on the remaining 13 control farms could not be verified. Moreover, due to the emerging nature of the disease, detailed information on the sensitivity and specificity of the test used is still incomplete. Therefore, in evaluating the serological picture of potential control farms, farms might have been misclassified due to false positive and/or false negative results (Martin et al., 1987). Due to the serological results from control farms, it could be concluded that at least a proportion of the potential control farms had been in previous contact with the EMCV. Therefore, analysis of the data for introduction of the EMCV was no longer valid. The research question that remained was why should some farms show clinical disease while other farms do not?

These sub-clinical findings from the field seemed to be in agreement with the findings from recent transmission experiments performed in Belgium. In those experiments, piglets either died

suddenly shortly after inoculation or after contact infection or, the piglets survived and only demonstrated a serological antibody response (Maurice et al., 2002). One farm without clinical signs, that was initially selected as a case due to a positive serological response for EMCV on samples from the ongoing Aujeszky's disease screening programme, was transferred to the control group.

With respect to the remaining research question of clinical disease versus non-clinical disease, the seropositive pig herds seemed especially suitable controls since they had at least been in contact with the virus. However, at the farm level the clinical picture due to EMCV varies considerably in the field, changing from a few dead animals to serious losses (up to 25% mortality) (Koenen et al., 1999). These findings, together with the non-specific nature of the clinical signs of EMCV (mortality amongst suckling piglets, abortions and stillbirths), may raise the question whether this emerging disease will be recognised (at an early stage) when a farm is only mildly infected. Therefore, by possibly overlooking mild clinical signs, some farms may have been misclassified as control farms. On the other hand, the presence of rodents may confuse the analysis of EMCV introduction and/or spread and can put the seronegative status of some control farms in another perspective. Although rodents were often suggested as a reservoir host for EMCV on pig farms (Acland and Littlejohns, 1981), it is difficult to assess whether the seronegative potential control farms were really at risk of getting the disease, since no details were available on the EMCV status of the rodents at any farms.

Risk factor analysis

One of the problems in the study of an emerging disease is where to start looking for potential risk factors. Initially, a number of potential risk factors may be deduced from studies on other viral diseases, such as contact patterns between animals, housing systems, feeding systems. The current study was based mainly on the available knowledge obtained from the literature, from field case reports and from experiments. The results of the univariate analysis, where clinical disease versus no clinical disease was the dependent variable, are given in Table 1. With respect to the exploratory character of the study, an arbitrary cut-off p-value ($p \leq 0.25$) was used to test the factors for their association with clinical EMCV. Attention was directed at finding possible associations more than on the point estimate of the OR (Table 1).

Since rodents are often mentioned as the natural reservoir of EMCV (Acland and Littlejohns, 1981), attention was focussed on rodents (especially rats) during initial data analysis. However, none of the rat related factors such as presence of rats on the farm at night time or day time, nor the presence of rats in the three months preceding the farm visit, was found to be significant. Should we conclude that the role of rats in the epidemiology of EMCV has been previously overestimated? As was seen from the data, rats appeared to be almost equally present on both case and control farms (approximately 60% of the farms), possibly indicating that the presence of rats alone is not enough to explain their role in the introduction or transmission of the EMCV. This finding also points to one of the drawbacks of a case-control design, which depends on variation in any factor between cases and controls in the study population.

More insight into the disease status of the rodent population on case and control farms may increase the understanding of the role of such populations in the course of this disease. The presence of mice as a 'risk' factor may support the hypothesis of Seaman et al. (1986), who stated that the infection of pigs may be an interaction between rat, mice and pig populations on the farm. In their hypothesis, mice may only get infected when they come into contact with

infected rats but they may amplify the infection and transmit it to pigs. This hypothesis is supported by experimental findings where piglets died after eating infected mouse carcasses (Littlejohns and Acland, 1975). In addition, Maurice et al. (2001) indicated that the EMCV spreads easily amongst rats held under experimental conditions, without any mortality, highlighting their potential role as reservoir host.

Table 1. 'Risk' factors associated with clinical appearance of EMCV ($p \leq 0.25$)

Risk factor	Description	Case	Control	P	OR	95% CI
Presence of mice at night	Presence is high/medium	17	4	0.001	10.1	2.8–36.8
	Presence is null/low	11	26			
Water supply mated sows	Group-system	10	4	0.04	3.9	1.0-15.0
	Individual system	14	22			
Water supply preg. sows	Group-system	9	4	0.08	3.3	0.9-12.7
	Individual system	15	22			
Food supply mated sows	Automated system	15	10	0.09	2.7	0.9-8.4
	Feeding by hand	9	16			
Food supply preg. sows	Automated system	16	10	0.05	3.2	1.0-10.2
	Feeding by hand	8	16			
Food supply weaners	Automated system	19	14	0.14	2.5	0.7-8.4
	Feeding by hand	6	11			
Check stable for dead pigs	Only once a day	4	1	0.14	4.8	0.5-45.8
	>1 a day	25	30			
Presence of cows	Cows present	13	9	0.21	2.0	0.7–5.8
	No cows present	16	22			

The finding of EMCV in rat faeces might point to another possible transmission route of the virus from rodents to pigs (Acland and Littlejohns, 1981; Maurice et al., 2000). Direct contamination of feed and water equipment by EMCV excreted by carriers or hosts seems likely to occur (Acland and Littlejohns, 1981). The finding of 'risk' factors related to the food and water supply in the current study, may be perceived in this perspective. Providing water in a group system may implicate a more 'open' drinking system, which could more easily be contaminated by rodents. Feeding pigs automatically may possibly lead to leftovers in the troughs, which subsequently attract rodents. Although EMCV was found in mice and rats on farms where clinical disease due to EMCV occurred in pigs, Acland and Littlejohns (1975) argued that mouse control which was apparently effective on one of those farms did not reduce the clinical incidence in the herd. Although one observation is obviously of limited value in testing the rodent hypothesis, maybe the exact mechanism is still not properly understood. On the other hand, since the basic reproductive rate (R_0) of EMCV amongst pigs is around 1, a single introduction of the EMCV into the pig population may, due to chance processes, lead to large outbreaks from pig-to-pig contacts alone (Maurice et al., 2002). In this respect, early removal of dead pigs could prevent pig-to-pig transmission from cannibalism. The status of

cattle as a 'risk' factor might possibly be explained by the more open structure of a mixed farm compared to a dedicated pig farm. Food is usually stored loosely on such farms, which may attract and support larger rodent populations. It can be concluded that in the case of an emerging disease the unfamiliarity with potential virus hosts complicates the analysis of data for risk factors. This analysis is further complicated by a poor understanding of the exact transmission routes (within rodent and pig population, between species, horizontal/vertical).

Table 2 gives an overview of the most important 'protective' factors identified in this study. Again, the factors seem to be related to hygiene or rodent control. Direct removal of dead rodents might prevent the transmission of the virus between the different animal species, whilst good hygiene in general may reduce the probability of porcine infection by reducing the number of rats or reducing the contact rate with infected rodent faeces. If the p-value for screening is lifted to 0.30, the presence of cats or of dogs were also indicated as a 'protective' factors, which may be related to their role in reducing the number of (effective) contacts between the pig population and the rodent population.

Table 2. 'Protective' factors associated with clinical appearance of EMCV ($p \leq 0.25$ or $p \leq 0.30$)

Protective factor	Description	Case	Control	P	OR	95% CI
Sanitation	Removal dead rodents	2	19	0.001	0.05	0.01-0.3
	No removal dead rodents	20	10			
Fence	Farm is surrounded by fence	6	14	0.04	0.3	0.1-1.0
	Farm is not surrounded by fence	23	17			
Transporter	Truck has to be clean / empty	3	27	0.001	0.02	0.0-0.08
	No demands	26	4			
$p \leq 0.30$						
Dogs	Dogs present at farm	18	24	0.27	0.5	0.2-1.6
	No dogs at the farm	10	7			
Cats	Cats present at the farm	14	21	0.28	0.5	0.2-1.7
	No cats at the farm	10	8			

Continued analyses

Since the research question was altered to the occurrence of clinical EMCV, it may be questioned whether the serological negative controls or controls with an unknown status should be included in the control group. Therefore, a second univariate analysis was performed in which only seropositive controls (n = 13) were taken into account. The same 'risk' factors were identified within this analysis except for the factor 'check stable for dead pigs'. This means that the current results may be interpreted with more confidence. With respect to the 'protective' factors, the factor 'presence of dogs' no longer showed a significant association with clinical EMCV.

CONCLUSIONS

It can be concluded that the case-control study for risk factors for (clinical) EMCV as an emerging disease was not 'mission impossible' after all. The experience gained from this case-control study concluded that careful attention has to be paid not only to the selection of case and control farms, but also to their final definition within the analysis. Although the factors have only currently been evaluated using a univariate analysis, the analysis showed clusters of factors that at least were associated with the occurrence of clinical EMCV on pig farms. The factors identified all related generally to the presence of rodents and poor hygiene. This seems to fit into the hypothesis which had previously been formulated about the role of rodents in the course of the disease and their potential role in the transmission of EMCV. Future research should aim to unravel the virus transmission mechanisms within and between the different animal species involved. However, controlling the rodent populations at farm level could be considered as a useful first practical step in controlling an EMCV outbreak. In this respect, more knowledge on the survival of EMCV in food, water and manure could also help in clarifying the ecology of the disease. The occurrence of clinical disease may be regulated by the amount of virus in the feed or the infectivity of the rodents present, which would further support research into the EMCV dose-response relationship in pigs. Alternatively, the variable clinical picture could be dependent on chance processes originating from the transmission characteristics of EMCV amongst pigs (R_0 around 1) and between rodents and pigs. More information on the disease status of the rodent populations on both case and control farms is needed to enable evaluation of their potential role in the course of the disease.

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THE IMPACT OF DIFFERENT HOUSING SYSTEMS ON THE HEALTH AND WELFARE OF GROWER AND FINISHER PIGS

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SUMMARY

The objective of this study was to evaluate the effectiveness of two different housing systems with regard to improving the health and welfare of grower and finisher pigs. Grower and finisher farms, stratified by type of housing and evenly distributed over the German speaking part of Switzerland, were examined. The health and welfare statuses of traditional pig farms were compared with farms participating in a subsidised programme aimed at improving pig welfare.

Farms participating in the study received four visits during two different fattening periods. A general herd health evaluation of all pig pens along with an individual clinical examination of 20 - 30 pigs from the herd was conducted during each visit. Indicators used to assess health and welfare were the presence of lesions on the snout, ears, shoulders, legs and tail. Whole herd clinical examination included assessment of lameness, respiratory disease, diarrhoea, tail and ear biting, skin lesions, abscesses, sunburn and behavioural abnormalities. In addition to the health and welfare parameters, information on management practices was collected by interviewing the farmer. Pooled faecal samples were examined to estimate the intestinal parasite burden. Farm records on treatments, deaths, slaughter checks and production data were collected at the end of the 17-month study period.

Preliminary results are available for the examination of individual animals from the first two farm visits and from the faecal sample examinations. Farms with improved welfare conditions had fewer animals with snout lesions and also fewer pigs with swellings of the carpal, tarsal and coronary joints than pigs held on traditional farms. The examination of pooled faecal samples showed no significant difference between the two systems regarding intestinal parasite burden. The influence of the different housing systems and management factors on animal health and welfare will be assessed by multivariable modelling. Results of the models will be presented as attributable risks for each type of housing.

INTRODUCTION

In Switzerland, all housing systems have to be evaluated before being marketed (Wechsler et al., 1997). This evaluation is required under the legislation on animal protection and welfare. In addition, the government provides incentives to farmers who keep their animals in

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housing systems that are especially well adapted to their behavioural needs. A subsidy is available to help support farmers who keep their pigs in stalls with multiple areas containing straw bedding, where pigs also have daily access to outdoor facilities. This is in comparison with traditional indoor housing in pens with slatted floors. The subsidised housing systems fulfil the welfare requirements of EU regulations for organic farming. To date, no information is available on the practical implications of these improved housing systems on the health and welfare of Swiss pig fattening farms. The objectives of this study were:

- To identify meaningful indicators of health and welfare that can be examined under field conditions;
- To evaluate the effect of the animal friendly housing systems on the health and welfare of grower and finisher pigs.

MATERIALS AND METHODS

Farms were recruited by the Swiss Pig Health Service. Four main selection criteria were established. Firstly, the farms had to be recognised as free from both enzootic pneumonia (EP) and *Actinobacillus pleuropneumoniae* infection. Secondly, the minimal herd size was 60 pigs for farms operating an all-in, all-out system, and 100 pigs for farms with a continuous flow system. In addition, the housing system had to be either a traditional indoor operation with slatted floor or an animal friendly system with multiple areas, straw bedding and daily access to outdoor facilities. Finally, the manager of the farm had to be willing to participate and to provide farm data, such as slaughter checks, treatment records, mortality records and production data, where such records were available. Staff of the Swiss Pig Health Service asked the farm managers whether they were willing to participate in the study. Even if the farm manager refused to participate, the basic farm data were collected.

From 72 farm managers on animal friendly systems who were asked to participate in the study, 61 agreed (85 % participation rate). For the traditional farms, 51 farm managers were asked, and 41 agreed (80 % participation rate). Geographically, the farms were situated in the central, German speaking part of Switzerland, which has the highest pig density. Fifty-five animal friendly farms were selected for participating in the study. Seven of these were then rejected during follow up investigations. Two farms did not fulfil the welfare requirements concerning straw bedding, four were re-infected with EP, and one had given up pig production. Of the traditional farms, 41 were selected and four were lost during follow up. One was reinfected with EP and three had changed the housing system or given up pig production.

A total of 85 pig farms (37 traditional and 48 animal friendly farms) were each visited four times. The average herd size for animal friendly farms was 150 pigs (standard deviation [s] = 71.8) and 307 pigs (s = 293.8) for traditional farms. The farm visits were conducted during two fattening periods from July 2000 until December 2001.

Within each fattening period, the farm was visited twice. The first farm visit was performed shortly after a new group of growers had been purchased by the farmer (25 - 35 kg pigs). The second visit took place two weeks before slaughter (90 - 100 kg pigs). The second cohort of fattening pigs was examined when the pigs were at the same weights as the first group, but during a different season of the year. Thus, problems of the housing system that only occur during specific weather conditions, such as sunburn or frozen water supply, could be described. A structured interview based on a questionnaire was performed at the first farm visit. A general

herd health evaluation of all pig pens along with an individual clinical examination of 15 - 30 pigs of the herd was conducted at each visit. At the herd level, lameness, frequency of coughing and sneezing, diarrhoea, tail biting, skin lesions, abscesses, sunburn, and behavioural abnormalities (such as dog sitting, excessive fear or aggression) were assessed as indicators of health and welfare.

In the study, the individual clinical examination of pigs was based on the method described by Mayer (1999). Ears and shoulders were examined for injuries and scratches. Swelling and lesions of the snout were recorded. Carpal and tarsal joints, coronary band, and tails were examined for loss of hair, hyperkeratosis and lesions. The method (Mayer, 1999) was modified by addition of the parameters ear biting and thickening of carpal, tarsal or coronary joints. Furthermore, tidiness of legs and body were assessed.

Intestinal parasite burden was assessed by collection of fresh faecal samples from the pen floor. Five faecal samples each were pooled and examined using a modified McMaster method (Ward et al., 1997). For all farms, two pools of faecal samples were examined from two pens each taken at the last farm visit. In order to obtain a baseline level of parasite burden of grower pigs entering the farm, faecal samples were also collected at the third visit on 21 animal friendly and 13 traditional farms. Slaughter check data, medical treatment records, production data and mortality records of both cohorts were collected, if the data were available.

For descriptive statistics, all indicators of health and welfare were summarised at the herd level for each farm visit. Within-farm prevalence was calculated for the herd level examination as well as for the clinical examination of individual animals. Statistical analyses consisted of initial bivariate screening for variables associated with each indicator of health and welfare. Factors significantly correlated with health and welfare will be determined in a hierarchical multiple regression model, with farm as a random effect and cohort and age of pigs (visit) as fixed effects (Snijders & Bosker, 1999).

PRELIMINARY RESULTS

Descriptive statistics are available for the clinical examination of individual animals and for faecal sample examination. For the individual clinical examinations, lesions were more frequently found in older pigs shortly before slaughter (Tables 1 and 2). An exception to this was scratches on ears and shoulders in animal friendly farms. The incidence of these lesions decreased between the first and second visit. In general, animal friendly farms had a lower prevalence of lesions than traditional farms in most of the parameters examined in this study.

In traditional farms with slatted floors, an average of 23.4 % of pigs had thickening of the edge of the snout (s = 24.1) at the first visit (30 kg weight), and 28.5 % (s = 16.5) at the second visit (before slaughter). However, in animal friendly farms, 9.8 % (s = 14.1) of pigs showed the such lesions at the first visit and 13.3 % (s = 15.9) at the second visit. Moderate scratches of the ears were seen in 7.7 % (s = 14.8) of the pigs in traditional farming systems during the first visit, and in 11.6 % (s = 19.7) at the second visit. In animal friendly farms, 4 % of the pigs showed scratches during the first visit, and 2.1 % (s = 6.3) at the second visit. Scratches on the shoulder were visible in 2.8 % (s = 4.8) of pigs at the first visit, and in 0.8 % of pigs at the second visit on animal friendly farms. In traditional farms, 3.3 % (s = 6.6) of the pigs showed scratches on the shoulder at the first visit and 3.4 (s = 5.9) at the second visit.

Table 1. Summary results for the individual clinical examination of piglets at 25-35 kg of weight (first visit) for the different housing systems (37 traditional farms and 48 animal friendly farms). On average, 21.8 animals per traditional farm and 23.1 animals per animal friendly farm were examined individually. Mean within-farm prevalence ($\overline{X}$ (p)), standard deviation (s) and median are given for each parameter.

	Traditional farms			Animal friendly farms		
	$\overline{X}$ (p)	s	Median	$\overline{X}$ (p)	s	Median
Snout lesions (%)						
Thickening of edge of snout	23.4	24.1	16.7	9.8	14.1	4.1
Scratches on snout	5.6	10.2	0	0.4	1.5	0
Injury covering large area or deep cut	1.7	4.4	0	0.2	1.1	0
Hyperkeratosis – Carpus (%)						
Beginning hyperkeratosis	29.5	23.3	25	25.7	21.3	27.6
Distinct hyperkeratosis	31.5	28.9	31.0	6.6	13.8	0
Distinct hyperkeratosis with deep cuts	2.5	7.0	0	0.8	4.0	0
Hyperkeratosis – Tarsus (%)						
Beginning hyperkeratosis	40.9	22.7	40	17.4	16.7	13.2
Distinct hyperkeratosis	9.0	13.4	0	2.7	5.7	0
Distinct hyperkeratosis with deep cuts	0.4	2.5	0	0	0	0
Hyperkeratosis – Coronary band (%)						
Beginning hyperkeratosis	14.1	20.0	0	1.5	2.9	0
Distinct hyperkeratosis	4.0	8.6	0	0.4	2.2	0
Swelling at carpal joint (%)						
Swelling present	7.1	9.0	3.7	3.8	7.6	0
Swelling at tarsal joint (%)						
Swelling present	38.7	23.5	37.5	20.2	18.5	18.1
Swelling at coronary band (%)						
Swelling present	0.5	1.6	0	0.4	1.7	0
Tail biting lesions (%)						
Top of tail is missing	4.7	10.3	0	0.4	1.6	0
Section of tail is missing	1.5	4.0	0	0	0	0

With respect to carpal joint lesions, the within-farm prevalence of distinct hyperkeratosis in animal friendly farms was 6.6 % (s = 13.8) for the first, and 19.7 % (s = 24) for the second visit, respectively. In traditional farms, 31.5 % (s = 28.9) of the pigs showed the same lesions at the first and 37 % (s = 26) at the second visit. The findings for the tarsal joints were similar; animal friendly farms: 2.7 % (s = 5.7) prevalence of moderate lesions at visit one and 7.7 % (s = 8.1) at visit two. Traditional farms showed 9 % (s = 13.4) and 39.4 % (s = 18.8) for the first and second visit, respectively. In traditional farms, an average of 7.1 % (s = 9) of pigs had swollen carpal joints at the first visit, and 21.8 % (s = 16.7) at the second visit. These alterations were found in 3.8 % (s = 7.6) of the pigs in animal friendly farms at first visit, and in 7.0 % (s = 10.6) at the second visit. Traditional farms had an average of 4.7 % (s = 10.3) of pigs with the top of

the tail missing at the first visit and 12.0 % (s = 20.6) at the second visit. In animal friendly farms, 0.4 % (s = 1.6) and 1.7 % (s = 6.0) of pigs had a missing top of the tail. Detailed results of the clinical examination of individual animals are presented in Table 1 for the first visit and Table 2 for the second visit.

Table 2. Summary results for the individual clinical examination of pigs before slaughter (90-100 kg; second visit) for the different housing systems (37 traditional farms and 48 animal friendly farms). On average, 19.9 animals per traditional farm and 22.9 animals per animal friendly farm were examined individually. Mean within-farm prevalence ($\overline{X}$ (p)), standard deviation (s) and median are given for each parameter.

	Traditional farms			Animal friendly farms		
	$\overline{X}$ (p)	s	Median	$\overline{X}$ (p)	s	Median
Snout lesions (%)						
Thickening of edge of snout	28.5	16.5	28.6	13.3	15.9	6.7
Scratches on snout	16.5	13.7	14.3	3.1	5.6	0
Injury covering large area or deep cut	17.0	19.7	13.6	0.8	2.4	0
Hyperkeratosis – Carpus (%)						
Beginning hyperkeratosis	15.7	16.9	9.1	32.1	23.3	28.9
Distinct hyperkeratosis	37.0	26.0	33.3	19.7	24.0	10.0
Distinct hyperkeratosis with deep cuts	31.2	30.0	23.8	2.4	8.3	0
Hyperkeratosis – Tarsus (%)						
Beginning hyperkeratosis	25.4	18.0	25	28.8	18.8	29.9
Distinct hyperkeratosis	39.4	18.8	45.8	7.7	8.1	5.6
Distinct hyperkeratosis with deep cuts	13.0	18.8	3.8	0.2	1.1	0
Hyperkeratosis – Coronary band (%)						
Beginning hyperkeratosis	22.8	23.1	20	9.2	16.9	0
Distinct hyperkeratosis	26.8	28.2	17.6	1.0	2.7	0
Swelling at carpal joint %						
Swelling present	21.8	16.7	20	7.0	10.6	0
Swelling at tarsal joint %						
Swelling present	66.1	22.5	66.7	30.9	20.3	25.8
Swelling at coronary band %						
Swelling present	7.9	9.0	4.8	3.6	1.2	0
Tail biting %						
top of tail is missing	12.0	20.6	3.7	1.7	6.0	0
Section of tail is missing	2.6	10.1	0	0.4	1.2	0

All of the 34 pooled faecal samples taken at the third farm visit were negative regarding intestinal parasite eggs. Furthermore, 37 out of 42 (88 %) pooled faecal samples collected during the fourth visit on animal friendly farms were negative for worm eggs. The result for the faecal sample examination at the fourth farm visit was similar for traditional farms with 24 out of 28 (85 %) samples were negative.

DISCUSSION

In order to assess health and welfare in the field, valid indicators are needed which can be measured with a minimal effort. During the individual clinical examination of pigs, observation of lesions on snouts, alterations at joints, such as hyperkeratosis or thickening of the skin, injuries of the skin and cleanliness, could be easily and accurately measured and analysed across housing systems.

The high participation rate of farmers was probably due to the personal contact by Swiss Pig Health Service staff. On the other hand, a positive pre-selection of farms by staff members can not be excluded. Even though the Pig Health Service people were instructed to ask all farmers whom they visited during a certain time period, it may have been tempting to predominantly inquire with co-operative farmers. For example, it may have been easier to convince managers on animal friendly farms rather than those on traditional farms. Most of the farmers with animal friendly systems have remodelled their stable recently and thus were probably highly motivated to show the benefits of their method of pig production. Therefore, the selection of farms was not completely random. For biosecurity reasons, it was necessary to exclude farms positive for EP and *Actinobacillus pleuropneuminae*. Due to a minimal cohort size for estimating disease prevalence, farms in this study were larger than the Swiss average of 61.5 pigs per farm (Swiss Federal Statistical Office, 2000). Also, farms volunteering to participate in a research study typically form a bias selection.

The preliminary results indicated differences between housing systems regarding the welfare status of finisher pigs. However to date, only data for one fattening cohort per farm and only one part of the indicators of health and welfare have been evaluated descriptively. Therefore, the data need to be interpreted with caution. Currently, data on clinical examination and on pooled faecal sample collection is available. From the results obtained for the individual clinical examinations, substantial differences could be seen from individual farms within each housing system. This is reflected in a skewed distribution for the prevalence of different lesions between farms along with a large standard deviation for each housing type. Because of the shape of the distribution of within-farm prevalence of lesions, the final analysis will not be performed at a farm level. Instead, data will be analysed at an individual animal level in a hierarchical logistic regression model with 'farm' being incorporated as a random effect.

An interesting tendency was observed for scratches to the ears and shoulders. These lesions were less frequent in older pigs on animal friendly farms, whereas their prevalence increased from the first to the second farm visit on traditional farms. One possible interpretation could be that scratches in young pigs are due to hierarchy conflicts after mixing of pigs. In traditional pens with less space per pig, fighting among pigs might continue for a longer period of time. In the clinical examination, symptoms such as swelling of joints were recorded rather than an exact diagnosis. Due to the large number of pigs and pens that were examined, it was impractical to distinguish between arthritis and thickening of the skin around the joints. However, swelling at the joints recorded in younger pigs is likely to be caused by infection with *Haemophilus parasuis* (Plonait & Bickhard, 1997)

Due to financial limitations, an inexact method had to be utilised for the determination of prevalence of internal parasite infestation. This is because a pooled faecal sample collected from the pen floor at one point of time has limited accuracy due to the intermittent excretion of parasite eggs. Data thus obtained may not allow assessment of whether there is a big difference in the intestinal parasite burden between the two management systems. In addition, egg counts

may depend on the consistency of the faeces. Pigs with either diarrhoea or a high amount of raw fibre in the diet may have a lower egg count due to a dilution effect. Therefore, the results of the faecal examination should only be interpreted in combination with results on liver damage (white spot liver) during the slaughter check.

In conclusion, the final results of this study should indicate whether there are significant differences regarding the health and welfare status of finisher pigs from the different housing systems.

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EPIDEMIOLOGICAL TOOLS

THE RELATIVE VALUE OF FARMER, VETERINARY PRACTITIONER AND DIAGNOSTIC LABORATORY RECORDS IN PROVIDING EPIDEMIOLOGICALLY SOUND ENDEMIC DISEASE SURVEILLANCE DATA

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SUMMARY

On-farm computer disease records collected retrospectively from forty New Zealand dairy farms serviced by five veterinary clinics, were compared with time-matched veterinary practitioner and diagnostic laboratory records as endemic disease surveillance techniques. Veterinary practices visited the farms on average 17.8 times per year.

Farmer records yielded the highest disease event incidence (14.7 events per 1000 cow-months at risk), then veterinary clinics (5.2) and laboratory submissions (0.58 for disease records, 2.6 for all records). Farmer recording focused on mastitis and lameness. Many events recorded by the attending veterinary practice did not appear. Over one third of farmers recorded no disease events. Veterinary practice records provided the best spectrum of different types of disease events.

Veterinary practice records offer a valuable tool for monitoring the temporal and spatial pattern of disease events on farms. A palmtop recording system (VetPAD) was designed, which offers easy standardised data capture without loss of client privacy.

INTRODUCTION

New Zealand's claims to freedom from all Office International des Epizooties List A diseases and many other important infectious diseases are underpinned by a veterinary infrastructure that would detect these specified diseases, were they to be introduced. The New Zealand Ministry of Agriculture and Forestry (MAF) has statutory responsibility for protecting animal health in New Zealand through border protection policies and animal health surveillance programmes. These are designed not only for early detection of disease but also for demonstrating continued freedom from exotic animal diseases to New Zealand's trading partners.

Private veterinary practices provide clinical services to farms in all parts of New Zealand that are commercially farmed. The frequency of veterinary involvement varies with intensity of

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livestock management, from very regular in intensive dairy farming to occasional for extensive pastoral enterprises. However, this comprehensive monitoring of the New Zealand livestock industries by the private sector is not fully exploited for the purpose of animal disease surveillance. It is employed for notifiable disease reporting but not for recording patterns of occurrence of endemic diseases, with the capacity which that would create for promptly recognizing atypical patterns.

Until the 1980s, MAF provided regional laboratory services at little or no cost to veterinary practitioners or farmers, thereby gaining access to a range of diagnostic material that underpinned disease surveillance activities. Submission rates from production animals declined dramatically after full cost recovery for non-notifiable disease investigations was adopted in the mid 1980s. At the same time, laboratory services became fragmented as various private laboratories were established and the national network shrunk substantially. This further depleted the supply of surveillance material to the state service, to the point where private laboratories were contracted to provide diagnostic laboratory surveillance services.

Currently surveillance information is sourced principally from submissions by veterinary practitioners to their preferred diagnostic laboratory. Laboratories approved by MAF Biosecurity as recognised providers of surveillance reports for appropriate diseased animal cases provide summaries of data to MAF on a quarterly basis. The approval process involves contractual obligation to operate to a specified standard. Briefly this covers issues such as diagnostic capability, availability of suitable case material, quality control systems, lines of reporting and accountability.

Dairy farmers are required by law to record diseases occurring in, and treatments given to, animals providing milk for human consumption. The computer programme, DairyWIN©, gives its approximately 2300 dairy farmer users the option of recording that information in electronic format. This source of data could usefully contribute to national disease surveillance. In this pilot project, we compared the suitability of animal disease event data from records of veterinary practices, computerised farm records, and laboratory submissions for endemic disease surveillance. We also developed a prototype system for electronic capture of veterinary interventions on farms.

MATERIALS AND METHODS

Practice recruitment

Practices and farms were selected at the end of a calving year, and data collected retrospectively for the two years to mid-2001. All participants were therefore unaware when collecting data that it would be used in such a study. Nine veterinary practices (four lower North Island, four upper South Island and one lower South Island) were asked to provide computerised clinic records pertaining to selected dairy clients who used the DairyWIN© program. The practices were chosen purposively based on location and past relationships with the authors, which gave some confidence that suitable data could be collected. All were staffed with veterinary practitioners with substantial (10-36 years) dairy cattle practice experience. Each practice was located distant to the others so there was no overlap of practice areas. Records of all diagnostic laboratory submissions and reports for participating farms were collated and compared with the other data sources.

Selection of farms

Dairy farmer clients in the five practice areas who were using DairyWIN© were sent an explanatory letter along with a request for authorisation for access to their clinical records held by the veterinary practice and their on-farm DairyWIN© records. Records requested were for the period 1 July 1999 through to 31 May 2001. A further condition for eligibility was that a farm used a single veterinary practice for all veterinary work.

Data collection

Participating veterinary practices were asked to provide clinic charging records for dairy cattle for the 1999-2000 milking season and the period from 1 December 2000 to 31 May 2001. Records provided by veterinary practices (laboratory records and clinic charging records) were printouts of computer records and photocopies of laboratory results.

Veterinary practice farm visit records were separated into those that pertained to sick or injured animals and those that pertained to healthy animal interventions, such as pregnancy testing. An intervention was defined as any examination or treatment of an individual animal on any given day. Occasionally multiple interventions such as pregnancy testing and vaccination for leptospirosis were performed on an animal on the same visit. If it was possible to identify the animals involved, they were treated as one intervention. Where animals could not be individually identified, they were treated as separate interventions. For example, a group of cows pregnancy tested and a group injected for early induction of calving were treated as separate interventions.

"Sick" animals included those cows examined and treated for "no observed oestrus". In some calculations these animals were excluded, and these instances are noted where appropriate in the results.

A pre-determined coding system was used to code the diagnosis provided by the veterinarian. Some interpretation of what the practitioner described was needed on many occasions to reach a coding conclusion. Sometimes the diagnosis was not stated, but could be readily inferred from the treatment provided. For example, uterine infection could be inferred from use of products licensed for uterine infusion. Where the diagnosis could not be determined with confidence, the record was coded undiagnosed / unspecified. Each individual animal intervention generated a case record consisting of a farm identifier, date, type of intervention or diagnosis, type of animal examined (e.g., cow or calf), and in a few instances the identity of the individual animal examined. Farm identifiers were related back to practices.

Animal type was as indicated in the records provided although there was probably some overlap between heifer and cow classifications. Where no indication was provided, this field was left blank.

Laboratory records were coded as either "sick animal" or "production profile" cases with separate records for each individual animal sampled. A record consisted of farm identity, date of sampling, sick or production coding, animal type and animal identity. "Production profile" included samples for trace element analysis and metabolic disease assessments of more than one cow, while "sick" applied to all other samples, including milk samples for bacteriology.

After farmers returned electronic copies of their DairyWIN© records, the disease and cow tables were imported into Microsoft Access and the records extracted to a new table.

DairyWIN© recorded herd sizes (number of milking animals) were likely to be slightly conservative as they reflected herd size at drying off, rather than at the start of the milking season. Records were checked against quantities of vaccine purchased to confirm that the value given was approximately correct.

Data analysis

Data were stored in Access 97 and descriptive statistics were calculated in Microsoft Excel 97 and SYSTAT 10.

Calculation of visits per farm per month was the sum of the clinic visits for that month, divided by the product of the number of years data contributing to that month and the number of farms contributing. To calculate monthly visits per 100 cows for each clinic, the mean monthly number of visits times 100 was divided by the sum of the participating herd sizes linked to the clinic.

RESULTS

A total of five clinics provided data for the study: one from the southern South Island, two from the northern South Island, and two from the central North Island. Three clinics provided records covering the 23-month period in its entirety. One provided 16 months of records, and the other could only provide 10 months of records.

Most farmers willingly assisted with provision of records once they understood what was involved. A total of 40 farmers provided records that could be used. Herd sizes ranged from 92 to 874 cows in milk towards the end of lactation, with a mean of 338 compared with the national average of 236 (1999-2000 milking season). The within clinic average herd size varied between 210 and 476 cows. Across the 40 farms and five clinics, the study population encompassed approximately 13,600 cows (steady state) and 267,461 cow-months at risk.

Veterinary clinic records

A database was created consisting of 41,675 veterinary interventions, 728 laboratory records of individual animals and 12,342 individual animal records extracted from farmer computer records. Of the 41675 veterinary interventions, 5638 pertained to sick animals. When cows examined because of no observed oestrus were excluded, 1402 remained as genuinely sick or injured. Ages of animals were not usually given in the clinic record, but could be inferred in some instances. As the animals grew older the reliability of this inference reduced. A diagnosis was not specified for 5.5 % of sick animals examined (22% if non-cycling cows are excluded). Practice principals reported that clinic staff involved in data entry were reluctant to enter any diagnosis with difficult spelling, pronunciation or similar attributes and often simply entered 'sick' on the computer records.

The frequency of visits to farms each month was calculated for each clinic, with data consolidated from multiple years when available. Because larger herds had higher frequencies of monthly visits than small herds (Visit frequency = 0.69+0.0026[Herd Size]), visit frequency was also expressed as visits per month per 100 cows to standardise for herd size. Four of the clinics had a mean number of monthly visits per farm per 100 cows in the range 0.04 – 0.06, the fifth clinic had a value of 0.11. Thirty-one percent of variation in visit frequency was explained by

herd size. There was no significant effect of clinic on visit frequency (p=0.18). As could be expected with seasonal calving systems, frequency of herd visits varied greatly among months, and visit frequency was 5.2 times higher in the busiest month (August) than in the quietest month (June), with two small autumn peaks in March and May. This pattern varied little among clinics.

After excluding non-cycling cows, the mean number of sick animals seen per farm per month by participating clinics ranged from 0.7 to 2.5. Among 1402 sick animal events, 80.7% were from milking cows, 6.9% from calves, 3.8% from heifers, 1.2% from bulls, and 7.4% were of unspecified cattle type. However, likely inconsistency among veterinarians in distinguishing between and recording animals as cows or heifers must be considered.

Disease of any type (excluding non-cyclers, i.e. cows not exhibiting oestrus) observed by veterinary practitioners occurred at a mean frequency of 5.2 cases per 1000 cow-months at risk. September had the highest percentage of disease cases (23.3%) and June the lowest (1.8%). Non-cyclers were excluded from these calculations since they affect just two months of the year, albeit spectacularly. Cows seen in October with reproductive problems increase 38 fold when "non-cyclers" are included and for November the figure is 126 fold. With respect to the potential for veterinary practice records to contribute to BSE surveillance, only three cases (0.21% of sick animals) were diagnosed with non-metabolic neurological disease, two as polioencephalomalacia (one cow, one calf), and one case as brain tumour or abscess in a cow. There were 74 cows, one heifer and one unspecified recorded as downer cows (5.4% of sick cows), another group considered at higher risk for BSE. It is likely that some of these were revisits to the same animal, although this could not be determined from the data provided. It is also likely that the great majority of these were metabolic disease or calving paralysis, but again it was not possible to confirm this from the data provided.

Laboratory records

A total of 728 laboratory records of individual animals pertaining to the time periods of interest for each of the clinics were available for analysis. These represented 157 laboratory submissions. Individual animals were identified in 49% of these cases. One clinic provided the laboratory records in a format that did not allow identification of individual animals. When this clinic was excluded, 70% of samples were individually identified. On average, 1.9% of all animals examined were sampled for further laboratory work. When only sick animals (excluding non-cyclers and animals tested under the enzootic bovine leukosis control scheme) were considered, the percentage rose to 9% (range 4-13%). Eighty-one percent of all the laboratory samples were for production profiling type assessments rather than diagnosis of the cause of ill health.

Of the sick animal laboratory reports examined, 48 of 115 animals from four clinics (one clinic excluded because of insufficient data to make this judgement) were able to have a diagnosis made or confirmed. Another eight were able to have a tentative diagnosis made, but not confirmed. The remaining 59 could not have a diagnosis made or confirmed from the laboratory result alone. Of the 48 positive diagnoses, 23 were milk samples submitted for routine culture and sensitivity. The remaining 25 included Johne's disease (4), hypomagnesaemia (3), hypocalcaemia (4), hypocupraemia (6, all on one farm), bovine virus diarrhoea (2), rotavirus diarrhoea (1), salmonellosis (4; 3 on one farm), and facial eczema (1)

Farmer Records

Of the 40 farmers providing computerised records for the study, only 25 recorded any disease events during the period of interest. Another 4 recorded only veterinary reproductive examinations. Of the 12342 records only 3930 were disease records, the balance being reproductive examination records. The great majority of disease events recorded by farmers were mastitis and lameness. Of all farmer-recorded disease events, 177 were reported as diagnosed by veterinarians, 1829 by the farmer and 1924 not specified. Seven (28%) farmers reported 94% of all recorded disease events.

Comparison of the numbers of sick animals the veterinarians reported with the numbers the farmers reported were examined indicated that farmers mostly recorded only their own diagnoses in the herd management software, not those made by veterinarians. Only one farmer recorded 100% of the veterinary examinations and the mean was 19% (median 4%). Interestingly, only one of the seven larger farms mentioned previously recorded an above average percentage of the veterinary diagnoses. One farmer from the lower South Island recorded five cases of ephemeral fever, an insect-borne disease exotic to New Zealand

DISCUSSION

Increasing recognition of the importance of animal disease surveillance can be partly attributed to greater movement of people, animals and animal products among countries, and ongoing problems with emerging (e.g. BSE) or re-emerging (e.g. FMD) diseases in many parts of the world (Doherr & Audigé, 2001). Credible documentation of regional or national freedom from specific diseases, and ability to detect and respond to changes in animal disease incidence rely on systems that enable timely collection, analysis and dissemination of animal disease data. One recommendation of a recent review of animal health surveillance in the United Kingdom (Meah & Lewis, 1999), was exploration of novel sources of validated information. There are precedents for using veterinary practices for surveillance of specific diseases (Johnson et al., 1995; Mellor et al., 2000), but the current study and that of Black et al. (2001) appear to be the only efforts to assess the usefulness of veterinary practice records for national disease surveillance.

A crucial step in designing a surveillance system is to define the objectives, which will depend on the animal disease profile of a region and the potential animal and public health, and economic impacts of particular diseases. Advances in information technology and uptake of new technologies by both the veterinary and livestock industries provide novel opportunities for capturing data of animal disease events for purposes of surveillance. This project was designed to assess and compare farmer records, veterinary clinic records, and laboratory submissions as indices of animal disease in a defined population of dairy herds. The dairy industry was chosen due to its national importance, relatively high veterinary inputs, and well-established use of computerised farm records. Only farms using a computerised herd management program enabling recording of disease and treatment events (DairyWIN©) were included, and all participating veterinary clinics were well experienced in dairy herd health. These preconditions for the study design arguably present an assessment of a 'best case' scenario for practice-based and farmer-based surveillance under prevailing industry conditions in New Zealand. Due to the purposive selection of both veterinary clinics and farms, these data cannot be portrayed to be nationally representative with respect to farm conditions (larger and likely more 'progressive'), or veterinary clinics and 'farm-clinic' interactions.

Farmer records yielded the highest rate of recorded animal disease events (14.7 disease events per 1000 cow-months at risk), followed by veterinary clinics (5.2), and laboratory submissions (0.58 for disease records and 2.6 for all records per 1000 cow-months at risk). Features of farm-based data were a high proportion of disease records being mastitis and lameness events (84% of farmer-recorded disease events compared with 28% of veterinary events), a low proportion of undiagnosed / unspecified events (0.3% vs. 22% for veterinarians), and a general failure to specify which diagnoses were made by veterinarians. Over one-third of farmer participants recorded no animal disease events despite a selection process that was deliberately biased to include farmers thought to be more likely to be recording disease events. Of the farmer recorded disease events, 98% were diagnosed by farmers or unspecified and only 2% (177) were recorded as based on veterinary diagnosis. These 177 events would represent only 20% of disease events recorded by practitioners on the 25 farms that recorded health events. Although it is possible that farmers kept additional data on disease events in other repositories, it is only data in electronic form that can be retrieved practically for surveillance purposes.

Reliability of farmer-based diagnoses is generally considered to be poor (Vaillancourt et al., 1993; Christensen & Svensmark, 1997), but is likely to vary greatly according to the conditions involved. Our data suggest a farmer recording bias towards the more common conditions endemic to dairy herds (mastitis and lameness problems), for which farmer interest may be highest, and also confidence in diagnostic specificity may be relatively high. If lameness and mastitis were excluded, the incidence of other farmer recorded disease events was 2.1 events per 1000 cow-months at risk, while the corresponding figure for veterinary clinics was 3.6. Thus for general surveillance purposes, the data indicate that veterinary clinical records currently offer a more balanced source of dairy animal disease data than farmer based records. The recent survey by Black et al. (2001) reported an average of 14.0 visits per year to dairy herds, similar to the figure of 17.8 farm visits per year in the current study. Black et al. (2001) categorised visits as either sick animal or routine farm visits. In our study many sick animals were examined at the same visit for routine work. The computational methods used in the two studies differ but some general comparisons of results can be made. The history code describing gastrointestinal signs was reported as affecting 7% of dairy animals (Black et al., 2001), while the corresponding figure for this study was 5%. For the history code for coughing, respiratory signs, similar values of 1.2% and 1% (this study) were found. The most striking difference was for the code for arthritis, lameness, musculoskeletal signs, where Black et al. (2001) found 5.7% affected while our study found 23%. The reason for this difference is uncertain.

This study was retrospective, and was designed to determine the patterns of disease events on the study farms as captured by the three different mechanisms, when participants did not know in advance that their records would be used for purposes beyond their own needs and had no motivation to put extra effort into keeping more complete records. If participants had been recruited for a prospective study and given financial or other motivation the results may well have been somewhat different.

In terms of pay-off per dollar invested in collecting endemic disease surveillance data from dairy farms, veterinary practice records would appear to offer the best value for money, if standardised diagnostic categories can be used. One of the primary objectives of such a system would be to detect unexpected temporal trends in particular categories of disease events (for example, a sharp increase in neurological diseases) either across the entire population under surveillance, or with evidence of spatial clustering. A second equally important objective, would be to provide reassurance that no such adverse trends were occurring. A third objective

would be to evaluate the scale of veterinary involvement in farms in an area, and the patterns of endemic disease events occurring on the farms under surveillance. While veterinary practice records offer clearly the single best information source, diagnostic laboratory records provide some additional assurance of the nature of unusual diseases occurring on farms. However in reality very few of the diagnostic laboratory submissions of material from these farms related to unusual diseases, and based on records available to us a substantial proportion of submissions failed to produce a definitive diagnosis. Farmer records produced the largest incidence of diseases, and without these records the total weight of disease on farms would be seriously under-estimated. It is a potentially very cheap to extract data from an on-farm recording system in dairy herds, since this data is routinely uploaded to the national dairy database, and with farmer approval could be extracted.

Considerable value was obtained from comparing the incidence of disease collected via the three different pathways, and it is suggested that a national surveillance system should allocate resources for endemic disease surveillance in a way which maximises the information payoff from use of the three different pathways. The data from this study would suggest that shifting expenditure towards extracting veterinary practice data would enhance the payoff.

For veterinary records to become a regular component of a national disease surveillance system, several obstacles will need to be overcome. A means of easily capturing the data in a consistent format needs to be developed. Ideally this would involve electronic capture by the diagnosing veterinarian in the field, so as to minimise non-specified diagnoses and inaccuracies involved in re-typing the data by lay personnel. Further, it would need to meet quality control system standards. Once captured, the data would need to be collected to a central database for epidemiological analysis. To encourage and maintain veterinary interest in the project, payment for reporting and a mechanism for feed-back of data in a useful and potentially profitable form for veterinarians and their clients would be required. However, there appear to be no serious obstacles and existing technology can adequately deal with all of those issues. To this end we developed a prototype data collection tool designed for use with palm held computers (VetPAD - Veterinary Practitioner Assisted Disease Surveillance) in conjunction with the analytical component of this project. To make such a tool appealing to veterinary practitioners, it was designed to provide parallel functionality to facilitate practice management with respect to record keeping, billing and inventory management. With rapid changes in technology, the technological barriers to electronic capture of veterinary clinical events are unlikely to present a long-term problem.

Broader issues involve the validity of veterinary clinical diagnoses and management of the volume of data potentially available. Some problems in interpretation of clinical records encountered in this study could be mitigated through a standardised collection mode (e.g. VetPAD) and through accreditation of practices supplying data (as currently done with diagnostic laboratories). The volume of data collected in this pilot project (over 40,000 veterinary interventions) indicates that, efficient means of extracting the appropriate customised data for all potential users would need to be devised. Again, this presents a technical barrier that is not insurmountable. For surveillance purposes, a structure for practice based sampling would need to be defined. For example, sentinel practices might be recruited in relation to livestock demographics. Privacy considerations at practice and farm level would need to be dealt with satisfactorily, but there is no need for such data to contain identifying details and these could be removed in processing the data.

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DIAGNOSING DIAGNOSTIC TESTS: EVALUATING THE PRECISION OF DIFFERENT APPROACHES FOR ESTIMATION OF SENSITIVITY AND SPECIFICITY

N. TOFT[1], E. JØRGENSEN AND S. HØJSGAARD

SUMMARY

When evaluating the performance of diagnostic tests there is traditionally a distinction between the cases where the test is compared to a known gold standard test, i.e., a test with perfect sensitivity and specificity, and the situation when there is an absence of such gold standard methods. However, it is possible to interpret the two cases in the same framework, where the only requirement imposed on the problem is that data must have at least the same degrees of freedom as the model has parameters. By unifying the different methods in a Bayesian framework, based on graphical models using animal specific registrations, we were able to demonstrate the effect of the different degrees of information available by the different situations. Using simulated data we investigated the effect of population characteristics such as prevalence on the precision of the estimates. The value of adding information using reference groups and informative priors was illustrated on a scenario with low prevalence and poor sensitivity of the test. This situation resembled that of evaluation of tests for screening purposes for chronic infections such as paratuberculosis.

INTRODUCTION

The performance of a diagnostic test can be evaluated in several ways. The traditional method is to establish a group of animals with known disease status (i.e., classify the animals as positive or negative by a "gold standard" method). The sensitivity of the test (true positive fraction) is then calculated using the diseased animals, and the specificity of the test (true negative fraction) using the animals classified by the gold standard test as disease-free. The problem with this approach is that the availability of a gold standard method with perfect sensitivity and specificity is somewhat questionable for a large group of diseases/infections. However, several other approaches has been developed for evaluation of tests in absence of a gold standard, see for, example, Enøe et al. (2000) for a review of existing methods.

The classic approach is to adopt the Hui-Walter method (Hui & Walter, 1980), which allows simultaneous evaluation of multiple tests by stratifying the population into subpopulations with distinct prevalence and estimate the diagnostic values and prevalences of the sub-groups using maximum likelihood estimation.

A Bayesian approach to avoid stratifying the population was proposed by Joseph et al. (1995). However, as pointed out by Andersen (1997) and recently emphasized by Johnson et al.

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(2001), even the Bayesian approach requires, in statistical jargon, identifiability of the problem, which requires the split into two or more subpopulations for evaluation of two screening tests in the absence of a gold standard.

In this paper, we elaborate on the Bayesian formulation of the problem to establish a unifying framework for evaluating the performance of diagnostic tests using all available information. As an example of available information, the specificity (Sp) of certain types of tests is known to be very high, hence an Sp of 95-99% is much more likely than one of, say, 45%. Another example could be that for a few animals the true state of the disease is actually known, but these have been subject to only one of the tests under investigation. Hence this information might be inadequate to determine the Se and Sp of that particular test using the classic method, but it may be worthwhile to include that additional information in the estimation based on the larger sample.

A GRAPHICAL MODEL BASED ON INDIVIDUAL TEST RESULTS

Traditionally the evaluation of diagnostic tests is performed on contingency tables with the counts of different outcome combinations of the various tests under evaluation. However, it is possible to use the registrations of the individual animals directly. Consider the model in Fig. 1 that illustrates a graphical model of the situation with one animal and two tests. The nodes (circles) are (dichotomous) variables.

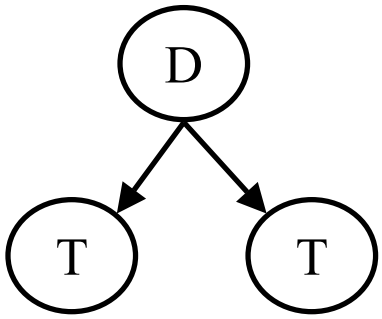

Fig. 1 The association between the disease status of animal and the outcome of two tests.

Note that the arrows indicate a causal relationship in the sense that the outcome of a test is affected by the state of the animal. The model assumes conditional independence between the tests, i.e., given that the true disease status of the animal is known, the probability of a certain outcome of one test is independent of the outcome of the other.

Now, assume we have 2 groups with N_1 and N_2 animals. Furthermore, assume that the two tests under evaluation have the same sensitivity and specificity in each group, and that the two groups have different disease frequencies (prevalences). This is the situation outlined as a graphical model in Fig. 2. The parameters of interest in this model are the Se and Sp for the two tests as well as the prevalences (P) in the two groups.

Information consists of two things: (i) observations on the individual test outcomes *and/or disease status of the individual animals*; and (ii) prior knowledge of test properties and prevalences expressed as prior distributions on the relevant parameters. If no information is available we refer to the priors as non-informative.

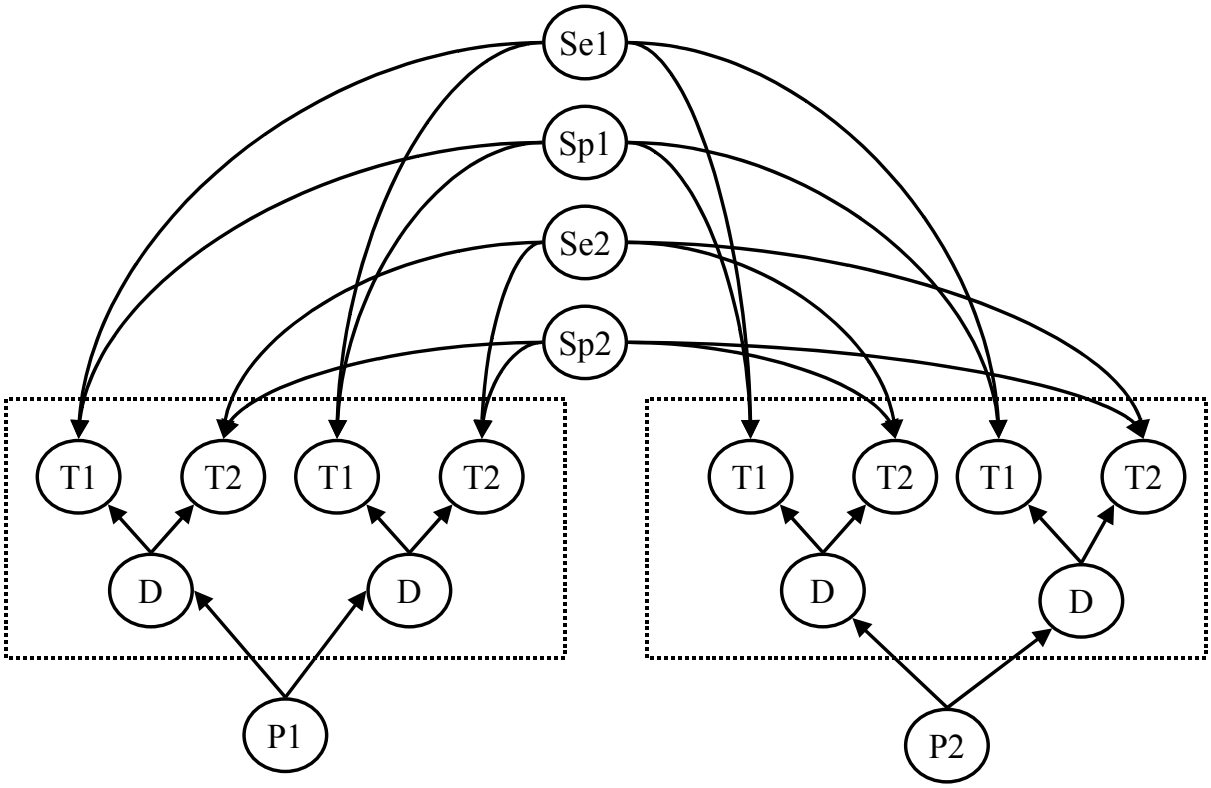

Fig. 2 The 2 groups, 2 tests problem as a graphical model (simplified by using only two animals in each group)

Using the model in Fig. 2, the joint distribution of the parameters can be specified in terms of the conditional distribution of each node given its parents. Thus, to specify the model we need the exact forms of the conditional probabilities implied by the model. Since we model the problem using registrations from individual animals the dichotomous variables describing the state of disease and test results can be assumed to have Bernoulli distributions, hence the likelihood terms of the model are (with some subscripts omitted):

$$
\begin{aligned}
D_i &\sim Bern(P_i) \\
T1_i &\sim \begin{cases} Bern(Se_1) & D_i = + \\ Bern(1 - Sp_1) & D_i = - \end{cases} \\
T2_i &\sim \begin{cases} Bern(Se_2) & D_i = + \\ Bern(1 - Sp_2) & D_i = - \end{cases}
\end{aligned}
$$

where Bern(π) indicates that the positive state has probability π. To complete the specification of the full probability model we require prior distributions on the nodes without parents, i.e. P_1, P_2, Se_1, Sp_1, Se_2, Sp_2 which we assume all have Beta distributions. The specific form of these distributions will be determined by any prior information.

The advantage of this formulation compared to the traditional idea of using the aggregate test results might become clear once we focus on the data required for this set-up. Full information regarding an animal consists of (besides an identifier): a disease state, result of test 1, and result of test 2. However, partial information where either the true disease state or one of the test results is missing can also be utilized in this set-up. The model in Fig. 2 can of course be generalised in both the number of groups and the number of tests. The lower limit is one group, one test. The only requirement lies in the nature of data in the sense that data must adhere to the Hui-Walter paradigm. This essentially states that data must have as many degrees of freedom as the model contains parameters. As an example: in a 2 groups, 3 tests situation, inference can be made from registrations where two or more tests are applied in both groups or one or more tests are applied to any animal with a known disease status. In general, care should be taken to assess how much information data contains and to avoid selection bias in the estimates.

Being able to add virtually all the registrations regarding the performance of the diagnostic tests into the same framework potentially reduces the uncertainty associated with the evaluation, which is usually expressed as confidence intervals. Being able to utilize additional information might further sharpen these bounds. As an example, the tests based on culturing faecal samples generally possess a very high specificity (often assumed perfect) since being able to grow the agent subject to testing indicates its presence in the original material. This kind of information is easily specified in terms of prior distributions. The Beta-distribution used in our model is very flexible and can adopt to virtually any prior knowledge regarding test or group characteristics. The special case where a test is evaluated against a known (but imperfect) test is one situation that is more adequately handled in the Bayesian framework. Rather than applying point estimates of the test diagnostic values, prior distributions indicating the range of the diagnostic values seems appropriate.

A generalisation of the 2 groups, 2 tests model from Fig. 2 is easy to implement in the WinBUGS (Spiegelhalter et al., 2000b) framework. Bayesian inference in WinBUGS is based on Markov Chain Monte Carlo methods. For an introduction to these methods see, e.g., Gilks et al. (1996).

ILLUSTRATION OF DIFFERENT LEVELS OF INFORMATION

As a starting point for analysis we consider Paratuberculosis and the diagnostic values for two enzyme-linked immunosorbent assays (ELISA) and one complement fixation (CF) test for detection of antibodies against *Mycobacterium paratuberculosis* cited in Nielsen et al. (2001). The sensitivities are all in the range of 38.4% to 58.8% and the specificities between 95.4% and 99%. These values are based on two groups of animals with known disease status: 150 or 196 negative animals and 196 or 177 positive animals. Actually, one study produced a specificity of 99.8% based on a sample of 1000 negative animals. To compare these results with diagnostic values obtained in absence of a gold standard, e.g., with two tests and multiple groups of animals with different prevalences, we use the sample sizes reported in Enøe et al. (2001). The tests are of the same type, e.g., ELISA and CF, but the disease in question is *Actinobacillus pleuropneumoniae* in pigs. In this study, a sample of 2369 was grouped in 6 sub-samples used for evaluation. The reported diagnostic values are of little interest to us, since they refer to a different disease, however, we may use the sample size as an indicator of appropriate sample sizes for evaluation in absence of gold standard tests.

In order to discuss the value of different levels of information we use simulated data where all information is known, i.e., the outcome of all tests as well as the disease status. Hence, by gradually reducing the available information we can explore how this affects the precision of the estimates. Under the full information the confidence intervals can easily be calculated by assuming Normal distribution of the parameters. However, we use the model for estimation of these as well to avoid ambiguity between assumptions.

The distinctiveness of prevalence in the subgroups

When stratifying the original population into sub-groups one must ensure that the prevalence differ among these groups. However, it is important that the stratifier does not interact with the test. Say, e.g., age is a poor stratifier when evaluating tests for chronic diseases like paratuberculosis. It is questionable whether or not the sensitivity and specificity of an ELISA-test is the same for heifers and multiparous cows.

The question is how much should these prevalences differ in order to be "distinct" enough to obtain valid estimates of the diagnostic values. In Enøe et al. (2001) the population is split into six groups, which should ensure that even by chance some of the prevalences must differ, thus yielding sufficient degrees of freedom for valid estimation. Here we consider 2 tests and 2 groups with varying prevalences. We simulate data from a situation where (Se_1, Sp_1) = (0.70; 0.99) and (Se_2, Sp_2) = (0.75; 0.95) and the total number of animals tested is 2400 with 1200 in each group.

In Table 1, the estimates of the diagnostic values and the associated 95%-credible intervals (which is the Bayesian equivalence of a confidence interval) are given for simulated data with different prevalences in the groups. The first three situations are chosen to ensure equal proportions of diseased and healthy animals. The last two scenarios are representative of the situation faced when evaluating tests for paratuberculosis (and quite likely other chronic infections).

Using the results in Table 1 several points can be made. Starting with the first three situations we are faced with 1200 sick and 1200 healthy animals distributed in three different ways with decreasing difference between prevalences (The notation (10;90) refers to a prevalence of 10% in group 1 and 90% in group 2, *etc.*). When the disease status is known there is no difference between the three situations. That the estimates differ is due to them being based upon three different simulations. However, in all three cases the estimates and their associated credible intervals are in accordance with what could be determined using a 2-by-2 contingency table of disease status and test results for the 2400 animals combined. For the three situations where nothing is known about the disease status of the animals, it is quite different. Consider, for example, the sensitivity of Test 1 represented in the third column of Table 1. The width of the 95%-CI is growing from 6.2 in the 10-90% case to 10.3 and 14.4 in the 40-60% and 45-55% respectively. Thus the value of full information (corresponding to a width of 5.2) depends on the difference in prevalences. This pattern is seen in the estimates and 95%-CI's of the other diagnostic values as well. Furthermore, the distributions, of the specificities especially, seem to skew towards the left as the difference in prevalence decreases (most obvious in the 40-60% case). For the 45-55% case it can even be questioned whether or not the estimates are still reasonable compared to the "true" values.

Table 1. The estimates of diagnostic values using full information and in absence of a "gold standard" using simulated data with known true disease status and varying disease prevalence in the different groups.

Group prevalences	Disease status	Test 1: Se (95%-CI)	Test 1: Sp (95%-CI)	Test 2: Se (95%-CI)	Test 2: Sp (95%-CI)
(10;90)	Known	70.2 (67.6;72.8)	98.5 (97.7; 99.0)	76.0 (73.2; 78.4)	94.7 (93.3; 95.6)
(10;90)	Unknown	70.5 (67.3; 73.5)	98.1 (96.8; 99.4)	76.7 (73.6; 79.6)	94.5 (92.7; 96.2)
(40;60)	Known	68.2 (65.7; 70.8)	98.9 (98.3; 99.4)	74.9 (72.4; 77.4)	95.2 (93.9; 96.3)
(40;60)	Unknown	67.5 (63.2; 73.5)	97.6 (91.9; 99.9)	77.1 (72.9; 85.5)	96.4 (91.2; 99.6)
(45;55)	Known	66.1 (63.5; 68.7)	99.0 (98.4; 99.5)	74.6 (72.1; 77.0)	95.6 (94.4; 96.7)
(45;55)	Unknown	69.5 (62.9; 77.3)	92.0 (86.0; 99.4)	85.3 (74.2; 96.3)	92.6 (86.7; 98.4)
(3;6)	Known	68.3 (59.2; 76.5)	99.3 (98.9; 99.6)	73.7 (65.5; 81.5)	95.2 (94.3; 96.0)
(3;6)	Unknown	53.9 (28.9; 97.2)	99.4 (98.4; 100)	75.3 (57.6; 97.2)	96.8 (94.0; 99.8)
(3;10)	Known	76.7 (69.6; 82.9)	99.1 (98.7; 99.5)	76.6 (69.7; 82.7)	94.8 (93.8; 95.7)
(3;10)	Unknown	71.2 (48.6; 98.4)	98.9 (98.0; 99.8)	82.2 (68.6; 95.7)	95.5 (93.4; 97.5)

Turning to the last four rows in Table 1, we are now concerned with cases where the difference in prevalence is even less than the 10%-point from the previous case. Because of the low prevalences in the groups, the sensitivity is estimated from rather small samples (98 and 156). This is seen by the very wide 95%-CIs given for the cases with known disease status. As a result of the low positive fraction of animals in the sample, the specificity is estimated from a very large number of animals. This implies that the difference between the estimation with and without information is negligible. Apart from the large number of negative animals, the fact that the specificities are high, helps to reduce the uncertainty about the estimate. Still, there is the problem of the sensitivity. One problem is that even though the total sample size used for evaluation is 2400 only (at most) 156 animals are sick, and hence used for evaluation. This is even less than the number used in studies where the status of the animals is known (Nielsen et al., 2001). The result is poor estimates with very wide 95%-CIs for the sensitivities. Thus, to improve the estimates in the case of low prevalence in both groups, there seems to be two reasonable paths. Either we add information regarding the sensitivity of the test or we try to establish a group of known positive animals and use these as a reference group.

Adding information using a reference group

Suppose we are given a gold standard test that can determine the true status of an animal, but this test has some properties which makes it unsuitable for general screening purposes: e.g. the test involves culling the cow; the test takes 4 months to produce a result; the test can only be carried out post-mortem etc. When choosing a reference group we are given 3 choices: establish

a group of (randomly selected) sick animals, establish a group of (randomly selected) healthy animals or use a random sample of the population. It is trivial to select a random sample from the population, but to establish similar random samples from the sick or healthy part of the population, might be difficult. A natural approach for the healthy sample is to sample from a known disease-free country with similar production traits. For the infected animals the use of culture-based analysis of faecal samples is often considered a perfect test with respect to specificity, i.e. a positive test result defines a positive animal with certainty. It is obvious that both approaches can be questioned with respect to the representativeness of the sample. However, here we ignore these problems and use a reference group of 180 animals, which are either all healthy, sick or a random sample from the population in question. We apply these reference groups to the usual test case with prevalences of 3-10% in the groups. The results are given in Table 2.

Table 2. The estimates of diagnostic values using full information, no information, a negative, positive and random reference group.

Simulated scenarios	Test 1: Se (95%-CI)	Test 1: Sp (95%-CI)	Test 2: Se (95%-CI)	Test 2: Sp (95%-CI)
Full information	72.9 (65.9; 79.0)	99.2 (98.7; 99.5)	78.7 (72.3; 84.7)	94.9 (93.9; 95.7)
No information	55.2 (38.7; 78.2)	99.3 (98.4; 99.4)	78.2 (64.9; 92.7)	96.9 (94.5; 99.4)
Positive reference	68.2 (61.4; 75.0)	99.6 (99.0; 100)	72.3 (66.5; 77.9)	95.5 (94.2; 96.6)
Negative reference	72.1 (64.4; 79.6)	99.4 (98.9; 99.7)	76.8 (68.9; 83.3)	95.0 (94.1; 95.8)
Random reference	64.4 (50.1; 78.8)	99.6 (98.9; 100)	74.9 (64.8; 85.6)	95.9 (94.5; 97.4)

In Table 2 we observe the usual pattern in the first 2 rows: using full information we can establish the usual estimates and the associated 95% credible intervals. When using only test results of the two tests, we see the now familiar pattern, where the estimates of the sensitivity of the tests are more or less useless, while the estimates of specificity are just as good as the ones obtained using full information. Again, this relates to the very large number of truly negative animals in the sample as well as the high values chosen for the specificity of the two tests.

In the last three rows, a third group with 180 animals of known status have been added. Starting at the bottom it can be seen that adding a random sample of animals from the population does help to reduce uncertainty about the sensitivity of the tests. However, the improvement is not nearly enough to produce reasonable estimates. The two other groups, where the reference group contains only sick or healthy pigs are more interesting. That the 95%-CI in some cases are narrower than the ones obtained using full information can be attributed to the fact that the estimates obtained using a reference group are based on more animals. However, the most interesting observation is that the 95% CI for the estimates of the sensitivities are about the same length for both reference groups as it is for the situation of full information. Furthermore, the estimates of Se using the negative (disease-free) reference group are closer to the "true" values in the case with full information. The reason is that the positive reference group contains more sick animals than the two original groups in total; hence the estimates are based more on the reference group than the two original groups. Apparently there was a bit difference in the two

realizations of simulated values of prevalence in the 2 cases. That there is no difference in estimated precision between the positive and negative reference in our cases, might prove extremely useful, since it can be considerably easier to establish absence than presence of e.g. paratuberculosis. The true effect of adding a reference population is to increase the knowledge of one of the diagnostic values. In our case we assumed that both tests where applied to the reference groups. Of course this can be generalized so that only a subset (e.g. one) of the available tests are applied to the reference group.

Adding information about the test using prior distributions

In the previous section, we used information about the state of a group of animals to deduce information about the properties of the test. There is, however, also the possibility to add information obtained by other means. In a Bayesian framework this is done by adding prior distributions. So far we have used non-informative priors on the diagnostic values and the prevalences in the shape of Beta(1,1)-distributions which are uniform in the interval [0,1]. Given information of the sensitivity and/or the specificity of a test, it is possible to specify other priors which reflects this knowledge. In the Bayesian framework there are no requirements imposed on the nature of this prior information. It can be obtained by means of expert information, pilot studies, literature or even from data itself. The only restriction is that information must be applied in the shape of distributions on the parameters of interest. In Table 3 we present results illustrating the effect of specifying priors where the precision is in accordance with the results cited in Nielsen et al. (2001). For a Se=70% based on 180 animals, the corresponding beta-distribution is approximately Beta(126,54). We omit listing the priors used since the distributions themselves are of limited interest.

Table 3. The estimates of diagnostic values using prior information on the data set from Table 2.

Priors defined for:	Test 1: Se (95%-CI)	Test 1: Sp (95%-CI)	Test 2: Se (95%-CI)	Test 2: Sp (95%-CI)
Both tests	68.9 (62.0; 75.4)	99.4 (98.8; 99.8)	76.3 (70.8; 81.4)	95.4 (94.3; 96.6)
Test 1	69.3 (62.6; 75.9)	99.3 (98.4; 99.9)	79.5 (67.9; 94.0)	95.4 (94.1; 96.5)
Specificities only	62.1 (46.3; 79.6)	99.3 (98.4; 99.9)	78.3 (67.1; 93.2)	95.9 (94.3; 97.6)
Sensitivities only	68.7 (61.9; 75.5)	99.5 (98.8; 99.9)	75.8 (70.4; 81.2)	95.4 (94.1; 96.6)

Comparing the first and last row in Table 3 we observe that the value of a prior on the specificity using this specific combination of test properties and group prevalences is limited. This is in accordance with the overall impression, that the large group of healthy animals is sufficient to establish good estimates of the specificity regardless of the problems imposed on the sensitivity estimates. The results using prior information on the specificities only show that the value of information regarding specificity is limited in our scenario. This is somewhat contradictory to the results from the previous section, where the negative reference group seemed to be just as informative as the positive reference group.

The situation in row 2 of Table 3 is a more realistic case of evaluation against a test with known diagnostic values. However, the approach referenced in Enøe et al. (2000) used the point

estimates of the diagnostic values of the reference test. Here we have applied prior distributions with a precision that conforms to what has been achieved by previous studies of ELISA-tests for paratuberculosis. The primary interest in row 2 lies in the estimates for test 2, but the precision attained here is below our usual standard for acceptance, which is somewhere around at least the precision obtained by the studies using full information and about 150-200 individuals.

DISCUSSION

As the simulation experiments in the previous section demonstrated there is a wide variety of problems to consider when evaluating diagnostic test in the population where it is intended for screening purposes. The absence of a gold standard when evaluating new tests has proven to be a difficult, but not unbeatable obstacle. The basic idea is to introduce more degrees of freedom in the data than parameters in the model. This is usually achieved by forming subgroups of distinct disease prevalences within the population in question.

We have presented experiments that clearly demonstrate that the difference between groups affects the overall precision of the estimates of diagnostic values. The situation with a low overall prevalence as well as a small difference between groups resembles, pretty well, the situation faced by many chronic infections. In our test scenario the low prevalence actually results in the evaluation of the sensitivity of the tests to be based on fewer animals than in the traditional set-up with evaluation based on samples with known infection status, i.e., evaluation against a gold standard test. This affects the quality of the estimates, as demonstrated by the simulations. It seems that there is no general objection to the established standard of using about 180 animals for evaluation, thus implicitly accepting that the standard deviation of an estimate of 70% is 3.4% which is equivalent to a width of the 95%-CI of 13.4. Whether or not this is an acceptable precision is not the topic of this paper. However, it seems problematic that once a study has been reported the usual approach is to apply the estimates and completely ignore the associated uncertainty.

To obtain reasonable estimates of the sensitivity of the tests in cases similar to our simulated scenario additional information is required. The experiments shown here suggest that the use of a reference population with confirmed positive or negative cases can improve the estimates. However, in practice it might be extremely hard to establish the true status of a group of animals. Applying priors might substitute the use of a reference group. But, applying informative priors can be dangerous. The priors need to be established by some sort of information. One method could be to adopt previous studies and use the reported results as priors. As demonstrated in Table 3, using the estimates and precision of previous studies might not prove very useful unless priors are applied to all parameters of interest, i.e., in our case at least both sensitivities. The problem is that these priors have more effect than the data. If the same prior is applied to both test sensitivities (say, the natural prior for test 2 applied to both) then the result will be that the posterior estimate of the sensitivity for test 1 is too high (73%, in an experiment excluded from the results presented here), with the same precision as if the correct prior had been used. Thus the prior becomes too important in absence of good data and the question is still how to obtain the prior distributions. The problems regarding prior specification are formally discussed in the context of Bayesian analysis in health technology assessment in (Spiegelhalter et al., 2000a)

In our formulation of the problem is it easier to utilise all available information, i.e., include situations where some tests have only been applied to a subset of the animals or groups in the

population. However, this situation might lead to considerable bias in the estimates so extreme care should be taken when designing such experiments.

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EXPLORATORY FACTOR ANALYSIS - AVOIDING MULTICOLLINEARITY IN RISK FACTOR STUDIES: AN EXAMPLE

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SUMMARY

The present study used factor scores from an exploratory factor analysis of 265 herds describing five underlying structures in typical Danish slaughter pig production. The objective of the present study was to investigate whether chronic pleuritis prevalence could be described in a simpler and more objective manner, using factor scores from an exploratory factor analysis, than by an ordinary risk factor study. The results showed that connections between type of herd, herd size, type of feed, all in all out production and type of floor were described in the risk factor study using factor scores, but could not be estimated in the ordinary risk factor study, due to multicollinearity.

INTRODUCTION

In ordinary risk factor studies the simultaneous characterisation of many risk factors may give rise to problems due to multicollinearity and confounding, which might influence the objectivity of the study. This can be avoided by using exploratory factor analysis, where all the original variables can be included, despite the presence of multicollinearity. The main objective of exploratory factor analysis is to describe intercorrelations among variables, describing the minimum numbers of latent factors or underlying structures, who are not directly measurable, but are responsible for the intercorrelation between variables (Scharma, 1996). Using orthogonal rotation ensures that the new latent factors are uncorrelated, thereby avoiding multicollinearity and confounding (Dohoo et al., 1996). Moreover, these latent factors determine those original variables explaining most of the underlying latent structure, thereby reducing the number of variables. Thus, factor analysis is also used as a variable reduction method. The estimated factor scores can be used in subsequent analyses without problems of multicollinearity and confounding, but with emphasis on the original variables describing most of the variance in the data (Dohoo et al., 1996). Exploratory factor analysis may thus result in simpler and more objective risk factor studies of complex surveys (Hatcher, 1994; Scharma, 1996; Dohoo et al., 1996).

The present study used results from an exploratory factor analysis of 265 herds describing the underlying structures in typical Danish slaughter pig herds, by data on management

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practices, neighbourhood factors herd and herd owner characteristics (Cleveland-Nielsen et al., 2001). These typical herd characteristics, presented as latent factors, were used as risk factors in a risk factor study of chronic pleuritis prevalence. The results from this risk factor study using factor scores, were compared to the results from an ordinary risk factor study of chronic pleuritis prevalence in the same herds and using the same variables.

Factor analysis of swine farm practices have been carried out in analysing the farm environment's role in respiratory disease (Hurnik et al., 1994), in categorising types of dairy cows (Sieber et al., 1987) and in describing the interrelationships between clinical variables in horses with colic (Thoefner et al., 2001).

However, to our knowledge, comparison between results for the same herds from an ordinary risk factor study and a risk factor study using factor scores from an exploratory factor analysis, have not previously been published.

Thus, the objective of the present study was to investigate whether chronic pleuritis prevalence in Danish slaughter pig herds can be described in a simpler and more objective manner, using factor scores from an exploratory factor analysis, than by an ordinary risk factor study.

MATERIALS AND METHODS

The 265 herds originated from a stratified random sample of Danish slaughter pig herds, where 141 herds were farrow to finisher herds and the remaining 124 were finishing herds. The herds were stratified by region, economic size (standardised gross margin), farmer's age and size of agricultural area and sampled by the Danish Institute of Agricultural and Fisheries Economics (Porskrog, 1986). Data on management practices and herd owner characteristics were collected by telephone interviews based on a questionnaire, whereas neighbourhood data, herd stem data and meat inspection data on chronic pleuritis prevalence were collected from databases at the Danish Bacon and Meat Council (DBMC). All data were collected in the year 2000. The questionnaire data had a response rate of 76% and were validated by visits from two veterinarians and two technicians to 92 of the herds, confirming the data obtained by the interviews.

The herd prevalence of chronic pleuritis were a monthly abattoir specific prevalence, calculated as the number of pigs detected with chronic pleuritis from a specific herd in a specific month at a specific abattoir at the mandatory meat inspection, divided by all pigs delivered by that specific herd in that specific month. The participating abattoirs were all 22 abattoirs under the auspices of the DBMC in the year 2000. Data from a total of 818,401 slaughter pigs from the 265 herds in the whole of year 2000 were used in the analyses.

A total of 28 original variables related to management practices, neighbourhood factors and herd and herd owner characteristics were analysed in the exploratory factor analysis. Variables related to management factors were: all in all out production (AI/AO), production of quality certified pigs, production of pigs above normal slaughter weight, mingling of pigs during the production period, feeding dry feed (opposed to liquid and wet feed), feeding homemixed feed, feeding *ad libitum*, acidification of drinking water with organic acids, number of drinking water suppliers per pig in a typical pen, using straw, having slatted floors, having solid pen dividers, having fully sectioned compartments (no passage of pigs or personnel and with separate

ventilation), changing clothes and boots before entering units, using a delivery room, using a delivery trailer by the road side, cleaning delivering facilities after each delivery. These management factors were selected due to their ability to describe underlying structures in typical herds and their relative reliability (i.e., only a few missing observations and the questions eliciting straightforward answers). Neighbourhood variables were: distance to nearest neighbouring pig herd, size of nearest neighbouring pig herd, health status (see description below) of nearest neighbouring pig herd, and pig density in a 5 kilometre radius. Herd and herd owner related variables considered were: type of herd (finishing or farrow to finisher), size of herd (annual deliverance of slaughter pigs), cattle present in the herd and health status of the herd. The herd owner variables included age, participating in study meetings and participating in pig production courses within the last year.

Health status is described as a conventional herd versus a SPF or MS herd. SPF herds are declared free of *Actinobacillus pleuropneumoniae* (AP) (except serotypes 6 and 12), *Mycoplasma hyopneumonia* (MYC), toxin-producing *Pasturella multocida*, *Barchyspira hyodysenteriae, Sarcoptes scabiei and Haematopinus suis*. SPF herds re-infected with MYC are designated MS herds. All other herds are designated conventional.

The following management factors were all analysed as continuous variables describing a percentage herd related factor, e.g., if three finishing units out of six practised AI/AO production, that particular herd had 50% AI/AO production: production of pigs above normal slaughter weight, AI/AO production, mingling of pigs, feeding dry feed, feeding homemixed feed, feeding ad libitum, acidification of drinking water, using straw, having slatted floors, having solid pen dividers, having fully sectioned compartments, using a delivery room, delivering the pigs on a trailer by the road side and cleaning delivering facilities after each delivery.

In order to secure the reliability of the variable AI/AO production, it was constructed from several questions from the questionnaire. The definition of AI/AO production in the present study was: AI/AO production on either unit or compartment level, no movement between batches, pigs below market weight were either slaughtered or moved to another unit, the units were always cleaned, dried out and had been empty for at least one day before new pigs were installed.

Pig density and size of nearest neighbouring pig herd were measured in heat producing units (HPU), in which a sow and a grower/finisher represent 0.45 and 0.17 units, respectively.

Before the exploratory factor analysis (Cleveland-Nielsen, 2001), optimal scaling of the original variables, using the maximum-total-variance method, was performed (PROC PRINQUAL) (SAS Institute Inc., 1989; Pheiffer,1999). The optimal scaling was done in order to obtain linear relationships between original variables, though they were recorded on different measurement scales (nominal, ordinal, continuous). Subsequently, exploratory factor analysis with principal component factoring (PCF) was performed (SAS Institute Inc., 1989; Hatcher, 1994). The analysis used the correlation matrix of the original variables and a linear transformation of the factor solution, an orthogonal rotation, which resulted in maximised explained variation, though maintaining uncorrelated factors (PROC FACTOR with VARIMAX rotation) (SAS Institute Inc., 1989; Hatcher, 1994).

Both the ordinary risk factor study and the risk factor study using factor scores from the factor analysis, were performed using a mixed model, PROC MIXED for continuous data,

(Littell et al., 1996), where the herd prevalences of chronic pleuritis were treated as a continuous variable. The mixed models were able to handle both fixed and random effects and the correlation structure between the repeated measurements of herd prevalences of chronic pleuritis. The correlation structure was modelled using a spatial power correlation structure.

In order to compare the results from the two mixed models, chronic pleuritis was estimated for different combinations of the original variables and compared. In order to estimate the impact of the original variables on the chronic pleuritis prevalence, the original variables were re-substituted in the factors estimated in the factor analysis. This was done for each factor by a linear combination of factor scores and standardised original variables as the sum of the products of factor score and standardised original variable. The original variables were standardised by subtracting the mean value and dividing by the standard deviation. The mixed model based on the factors estimated in the factor analysis was re-written with chronic pleuritis as a function of the re-substituted factors and mean estimates of months and abattoirs. The chronic pleuritis was estimated for a combination of the levels of the original variables when all other variables were held constant at mean values. Original variables with a high loading for the same factor were varied simultaneously. This resulted in a mean herd prevalence for combinations of levels of the original variables and these results were then compared to the least square means estimated for the variables in the ordinary risk factor study.

RESULTS

In the ordinary risk factor study, there was no confounding identified between variables, but multicollinearity between type of herd and the management factors AI/AO production, mingling of pigs and production of pigs above normal slaughter weight were demonstrated, i.e., for herds practising all in all out production, 72% were finishing herds. Type of herd was therefore excluded from the final analysis. Multicollinearity was also considered for the variables herd size and slatted floors, compared to the two management factors, all-in all-out production and dry feeding. Herd size and slatted floors were not risk factors in the resulting model, nor confounders for the management factors. However, the mean herd size differed according to the level of the two management factors. Herds having some all-in all-out production had a mean herd size of 6,266 pigs, whereas herds with no all-in all-out production, had a mean herd size of 2,819 pigs. Herds feeding only dry feed and having solid floors had a mean herd size of 2,123 pigs, compared to a mean herd size of 5253 pigs in herds with some or all wet feeding and slatted floors.

The result of the exploratory factor analysis is shown in Table 1, where 21 variables, describing most of the variance in the data of the originally 28 variables, were retained in the final model.

The analysis resulted in five factors describing typical Danish slaughter pig herds: Factor 1 was interpreted as herds with an old-fashioned management practice and being smaller herds. Factor 1 herds were described by the variables: dry feeding, use of straw, feeding ad libitum, not having slatted floors, feeding purchased feed and being a smaller than average herd. Factor 2 herds were herds with modern management practices and being large finishing herds, as factor 2 herds were described by the variables: practising AI/AO production, having fully sectioned compartments, being a larger than average herd and a finishing herd (opposed to a farrow to finisher herd). Factor 3 herds were characterised as herds with elderly herd owners not interested in ongoing education, as the herd owner did not participate in study meetings or pig production

courses and were an elderly pig producer having a high number of drinking water suppliers per pig (perhaps a consequence of elderly constructs of buildings). Factor 4 herds were interpreted as SPF herds focusing on bio-security and production of quality certified pigs, as factor 4 herds were characterised by the following variables: SPF herds with high health status and more often cleaning the delivering facilities than an average pig producer, having a SPF herd as a neighbouring pig herd, more often delivering pigs on a trailer by the road side and often producing quality certified pigs. Factor 5 herds were characterised as herds having dairy or beef cattle in the herd besides pigs and having herd owners without interest in bio-security, as factor 5 herds were characterised by the following variables: cattle (dairy or beef) in the herd, the herd owner and staff not changing clothes or boots before entering a pig unit and the herd situated at a further distance from other pig herds, than an average pig herd.

Table 1. Results of factor analysis using management variables, herd and herd owner characteristics to identify the underlying structures in Danish slaughter pig herds.

FACTOR 1	FACTOR 2	FACTOR 3	FACTOR 4	FACTOR 5
Old fashioned, smaller herds	Modern larger finishing herds	Elderly owner, no education	SPF herds, biosecurity	Also cattle, no biosecurity
Dry feeding	AI/AO	No study meetings	SPF status	Cattle in herd
Use of straw	Fully sectioned	High age of owner	Cleaning delivery	No changing of clothes/boots
Ad lib. feeding	Large herd size	Many water suppliers/pig	Neighbour SPF status	Larger distance to other herd
Purchased feed	Finishing herd	No courses	Trailer as delivery	
No slatted floors			Quality pigs produced	
Small herd size				

Results from the risk factor study using factor scores from the factor analysis showed, that Factor 1, Factor 2 and Factor 4 all had significant ($P<0.05$) impact on the chronic pleuritis prevalence. Moreover, the combined effect within these latent factors were all protective against chronic pleuritis (Table 2A).

Table 2A. Results using factor scores in a risk factor study of chronic pleuritis (CP) prevalence in Danish slaughter pig herds.

	FACTOR 1 Old fashioned smaller herds	FACTOR 2 Modern larger finishing herds	FACTOR 3 Elderly owner, no education	FACTOR 4 SPF herds, biosecurity	FACTOR 5 Also cattle, no biosecurity	Month	Abattoir
Estimate	-0.03	-0.02	<-0.01	-0.05	<0.01	<0.01[1]	-0.15[1]
P-value	0.01*	0.02*	0.73	<0.01*	0.89	<0.01	<0.01

*P-values < 0.05 were significant ; [1] Mean value

The results from the ordinary risk factor study of chronic pleuritis (Table 2B), showed that interaction between health status and pig density, dry feeding, AI/AO production, cleaning the

delivery facilities, mingling of pigs during the production period, production of pigs above normal slaughter weight, month and abattoir all were significant risk factors for chronic pleuritis prevalence. Health status interaction with pig density, dry feeding, AI/AO production and cleaning the delivery facilities were all protective factors for chronic pleuritis prevalence, whereas mingling of pigs during the production period and producing pigs above normal slaughter weight increased the risk of chronic pleuritis. The effect of different months and abattoirs differed, but the results are not shown here.

Table 2B. Results from an ordinary risk factor study of chronic pleuritis (CP) prevalence in Danish slaughter pig herds.

Variable	Estimates	P-value
SPF-status*Pig density		<0.01
SPF/MS*Pig density	-0.2418	
Dry feeding	-0.05	0.01
AI/AO	-0.05	0.02
Cleaning delivery	-0.05	0.02
Mingling	0.04	0.03
Prod. of pigs above weight	0.05	0.03
Month	Range –0.01 to 0.03	<0.01
Abattoir	Range –0.52 to 0.03	<0.01

*P-values < 0.05 were significant

The comparison of the results from the two risk factor studies are shown in Table 2C, where the mean difference on the chronic pleuritis herd prevalence due to the impact from a variable or factor are shown. In the ordinary risk factor analysis, the interaction of herds with SPF/MS health status with an increase of 100 HPU in pig density had a mean chronic pleuritis prevalence of 0.02 %, whereas a conventional herd with the same increase in pig density had an increased prevalence of chronic pleuritis of 21.97% compared to an SPF/MS herd. The effect of type of feed showed a decrease of 4.98% in the prevalence by only dry feeding, from 22.84% in herds using some or all wet feed. Practising some all-in all-out production decreased the prevalence by 5.07%, from 22.88%. Cleaning the delivery facilities decreased the prevalence by 4.72% from 22.71%. Mingling of pigs during the production period increased the prevalence by 4.12%, to 22.41%. Production of pigs above slaughter weight increased the prevalence by 5.12% to 22.91%. In the risk factor study using factor scores from the factor analysis, a mean herd prevalence of chronic pleuritis was estimated to be 21.62%, when all variables within factors were expressed as mean values. The significant Factors 1, 2 and 4 (Table 2A) had a mean chronic pleuritis prevalence of 18.17%, 18.27% and 10.28%, respectively. As shown in Table 2C, this resulted in a Factor 1 herd, with old fashioned management and with the highest impact from type of feed being dry feeding, having a significant reduction of the mean chronic pleuritis prevalence of 3.45%. Factor 2 herds, described as herds with modern management as practising AI/AO and being a large finishing herd, decreased the chronic pleuritis prevalence by 3.35%. Factor 4 herds, being SPF herds, decreased the prevalence of chronic pleuritis by 11.34%.

Table 2C. Mean difference of the chronic pleuritis prevalence in % in Danish slaughter pig herds.

Variable	A Risk factor study using factor scores	B Ordinary risk factor study
Factor 4	-11.34[1,2]	
SPF/MS*Pig density		-21.97[5]
Cleaning delivery facilities		-4.72
Factor 1	-3.45[3]	
100 % dry feeding		-4.98
Factor 2	-3.35[4]	
AI/AO		-5.07
Mingling	Not in final analysis	4.12
Prod. of pigs above weight	Not in final analysis	5.12

[1] Both MS and conventional herds, as compared to SPF health status of Factor 4 herds.
[2] Combined effect of Factor 4. [3] Combined effect of Factor 1. [4] Combined effect of Factor 2.
[5] Increase of 100 HPU in pig density.

In order to compare the results of the two risk factor studies, the herd size for a small herd was set to 2,123 pigs in the analysis using factor scores, as this was the mean herd size for herds feeding dry feed and having solid floors. The herd size for larger herds in Factor 2 was set to 6,266 pigs, as found for herds with AI/AO production.

DISCUSSION

In the ordinary risk factor study there were problems with multicollinearity concerning the variables type of herd, mingling of pigs, production of pigs above normal slaughter weight, herd size, type of feed, AI/AO production and type of floor. This led to the subjective exclusion of type of herd in the final analysis in the ordinary risk factor study. The combined effects of herd size, type of feed, AI/AO production and type of floor, presumably resulted in herd size being substituted by AI/AO production in larger herds and dry feeding in smaller herds. The effect of type of floor was presumably explained away by the dry feed effect. Herds feeding only dry feed had partially or solid floors and were only about half the size of herd, compared to herds with some or all-liquid feeding and slatted floors. This might be a consequence of larger herds more often having a fermenting system and thereby feeding liquid feed and having slatted floors, as having a fermenting system is more economically favourable in larger herds. The fact that AI/AO producing herds had twice the herd size and were most often finishing herds, might be a result of the better possibilities of applying all-in all-out production in larger herds. But due to problems of multicollinearity, these connections between variables could not be shown in the ordinary risk factor study.

The exploratory factor analysis resulted in five latent factors, that seemed to represent Danish slaughter pig herds in operation at the time of the study.

In the exploratory factor analysis the connections between type of herd, herd size, type of feed, AI/AO production and type of floor were illustrated. It was demonstrated that Factor 1

herds were herds with dry feeding and partly slatted or solid floors and were smaller herds, whereas Factor 2 herds were practising AI/AO and were larger finishing herds. Moreover, these intercorrelated variables were the ones describing most of the variation in the data in the exploratory factor analysis. Furthermore, type of herd did not have to be excluded from the factor analysis.

In the risk factor study using factor scores almost all original variables, 21 out of 28, were represented in one of the factors resulting from the factor analysis (Table 1) and of these, 15 contributed to the description of the chronic pleuritis prevalence in the subsequent analysis using factor scores, as Factor 1, 2 and 4 were significant risk factors for chronic pleuritis prevalence (Table 2A). This was in contrast to the ordinary risk factor study, where only 7 variables contributed to the description of chronic pleuritis, when month and abattoir were excluded.

Comparing mean prevalences from the study using factor scores and the ordinary risk factor study in Table 2C, showed that in general, the two different types of analysis both found the same protective factors for chronic pleuritis prevalence: Factor 1 herds were compared to the dry feeding effect, Factor 2 herds were compared to the AI/AO production effect and Factor 4 herds were compared to the effect of SPF health status and cleaning of the delivery facilities. However, the mean herd prevalences for the factors were generally smaller than the effects from the ordinary risk factor study. This is presumably due to the non-loading variables contribution to the impact of the factors.

The effect of the management variables AI/AO production and feeding dry feed in the ordinary risk factor study and being a Factor 2 herd or a Factor 1 herd in the study using factor scores were all of moderate impact on the chronic pleuritis prevalence. The effect of practising AI/AO production is most likely a consequence of disconnected transmission of pathogens from older animals to younger animals and the cleaning and disinfecting procedures applied in all-in all-out production, reducing the level of pathogens in the environment in general. As mentioned, the effect of dry feeding might be linked to type of floor and herd size. The interaction between SPF-status and pig density is most likely due to the fact that SPF and MS herds have restrictions of a minimum distance of 300 meters to a neighbouring pig herd, whereas this restriction does not apply to conventional herds.

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BOVINE EPIDEMIOLOGY

THE IMPACT OF CLINICAL LAMENESS ON THE MILK YIELD OF DAIRY COWS

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SUMMARY

This paper investigates the impact of lameness on milk yield. The dataset used included approximately 8,000 test day milk yields from 900 cows collected over 18 months from 1997 to 1999. The data were analysed to account for this auto-correlation. Farm of origin, stage of lactation, parity and whether a cow ever became lame influenced milk yield. In clinically lame cows, milk yield was reduced for up to four months before a case of lameness was diagnosed and treated and, for the five months after treatment. The total mean estimated reduction in milk yield per 305 day lactation was approximately 360kg. It was concluded that clinical lameness has a significant impact on milk production. This is important information for assessing the economic impact of clinical lameness and its impact on cow health. This finding adds weight to the importance of early identification of clinical lameness and the urgency required for techniques to improve the definition of this highly subjective diagnosis.

INTRODUCTION

Clinical lameness is of concern because of its high prevalence (Clarkson et al., 1996), association with pain (Whay et al., 1997) as well as with other conditions (Lucey et al., 1986; Barkema et al., 1994), and with economic loss (Whitaker et al., 1983; Enting et al., 1997; Kossaibati and Esslemont, 1997).

The definition of clinical lameness in cattle is fraught with difficulty, even amongst specialists. Currently, cows can be ‘locomotion scored' (Manson and Leaver, 1988; Whay et al., 1997). These scores include a category for 'imperfect locomotion' or 'uneven gait' to define a cow that is unsound (favouring one leg) but not clinically lame. Whether these cows will become lame or are recovering from an episode of lameness or are transiently unsound, is unknown. The importance of this state for the health, welfare and production of the cow is also unknown. This clearly indicates that a gold standard for 'clinical lameness - yes/no' has not been achieved. Despite this, the outcome, clinical lameness, is the best available measure and is frequently used in observational research throughout the world (Whitaker et al., 1983; Miller and Dorn, 1990; Tranter and Morris, 1991; Barkema et al., 1994; Hedges et al., 2001).

The difficulty in defining clinical lameness may partially explain the high variability in the reported incidence of clinical lameness in dairy cows. Estimates of between 5 (Eddy and Scott, 1980) and 70 cases per 100 cow years (Hedges et al., 2001) have been reported in the UK. This variability in incidence is reported world-wide. For example, Harris et al. (1988) reported 0 to

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50% in Australia and Barkema et al. (1994) reported 9 to 50% in the Netherlands. Part of the variation may also be attributed to the different skills of personnel responsible for identifying lame cows. Parlour workers, farm managers, veterinarians and research workers have been used to identify lame cows both within (Barkema et al., 1994; Clarkson et al., 1996) and between farms (Lucey et al., 1986; Hedges et al., 2001). There is also clearly a large variability in the incidence and types of lameness between farms (Barkema et al., 1994; Hedges et al., 2001).

The imprecise definition of lameness causes misclassification (Martin et al., 1987): lame cows defined as non-lame and vice versa. It also possibly causes bias (Martin et al., 1987) since under-diagnosis seems empirically more likely. If there is a downward bias in identifying whether or when a cow becomes lame, the impact of lameness on health, production and, therefore, the consequential economic loss is likely to be underestimated.

Sensitivity analysis indicates that the reduction in milk volume and quality is highly influential on estimates of economic loss from clinical lameness (Enting et al., 1997). However, the evidence for the impact of lameness on milk yield is conflicting. Some authors report a decreased milk yield after diagnosis (Whitaker et al., 1983; Tranter and Morris, 1991; Warnick et al., 2001), while others report a decrease in milk yield before a cow was treated as well as after (Lucey et al., 1986) and others that there is no change in milk yield (Cobo-Abreu et al., 1979). However, Barkema et al. (1994) reported an increased milk yield from 100 to 270 days in milk during the same lactation for cows with sole ulcers. These authors also reported an increase in the 100 day cumulative milk volume in the previous lactation for cows with any cause of lameness.

This finding led Barkema et al. (1994) to conclude that an estimate of milk loss by calculating the deviation from the lactation curve of daily yields was necessary to assess the impact of lameness on milk production, rather than a comparison of cumulative milk yields. This is particularly true for cows with higher than average yield since a reduction in total yield may bring these cows to the average, not below it, and consequently no difference in volume between lame and non-lame cows will be detected (Lucy et al., 1986).

It is therefore important to improve on current estimates of the impact of lameness on milk yield. Grohn et al. (1999) used an elegant technique with repeated measures of monthly test day yield (TDY) to demonstrate that cows produced less milk immediately before and after an episode of ketosis. These cows did not produce significantly less milk than unaffected cows over 305 days of lactation and a more simple analytical technique would not have detected the loss in milk volume.

Test day milk yields are repeated measures from one cow. The volume of milk produced at one test is dependent in part on the volume of milk produced at the previous test day and will influence the following TDY. These repeated measures can be analysed using hierarchically clustered mixed models with fixed and random variables (Goldstein, 1995). This paper utilises this technique and presents a multilevel model of the impact of lameness on the milk yield of dairy cows.

MATERIALS AND METHODS

The data came from Friesian/Holstein dairy cows on five farms in Gloucestershire (UK) from a study that investigated the effect of biotin supplementation on the incidence of lameness in dairy cows (Hedges et al., 2001). The herds were autumn calving. Cows were at pasture in

the summer months and fed on grass with concentrate ration being fed in the parlour. In the winter, cows were housed in cubicles and were fed a concentrate ration in the parlour and conserved forage (grass or grass and maize silage) in yards. The mean herd lactation 305 day yield ranged from 5500 to 7500 litres per cow.

The dataset included approximately 8000 TDY (one per cow per month in milk) from 900 cows over 18 months during 1997 to 1999. Clinical lameness was identified by the farmer and diagnosed and treated by one of six veterinarians who recorded the site of the lesion, the cause, treatment given and date of treatment on a standard form. There was no financial cost to the farmer for this treatment (Hedges et al., 2001).

The data were structured (Table 1) so that each TDY, for each cow formed one row of data. The TDY dates were repeated measures through time and these repeated measurements were coded 1 to 10 from calving to 300 days of lactation. TDY > 10 were not used in the analysis. The number of days from lameness to or from a TDY was estimated by subtracting the date of diagnosis of lameness from the test day date. This variable was converted to a factor variable coded as months before or after a diagnosis of lameness (-5 to +5) for each TDY. Only the first occurrence of clinical lameness in a lactation cycle was used to estimate the impact of lameness on milk yield. Stage of lactation was modelled as days in milk (DIM) using an exponential function to the power -0.05 days in milk (Wilmink, 1987). The dataset also contained the following factor variables: cow identity, farm of origin (1 to 5), parity (1 to 4+), first or second lactation in the study, ever lame (1 = yes, 0 = no). and whether the cow received a biotin supplement of 20mg per day during the study period (1 = yes, 0 = no).

Table 1. Example of the data structure relating to test day (TD) yield

Farm ID	Cow ID	Lactation in study	Parity	TD ID	TD date (mm/dd/yy)	Ever Lame	TD lame	lame +1 month	lame -1 month	lame -2 month
1	1	1	3	1	7/3/97	0	0	0	0	0
1	1	1	3	2	8/4/97	0	0	0	0	0
1	1	1	3	3	9/2/97	0	0	0	0	0
1	1	1	3	4	10/24/97	0	0	0	0	0
1	1	1	3	5	11/25/97	0	0	0	0	0
1	1	1	3	6	12/19/97	0	0	0	0	0
1	1	1	3	7	1/22/98	0	0	0	0	0
1	1	1	3	8	2/23/98	0	0	0	0	0
1	1	1	3	9	3/28/98	0	0	0	0	0
1	1	1	3	10	4/27/98	0	0	0	0	0
1	1	2	4	1	5/5/98	1	0	0	0	1
1	1	2	4	2	6/2/98	1	0	0	1	0
1	1	2	4	3	7/4/98	1	1	0	0	0
1	1	2	4	4	8/6/98	1	0	1	0	0
1	1	2	4	5	9/3/98	1	0	0	0	0
1	1	2	4	6	10/2/98	1	0	0	0	0
1	1	2	4	7	11/5/98	1	0	0	0	0
1	1	2	4	8	12/6/98	1	0	0	0	0
1	1	2	4	9	1/5/99	1	0	0	0	0
1	1	2	4	10	2/7/99	1				

The data were analysed in MlwiN version 1.1 (Rasbash et al., 1999). TDY was the outcome variable and the data were distributed normally. A two level general linear model with restricted iterative generalised least squares (RIGLS) procedure was used to analyse these hierarchically clustered data. Level 2 was the cow identity and grouped within this at level 1 were the TDY repeated measures sorted by month from calving. Each cow contributed a maximum of 20 TDY events and a minimum of one. Farm of origin and biotin supplementation were forced into the model as fixed effects.

The model was:

$$y_{ij} = \alpha_{ij} + \Sigma\beta_{ij}X_{ij} + \Sigma\Delta_j Z_j + u_j + e_{ij}$$

Where y_{ij} = milk yield on test day i for cow j, α_{ij} = intercept value for test day i for cow j, β_{ij} = coefficients for X_{ij}, X_{ij} = exposure for TDY ij, Δ_j = coefficients for Z_j, Z_j = exposures for cow j, Σ = sum of 1 to n exposures, u_j = error term for between cow variation, e_{ij} = residual level one error.

Complex variation (where the intercept and the slope of the lactation curve varied between cows) was tested. As a consequence u_j, between cow variation, was dependent upon $\gamma + \delta_j A_j$, where γ = intercept variance, $\delta_j A_j$ = variance for exposure Z_j, and e_{ij} was dependent upon $\eta + \theta D_{ij}$, where η = intercept variance of repeated measures and θD_{ij} = variance for exposure D_{ij}.

The occurrence of first lameness by month in milk was plotted and the mean lactation curve for cows that were never lame and cows that were clinically lame during a lactation was compared visually in MS Excel 97 (Microsoft Inc.). The distribution of standard residuals of the multilevel model was plotted to check the fit of the model.

RESULTS

Over 70% of cows became lame at least once. The four most frequent diagnoses of lameness were sole ulcer, white line disease, foul and digital dermatitis. These had an incidence of 9 - 11 cases per 100 cow years. The incidence of first episode of lameness peaked three months after calving.

High yielding cows were more likely to be lame and produced more milk throughout lactation than cows that were never lame. As a consequence, the dummy variable 'ever-lame' was put into the model (Table 2). These cows produced a mean of 1.12 (±0.34) litres per day more milk than cows that were never lame. This was a mean of 342 extra litres of milk over 305 DIM (95% CI: 135 to 549 litres). Farm of origin, stage of lactation, parity and whether this was the cow's first or second lactation in the study, influenced milk yield (Table 2). Interactions between farm of origin and parity with stage of lactation were significant but had no impact on the estimated milk loss attributable to lameness. These have not been presented.

Clinically lame cows had a reduced milk yield from up to four months before a case of lameness was diagnosed and treated, and for five months after treatment (Table 3). The total mean estimated reduction in milk yield for a cow lame in the fifth month of lactation onwards was 357 litres (95% CI 163 to 552) per 305 day lactation (Table 3).

There was complex variation in the random structure of the model with (DIM) and whether the cow was in her first or second lactation in the study accounting for some of the between cow

variation. This indicated that the slopes of the lactation curves varied between cows because of these exposures. The residual plots indicated that the model assumptions were correct.

DISCUSSION

This analysis has identified that there was a higher mean lactation yield in cows that were ever-lame compared to those cows that were never-lame. This has been previously postulated from other studies (Hansen et al., 1978; Lucey et al., 1986; Barkema et al., 1994). As a consequence, a level 2 dummy variable that coded cows as ever-lame was created and put into the model. Ever-lame cows would have produced a mean increased milk yield of 1.12 litres per day. This has implications for the health of high yielding dairy cows. Such cows are at greater risk of ketosis (Grohn et al., 1999) as well as other health disorders (Hansen et al., 1979) and, this study has confirmed that such animals are at a greater risk of lameness. This increased risk may arise because their nutritional demands are not met. If a farmer feeds his cows to an average, these high performers are at risk of malnutrition. Well-fed high yielding cows must stand for long periods to eat and this too may increase their risk of lameness. It may also be that these cattle have a greater innate risk. However, genetic studies indicate that high milk yield is negatively correlated to lameness (Hansen et al., 1979).

Table 2. The impact of fixed effects on milk yield

Exposure	Mean effect	S.E.	Lower 95% CI	Upper 95% CI
Intercept	26.578	0.697	25.184	27.972
Ever lame	1.123	0.343	0.437	1.809
Days in milk	-0..49	0.002	-0.053	-0.045
Wilmink function	-9.169	0.424	-10.017	-8.321
2nd study lactation	2.777	0.312	2.153	3.401
Farm 2	4.219	0.631	2.957	5.481
Farm 3	0.983	0.578	-0.173	2.139
Farm 4	5.907	0.510	4.887	6.927
Farm 5	-2.143	0.769	-3.681	-0.605
Parity 2	0.493	0.368	-0.243	1.229
Parity 3	1.022	0.426	0.176	3.314
Parity 4+	2.462	0.413	1.636	3.288
April – June	0.179	0.732	-1.285	1.643
July – Sept	0.866	0.666	-0.466	2.198
Oct – Dec	0.737	0.546	-0.355	1.829
Biotin – yes	-0.008	0.0.302	-0.612	0.596

DIM = days in milk; S.E. = standard error; CI = confidence interval

Table 3. Mean daily reduction in milk yield in lame cows in the months before and after diagnosis

Exposure (months)	Mean effect	S.E.	Lower 95% CI	Upper 95% CI	Cumul. mean loss in yield*	Cumul. lower 95% CI loss in yield	Cumul. upper 95% CI loss in yield
Before diagnosis							
-5	-0.255	0.352	0.449	-0.959	-7.65	13.47	-28.77
-4	-1.065	0.353	-0.359	-1.771	-31.95	-10.77	-53.13
-3	-0.85	0.355	-0.14	-1.56	-25.5	-4.2	-46.8
-2	-1.598	0.374	-0.85	-2.346	-47.94	-25.5	-70.38
-1	-1.729	0.363	-1.003	-2.455	-51.87	-30.09	-73.65
After diagnosis							
1	-1.706	0.394	-0.918	-2.494	-51.18	-27.54	-74.82
2	-1.885	0.422	-1.041	-2.729	-56.55	-31.23	-81.87
3	-1.228	0.466	-0.296	-2.16	-36.84	-8.88	-64.8
4	-1.847	0.514	-0.819	-2.875	-55.41	-24.57	-86.25
5	-2.028	0.563	-0.902	-3.154	-60.84	-27.06	-94.62
Total#					-357.24	-162.78	-551.7

S.E. = standard error; CI = confidence interval
*Assuming 30 days per month.
excluding 5 months before diagnosis because CI include unity

Table 4a. Random effects: level 2

Exposure	Variance	S.E.
Intercept	42.385	2.575
DIM	0.001	0.000
covariance DIM/Intercept	-0.177	0.013
2^{nd} study lactation	29.025	2.669
covariance: 2^{nd} lact./Intercept	-14.629	2.228
covariance: 2^{nd} lact./DIM	0.053	0.012

S.E. = standard error; DIM = days in milk

Table 4a. Random effects: level 1

Exposure	Variance	S.E.
Intercept	15.655	0.287

S.E. = standard error

Total log likelihood from model (Tables 2 - 4) = 47412.3, null model 52434.4

The availability of is information increases the complexity of decision making on culling for lameness. Total yield needs to be considered before lame cows are culled. This may be why there is not always a positive association between culling and lameness (Barkema et al., 1994). It appears that farmers are already aware of this positive association between milk yield and lameness when they decide whether or not to cull a cow (Barkema et al., 1994). However, this in no way indicates that clinical lameness is acceptable, or even tolerable, in these cows. The estimates from this paper indicate that lame cows fail to produce an average of approximately 350 litres of milk and, therefore, any advantage from higher yields is lost. The conclusion is that to benefit from high yielding cows, extra attention to management is required. If this is not possible, then rather than aiming for maximum milk yields, farmers should define an optimum yield suitable for their system that maximises cow health and productivity and use the appropriate genetic stock.

Lame cows produced significantly less milk from up to 4 months before and up to 5 months after a diagnosis of lameness. This reduced milk yield before a diagnosis of lameness could arise from a confounding effect that was associated with lameness later in lactation and reduced milk yield. For example, it is possible that these cows had an insult early in lactation (e.g. ketosis) that reduced their milk yield and this was correlated with, or increased their likelihood of, becoming lame later in lactation. In which case, this early reduction in milk yield was caused by a separate, earlier insult and not by the lameness. Also, since 66% of sole ulcers occur by 100 DIM (Collick et al. 1989) and white line lesions were most severe at 63 DIM (Leach et al., 1997), it is possible that this reduction in milk yield is a result of undetected clinical lameness. These animals may have gone undiagnosed until later in lactation. It has been recently indicated that farmers grossly underestimate the prevalence of lameness in their cows. In a study of 53 herds, the mean estimate of lame cows was 5% by farmers versus 25% by the researcher (Whay et al., 2002).

However, the data for this paper come from a study where the farmers detected lameness and cows were treated at no cost. The veterinarians diagnosed the cause of lameness, locomotion scored the herds every two months and identified all lame cows not already under treatment (there were rarely any undetected cases). This may have been because the cows were treated at no cost to the farmer. The very high incidence rate of 70 cases per 100 cows per year also indicated that lameness detection rates were high. Assuming that relatively mild cases of lameness were included as well as more severe cases, the data presented in this paper would be an underestimate of milk loss effects in herds where only more severe cases are diagnosed.

A more likely possibility for the delay in treatment may be that these cows were unsound but not clinically lame in early lactation. However, they did eventually become clinically lame. This highlights the important issue of case definition. There is clearly a need to improve the detection of clinical lameness and to remove the subjective assessment of the human observer, whether it be the farmer, veterinarian or agricultural consultant. There have been attempts to do this using rising position and limb placing, but none of these techniques have achieved commercial development. A reliable and repeatable objective assessment of lameness is required. Until such a measure is in place, all estimates of the impact of lameness will be imprecise and therefore, will provide inaccurate estimates of the effect of lameness on milk yield, despite the improved precision of estimates of milk lost.

Lucey et al. (1986) reported a reduction in yield from nine weeks before an episode of sole ulcer or white line separation lameness (this was the maximum time that milk loss was investigated in this study). Both sole ulcers and white line lesions result from an insult to the

corium. The defective horn, which is produced as a consequence of this insult, may not become visible (on the sole surface) until two-to-three months later (Lischer et al., 2000). Therefore, such lesions may affect milk production over a period of time prior to any signs of injury. Warnick et al. (2001), using daily milk recording, reported that acute and severe lameness cases were quickly resolved (e.g. interdigital necrobacillosis) and had a short impact on milk production. In the present study, TDY were recorded each month and such short-duration changes in milk production may have been missed.

Unfortunately, in this study, there were not enough cases of each individual cause of lameness to test their specific impact on milk yield. However, it is an important issue and it should be the subject of future research. To facilitate this without resorting to expensive prospective studies, current recording systems that are utilised in herd health programmes, need to move away from recording 'lameness' as a single entity and towards recording individual causes of lameness.

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USING A SYSTEMATIC REVIEW OF LAMENESS IN CATTLE TO DEVELOP AN INTERVENTION STUDY

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SUMMARY

This paper presents an information extraction method, called systematic review, which can be used to identify risk factors that can be modified to provide practical intervention strategies. A systematic review of lameness in cattle (http://cattle-lameness.dhs.org/) was carried out and yielded 1,007 references, written in English. Almost half of these references (n = 445) involved epidemiological investigations of at least one risk factor for lameness. References that investigated 'biological' risk factors (n = 259), were used for illustrative purposes. Relevant information was found in 85 of these references. The majority of these had appropriate statistical analyses. To highlight pertinent points, evidence for the relationship between the time of year that calving occurred and lameness was obtained from 22 references that covered 47 investigations. There were 23 different definitions of calving season and 18 different lameness and lesion outcomes considered. This demonstrated that meta-analysis was not feasible. Overall, there was little evidence that calving season had a consistent effect on lameness and lesion outcomes, and the effects that were seen, were small.

INTRODUCTION

Lameness in cattle is a source of economic loss to the farmer through both direct and indirect costs. The direct costs include labour, through the herdsman, farmer and veterinarian, materials such as drugs and dressings, and the loss of milk discarded due to antibiotic treatment. Indirect costs include an increased culling rate because of poor performance or for welfare reasons, additional replacement stock and reduced fertility.

At a workshop on the welfare of the dairy cow held in 1996, it was agreed that it would be possible to halve lameness within 10 years. In 1997, the 'Report on the welfare of the dairy cow' (Farm Animal Welfare Council, 1997) and in 1998, the 'Report on lameness in dairy cattle' were subsequently updated (MAFF Publications, 1998). However, it was recognised that a comprehensive review of all current and previous research work on lameness was needed as part of achieving the targeted reduction that had been set. This review would help to identify the many risk factors for lameness and subclinical claw horn lesions. Following this, it was anticipated that intervention studies would be required to investigate the key risk factors, evaluate their usefulness in controlling lameness and to help in yielding a better definition of

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potential aetiologies. The results of these intervention studies would then be passed on to farmers in the form of decision support.

The collation of work published on lameness would be achieved through use of a systematic review. Although this technique is widely used as an epidemiological tool in human medical research, this would be one of the first times it has been used in veterinary science. Some of the principles of Cochrane Collaboration Reviews were used, for instance utilisation of a pre-defined written protocol. However, the topic of lameness is much broader than a typical Cochrane review since the latter normally only encompasses one intervention for one condition. For example, one abstract in the Cochrane Collaboration is 'Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis' (van der Schans et al., 2002). In contrast, lameness encompasses a myriad of conditions and has many risk factors. In addition, some risk factors may affect dissimilar types of lameness differently.

The systematic review covered the many facets of lameness, including clinical and subclinical claw horn lesions, infectious conditions of the feet and legs, and leg injuries. Locomotion as a measure of lameness was also incorporated. The aim of this paper is to illustrate the use of the references identified by this systematic review to establish practical, modifiable risk factors (interventions) and to demonstrate a method for evaluating the weight of evidence for their use.

MATERIALS AND METHODS

The systematic review

The full methodology of the systematic review is described by Hirst et al. (2002). Briefly, four online reference databases (BIDS ISI, BIOSIS, CAB Abstracts and MEDLINE) were searched for records between the years 1981 and 2000. A list of search terms that were the same for each online reference database meant that references had to contain both a term for 'cow' and a term for 'lameness'. A total of 914 English references were obtained and these can be accessed on the web site, http://cattle-lameness.dhs.org/. Non-English language references (n = 435) are also available from this web site. In addition, 93 papers from one of the most recent international lameness conferences are also listed, as these references may contain valuable information that has not yet been published. Only English references (n = 1007) were considered in this paper.

The research group, through group discussion and pilot studies, devised a general classification scheme for these references. The scheme covered areas that included risk factor categories, the type of study, the statistical analyses and the outcome variable(s). This involved adding new keywords to each reference. One researcher classified all references within this broad scheme in order to eliminate between-observer variation.

Extracting relevant references

Initially, the new keywords of risk factor category and type of study were used to identify the most relevant references. There were seven broad risk factor categories and these are described in Table 1, along with examples. A study of 'epidemiological investigations' within the systematic review identified possible risk factors. These included both observational studies and experimental studies but did not include references such as reviews, case reports and letters. Observational studies could include surveys and small scale investigations where no interference

was made by the researchers which influence the outcome or the explanatory variables, and these included both descriptive and analytical accounts. Experimental studies included studies where the researchers either randomised for treatment or disease groups.

Table 1. Description and examples of the new classification of risk factor category

Risk factor category	Description	Number of references	Examples
Behaviour	Conduct of an animal in response to external and internal stimuli	35	Lying time, milking behaviour
Biological	Biomechanical and physiological influences	259	Calving season, milk yield
Environment	External physical conditions affecting the animal	167	Type of housing, Track maintenance
Genetics	Inherited characteristics and their variation	77	Breed, Conformation
Management	External controls of the animals within their environment	63	Stockmanship, Exercise regime
Nutrition	Feeding practices and regime	89	Frequency, Transition diet
Therapy	Treatments applied in an attempt to control or cure disease	50	Bandage

Databases of relevant references were established for each risk factor category. These incorporated epidemiological investigations, namely descriptive-observational, analytical-observational, semi-experimental, full-experimental or case-control studies. Out of the 1007 references, 445 (44%) were epidemiological studies that investigated at least one risk factor.

Initial assessment

A one-day workshop, consisting of nine researchers comprising of veterinarians, animal scientists, epidemiologists and statisticians, was held in April 2001. By this stage, virtually all references were broadly classified or had been obtained in hard copy and were on-site for perusal. Three groups of three people (including in each case a statistician and/or an epidemiologist) were given a similar number of references to examine. This process made the challenge of collating the information clear. Using both the results of this meeting and subsequent work, categories of risk factors were identified over the many types of lameness and outcome variables (Table 2). In some cases, these are still not precise. For example, the category ‘tracks’ covers a number of characteristics. Hence, the terms are still being developed. Evidence for risk factors generally arose from well-designed, small-scale, experimental studies or from large-scale, observational studies.

An ‘intervention’ is a modifiable risk factor. The most useful interventions are those that can be easily controlled with minimal effort and will reduce the extent of disease by a large amount. Some of the risk factors described in Table 2 will be easier to implement than others, both in terms of practicality and cost-effectiveness. However, no attempt has been made to order these in Table 2.

Table 2. Risk factor category and explanation

Risk factor	Explanation
Age at first calving	Age of cow at the time of first calving
Bedding	Material used for the animal to lie on
Biotin	Addition of biotin to the diet
Breed	Genetic strain
Calving season	Time of year calving occurs
Change in diet	Rate at which the constituents of the diet are changed at any time, in particular around calving
Closed herd	All stock reared on farm
Cubicle environment	Size and design of cubicles
Exercise	Encouragement of movement beyond standing
Feeding frequency	Number of meals of concentrate per day
Footbath	Walking animals through solutions selected to clean/treat their feet
Forage	Type and characteristics of the bulky, fibrous constituents of the diet
Grazing	Eating natural forage *in situ*
Herd size	Number of animals in the herd
Housing floors	Physical characteristics of walking surfaces within (and surrounding) the housing system
Level of concentrate	Amount of high density energy or protein supplement to forage
Lying time	The amount of time spent recumbent
Milking behaviour	Conduct of the animals at collection for and during milking
Other micro-nutrient supplementation	Addition of micro-nutrients to the diet other than biotin, for example zinc methionate
Pre-calving diet	Characteristics of the diet fed when not lactating
Protein	Level of crude protein in the diet
Starch:Fibre ratio	Proportion of starch to fibre in the diet
Stocking density	Available room in environment
Stockmanship	Knowledge and application of animal care by farm staff
Tracks	Physical characteristics of walkways out-with the housing system, generally to grazing
Trimming	Removal of excess hoof horn

Spreadsheet standardisation

While the workshop initially identified risk factors and definitions, these were identified rather subjectively. To gain evidence for or against use of the various risk factors in an intervention study, the retrieval and collation of more precise and consistent information was required from the references. The information contained within the references followed a hierarchical structure as shown in Fig. 1. Some information would be the same for each

reference, some for each risk factor and some only for each outcome. A spreadsheet was designed to store this information, with a separate worksheet for each level of the hierarchy.

Each reference was given a unique number and this was also recorded on the hard copy. At the reference level, information was recorded on whether a risk factor was identified in the reference, and whether a reference was a repeat of earlier work.

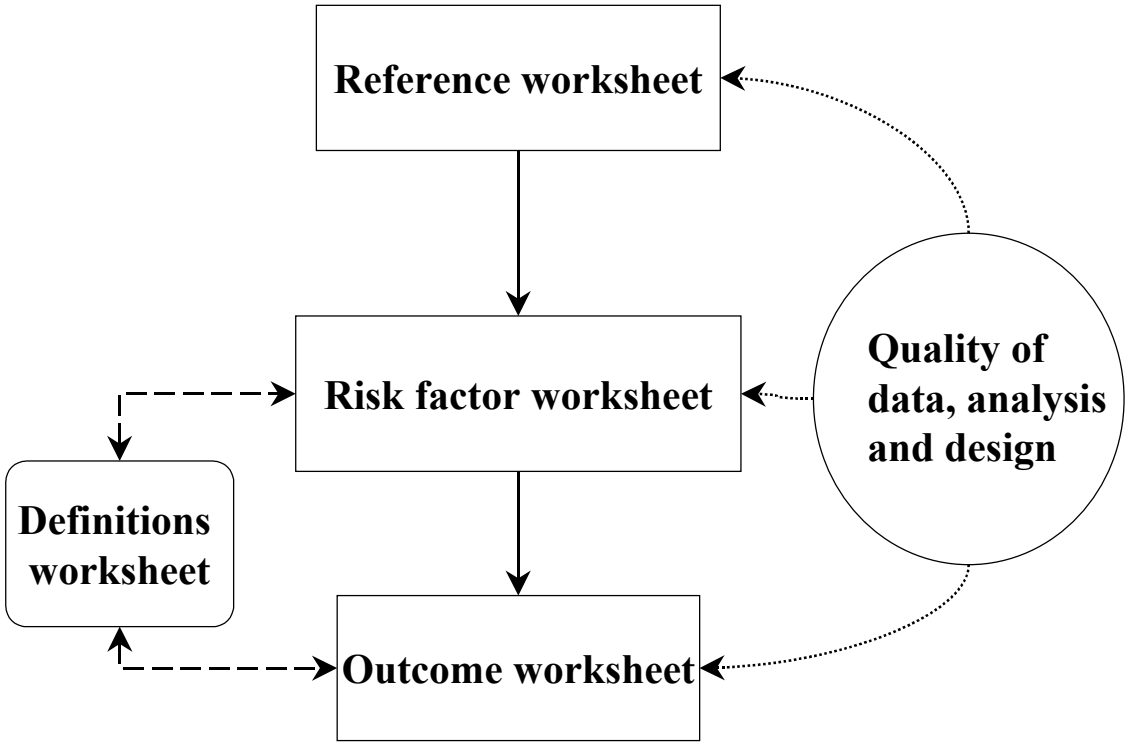

Fig. 1 Hierarchy of information retrieval

At the risk factor level, information was recorded on the specific risk factors that were examined within the reference and these were defined in a corresponding worksheet. In general, the risk factor terms (Table 2) were broad-based so as to encompass a variety of individual risk factors but the specific treatment groups or continuous risk factor, for example milk yield, were defined in a separate column.

In addition, at the outcome level, the outcome was defined and a treatment effect was entered. This was negative, null, positive or not determinable. A separate column defined the treatment direction in relation to the treatment groups or continuous variable. There was also space to record statistics such as means and odds ratios. Additionally, a column, which could apply at any one of the three levels, provided a subjective score of whether the results were based on quality data, quality statistics and a well-designed study. This was coded as poor, satisfactory or good.

RESULTS

Types of study and statistical methodology

The 259 epidemiological references that were classified as 'biological', are used to illustrate the extraction of information. Out of these references, 13 were identified as repeated information (for example, conference proceedings that were subsequently published in a refereed journal). Of the remaining 246 references, 85 (34.6%) contained relevant information on a biological risk factor, that is to say, information on changeable interventions or important risk factors. Many references that were not relevant, investigated the properties of the foot or joints, such as the mineral composition. In addition, a number of references described the bacteria associated with digital dermatitis and some references investigated growth hormones, which are not licensed for use in the UK.

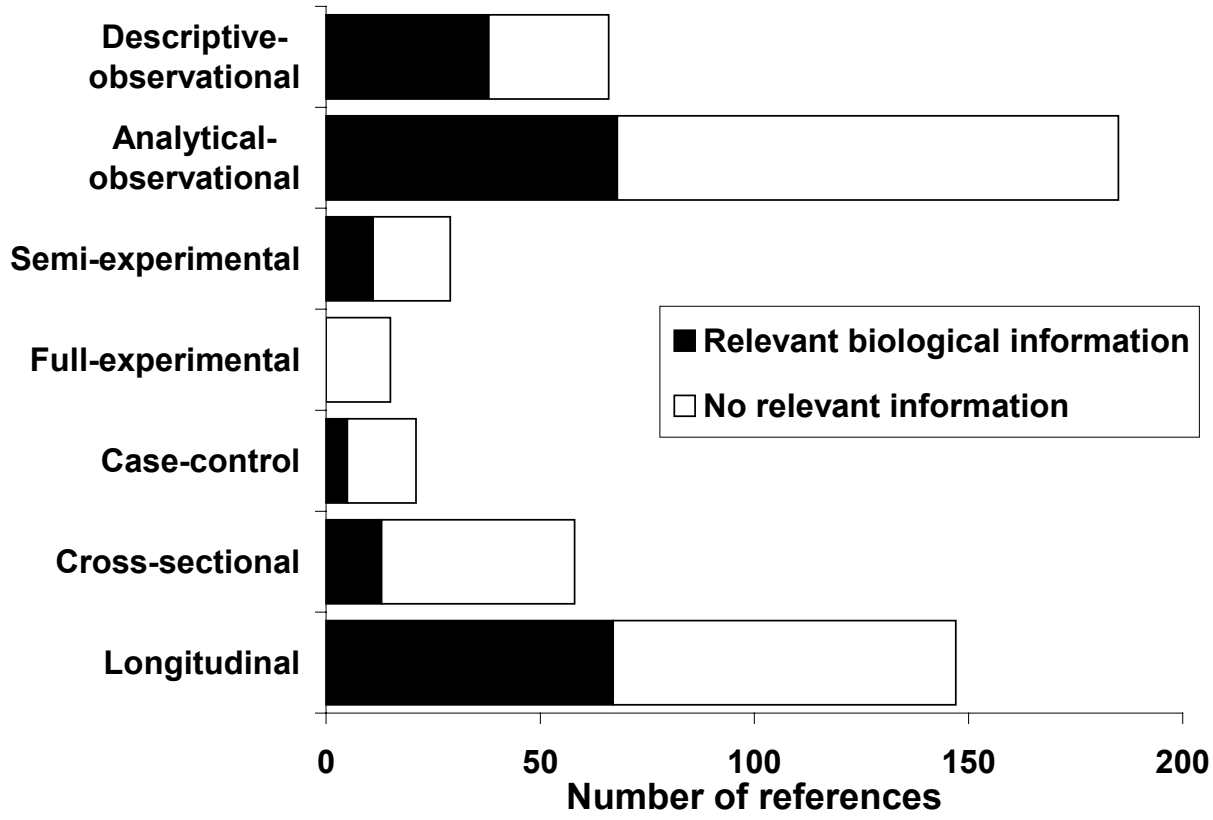

Fig. 2 Number of references containing either relevant biological information or no relevant biological information by type of study

The numbers of biological references of each study type are shown in Fig. 2 with those that contained relevant information and those that contained no relevant information identified separately. The majority of the references classified as 'biological' were analytical-observational studies (n = 185). Descriptive-observational studies were the next most commonly found study type (n = 66), whilst semi-experimental (n = 29), case-control (n = 21) and full-experimental (n = 15) were the least frequently occurring types of study. Most of the relevant information on interventions was contained within the analytical-observational studies

(n = 68), though proportionately, a greater number of the descriptive-observational studies contained relevant information (n = 38). The majority of references that contained relevant information were of a longitudinal study design.

Fig. 3 summarises the numbers of references containing information collated by statistical methodology. Only 38 'biological' references contained no statistics and of these 8 contained relevant information on risk factors. Most references contained univariate statistics (n = 142) but proportionately few contained relevant information (n = 44). Although fewer 'biological' references contained multivariable statistics (n = 103), a greater proportionate number contained relevant information (n = 54). Hierarchical data that had appropriate analysis was contained within 52 references. Inappropriate analysis of hierarchical data was found in 63 references. The sub-optimal analysis of longitudinal data occurred in 23 references. Only a small number of references had reported the use of power calculations (n = 2), randomisation (n = 13), and regression diagnostics (n = 20).

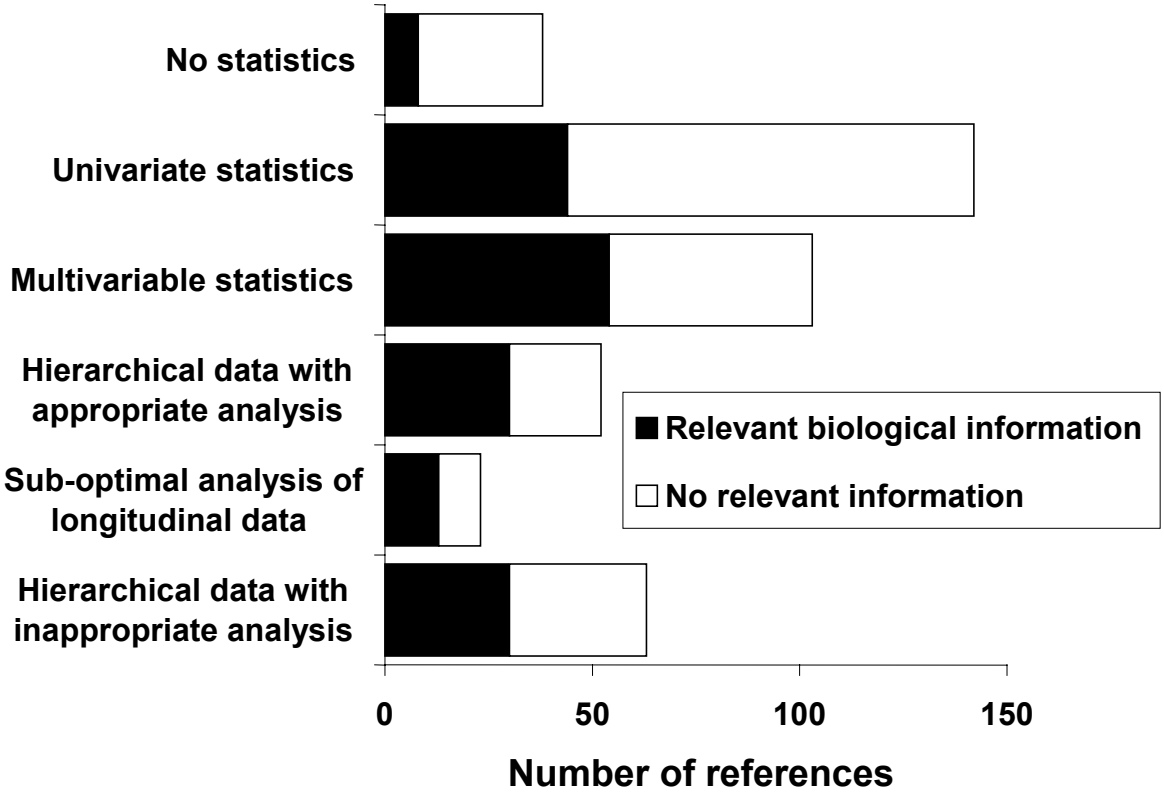

Fig. 3 Number of references containing either relevant biological information or no relevant biological information by statistical methodology

Number of references for each biological intervention or risk factor

The number of references that examined specific 'biological' changeable interventions or important risk factors is shown in Table 3. Some of these risk factors cannot be directly controlled. However, they are important in achieving a better understanding of the aetiology of lameness and identifying high risk groups of animals or high risk times of year. The most studied of these was parity (or age), which was examined in 56 (66%) references. It was not possible to consider meta-analysis of the overall effect of each risk factor due to differences in the definitions and also due to the use of a wide variety of outcomes.

Table 3. The number of references that investigated each important risk factor

Biological risk factor	Number of references	Percentage
Age at first calving	8	9.4%
Calving season	22	25.9%
Milk yield	14	16.5%
Parity/Age	56	65.9%
Prior lameness	8	9.4%
Stage of lactation	33	38.8%
Time of year	13	15.3%

Calving season

To illustrate the issues involved with the data extraction process, the risk factor, calving season, is used. This highlighted the many outcomes of lameness and lesions, the many definitions of risk factors and the type of information presented in the references.

It has been hypothesised that the hormones of late pregnancy and early lactation affect the internal structure of the foot (Holah et al., 2000). In this case, the foot would be more susceptible to other challenges that occur at the same time as calving. For this reason, in countries that graze in the summer and house in the autumn, it is anticipated that cows calving in autumn will suffer more lameness and claw horn lesions than cows calving at other times of the year. Calving could coincide with the early housing period and its hard underfoot environment.

This hypothesis was examined using the 22 references that were identified (by the systematic review and assigned the keyword 'biological') as studying, calving season. In these 22 references, 47 different investigations were undertaken so that some references examined a number of lameness outcomes while others examined only one. In these investigations, there were 23 different definitions of calving season. This was due to calving season being broken down into months of the year and then categorised into groupings of months that could include two, three or six months. For example, in one investigation, September and October were compared against the months from November to August, but in another investigation, August through to December was compared against January to March. Moreover, in yet another investigation, November through to February was compared with March to October. In addition, 18 different lameness and lesion outcomes were considered ranging from the incidence of foul in the foot, hock lesions, sole ulcer, white line disease, through to the incidence of all types of lameness. It soon became clear that a meta-analysis was not feasible on such varied sources of information.

Housing conditions and country of study also varied across the studies. Apart from four studies that were conducted in Mexico, India, Chile and New Zealand, all other studies were conducted in Europe. In some cases, the cattle were housed all year round and in other cases the cattle were never housed. It was not possible to establish the management of the cattle in all studies. In permanently housed herds, it may be less likely that calving season would have an effect.

In summary, 14 investigations found no significant effect of calving season and in four cases it was not possible to assess the statistical significance from the information contained in

the reference. Excluding the two studies from the Southern Hemisphere, of the remaining 27 investigations, the only possible way to collate the information was to compare the category or two categories that included the months of November and December against the other categories (Table 4). The cows that calved in November and December in the Northern Hemisphere had more lameness or claw horn lesions than the other categories in 10 investigations, but in 17 investigations there was a decrease in the level of lameness or claw horn lesions. The majority of investigations were of satisfactory or good quality in their data analysis and design. Those investigations that were assigned a poor rating had not defined calving season within the reference, or failed to give a good reason for the way in which it was chosen.

Table 4. Number of investigations, by the effect of calving during the months of November and December in the Northern Hemisphere, and the quality of the investigation

Effect of calving season	Poor quality	Satisfactory quality	Good quality	Total
Less lameness in cows	3	13	1	17
No effect of calving season	2	10	2	14
More lameness in cows	1	6	3	10
Unable to establish effect	1	2	1	4
Total	7	31	7	45

Overall, in those cases where there were statistically significant results (regardless of month categorisation), these were not usually large effects. In Alban (1995), in a study investigating the incidence of all types of lameness, cows calving in May through to September (referent group) were compared to cows calving between October and April. An OR of 1.2 with a confidence interval from 1 to 1.43 was found. However, when the same study was used for the incidence of hock lesions alone (Alban et al., 1996) and cows calving in the months November to January (referent group) were compared to cows calving July to September, an OR of 0.74 was found with a confidence interval from 0.59-0.94.

However, in Enevoldsen et al. (1991a), which in one case analysed the incidence of heel erosion in second lactation cows, those calving in November to February (referent group) were compared with cows calving from March to October. An odds ratio of 76.7, with a confidence interval from 4.1 to infinity, was found. On the other hand, in the same reference, the incidence of heel erosion in cows from later lactations calving during the same months showed no significant result. In the same study (Enevoldsen et al., 1991b), the incidence of sole ulcer in one foot during the first lactation compared the months May, June, September and October with all the other months (referent group) and found an odds ratio of 1.4 with a confidence interval from 1.1 to 1.8.

Rodriguez-Lainz et al. (1999) studied the incidence of lesions of digital dermatitis. This study was conducted in Chile and defined calving season as spring (referent group), summer, autumn and winter. In this case, an odds ratio for cows calving in winter was found to be 1.4 with a confidence interval from 0.98 to 2.05.

DISCUSSION

It is important to note that the identification of relevant references relied heavily upon those identified in the systematic review. Additionally, they must have had the appropriate new keyword assigned. In this case, we are aware that not all the relevant references would have been obtained but, to the best of our knowledge, the review located the vast majority of references.

Overall, relevant references were of good quality, with many incorporating appropriate statistical analyses for hierarchical data. A common form of analysis was mixed effects logistic regression on the incidence of a lameness or lesion outcome.

The use of a hierarchical spreadsheet was a valuable exercise. It allowed data extraction in an accurate and systematic manner. When the direction of effects was the same for each investigation of that risk factor, it was easy to extract evidence for each risk factor.

It became apparent that the multitude of lameness conditions and risk factors meant that there were very few investigations into the same condition using precisely the same risk factor. In addition, the categorisation of calving season was distinct for each reference and indeed, in some cases had more than one definition in the same reference. In some cases, it appeared that the categories were chosen for statistical significance with little thought given to biological reasoning.

In general, when calving season was found to be not statistically significant, it was either not defined or the odds ratios or incidence were not presented. This is to be expected as traditionally only the significant results are presented in publications. However, in these cases, it was unclear whether the null result was due to the lack of power because of a small sample size relative to inherent variability or a true lack of effect. The examples shown in the results where calving season was found to be significant generally showed only a small effect. However, the months of greatest risk differed between the studies. Overall, there was little evidence that calving season had a consistent effect on lameness and lesion outcomes, and the effects that were seen were small. However, in general, those animals with more lameness and lesions tended to calve sometime over the autumn and winter period, but it was not possible to quantify this result with meta-analysis due to the myriad of definitions for calving season and the number of different outcomes measured.

This exercise has been the culmination of many months of work. It has highlighted the need for more co-ordinated future research on lameness and reinforced the need for testable hypotheses. There is a real need for the use of standardised approaches, in definitions of risk factors, definitions of lameness and lesion outcomes and in the analysis and presentation of results. If this was achieved, meta-analysis could be used in the future to identify the most important risk factors for lameness.

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SIMULATION OF ALTERNATIVE FOR THE DUTCH JOHNE'S DISEASE CERTIFICATION PROGRAMME

M.F.WEBER[1], H. GROENENDAAL, H.J.W. VAN ROERMUND AND M. NIELEN

SUMMARY

Certification schemes for *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) unsuspected herds were studied using a stochastic simulation model. The model, called JohneSSim, simulated the within herd transmission and economic aspects of *Map* in closed Dutch dairy herds. The model was validated by comparison with field observations on *Map* unsuspected herds. The current Dutch certification scheme was compared with alternative test schemes in which the individual and pooled faecal culture, ELISA, Johnin intradermal test and gamma interferon ELISA were employed, whilst the test frequency, age groups tested and number of animals tested, were varied.

On reaching the '*Map* free' status with the standard certification scheme, 11% of the simulated herds was not truly free of *Map*. Therefore, the name of the '*Map* free' status should be changed to, '*Map* unsuspected'. An alternative certification scheme in which the '*Map* free' status was reached after four herd examinations, at two year intervals, consisting of serial testing of all cattle ≥ 2 years of age by pooled faecal culture followed by individual faecal culture for positive pools, seemed to be the best option. This scheme resulted in lower total and annual discounted costs and a lower prevalence when reaching the '*Map* free' status compared to the standard scheme, assuming that there was no new introduction of the infection.

INTRODUCTION

Certified *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) free cattle herds are important in a national control programme for Johne's disease, as a source of non-infected cattle. In the Netherlands, herds can obtain a '*Map* free' status following five annual herd examinations for which all the results are negative. The first herd examination consists of serial testing of all cattle ≥ 3 years of age by serology (ELISA) and individual faecal culture. The subsequent annual herd examinations (years 2 – 5) each consist of serial testing of all cattle ≥ 2 years of age with pooled faecal culture with individual faecal culture of positive pools. The status of certified '*Map* free' herds is then monitored by annual herd faecal examinations, using the same protocol. For the pooled faecal culture, all animals ≥ 2 years of age are stratified by age. A pooled faecal sample is then obtained from each group of five animals and cultured as a single sample (Kalis et al. 2000). If a pooled sample is culture positive, the five animals are examined by individual faecal culture. If all individual faecal samples of a positive pool are negative, then this pool is regarded as culture negative. In order to reduce the risk of

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introduction of a *Map* infection in '*Map* free' herds, cattle may only be added to these herds if they originate from another '*Map* free' herd. Furthermore, cattle may only be added to herds that are in the process of achieving '*Map* free' certification if they originate from a herd with an equal or higher number of negative annual herd examinations.

In September 2000, the first Dutch dairy herd obtained '*Map* free' status, and at the end of 2001 there were approximately 60 '*Map* free' herds in the Netherlands. However, as the current certification and monitoring scheme was felt to be too expensive, especially for closed herds, a study of alternatives was required. Alternative certification and monitoring schemes had to fulfil three requirements: (1) the costs of obtaining and monitoring a '*Map* free' status were reduced, (2) the prevalence of pre-existing *Map* infections in closed '*Map* free' herds that were not detected by the herd examinations was low, and (3) transmission of *Map* infections between '*Map* free' herds was limited. The monitoring of '*Map* free' herds was studied in a separate study with two models (van Roermund et al., in preparation; Weber et al., in preparation). In the present study, alternative certification schemes were simulated to study their effect on the prevalence of pre-existing infections in closed dairy herds, including the costs.

MATERIALS AND METHODS

The current test scheme and various alternative test schemes for the certification of '*Map* free' herds were simulated with the JohneSSim model (Groenendaal et al., 2001, 2002). The scheme is described briefly below.

JohneSSim model

The JohneSSim model is a stochastic and dynamic simulation model that simulates (a) herd dynamics, (b) the disease dynamics, (c) the control of Johne's disease and (d) the economic consequences at herd level at six month time steps for a period of 20 years. The herd dynamics of a typical Dutch dairy herd are simulated, including all calves and replacement heifers. All animals in the herd have various attributes, such as, parity, stage of infection, month in lactation, and milk production. The model contains many probability distributions for uncertain events, such as infection, progression of the stage of infection and culling. In the model, five infection routes are considered: (1) intra-uterine infections, (2) infections at birth, (3) infections due to drinking colostrum, (4) infections due to drinking whole milk, and (5) infections due to environmental contamination with Map. Six stages in the infection and disease process are distinguished: (1) susceptible, (2) non-susceptible, (3) latent infected, (4) low infectious, (5) high infectious and (6) clinical disease. Both voluntary culling and involuntary culling are considered. The probability distributions for uncertain events are used through random sampling; repeated runs of the model provide insight into the variation in outcome at farm level.

In both the former and present study, insight into results of the certification schemes on a national level could be obtained by simulating different dairy herds. Both infected and non-infected herds were simulated. To represent the difference between preventive management on individual farms, eight different herd risk-profiles were defined (van Roermund et al., 1999) and simulated with the JohneSSim model. After simulating the eight risk-profiles separately, the results of the different profiles were aggregated to results at a national level, according to each profile's proportional presence in the Netherlands. The assumptions made in the present study on parameters such as herd size, herd prevalence, distribution of within-herd seroprevalence at

Table 1. Assumptions on sensitivity (SE) and specificity (SP) of different tests in simulations of the certification of '*Map* free' herds.

	Age group (months)	Stage of infection	Individual faecal culture	Pooled faecal culture	ELISA	Intradermal test[1]	Gamma interferon (γ-IFN) ELISA	Serial testing with intradermal test and γ-IFN ELISA
SE	12 – 36	Latent infected	0%	0%		60%	60%	36%
		Low infectious	40%	36%		60%	60%	36%
		High infectious	95%	95%		50%	50%	25%
		Clinical disease [2]	90%	90%		30%	30%	9%
	> 36	Latent infected	0%	0%	5%	60%	60%	36%
		Low infectious	40%	36%	10%	60%	60%	36%
		High infectious	95%	95%	60%	50%	50%	25%
		Clinical disease	90%	90%	80%	30%	30%	9%
SP	***All***	***Not infected***	100% [3]	100%	99.7% [4]	88.8% [5]	96.0% [5]	98.6 % [5]

[1] Intradermal test = Johnin skin test;
[2] In JohneSSim simulations, animals do not become clinical diseased before two years of age;
[3] Reinders (1963);
[4] van Maanen (1999);
[5] Kalis et al., in preparation.

the start of simulations and probability distributions for uncertain events were described previously (Groenendaal et al., 2002). At the start of the simulations, the assumed herd prevalence of the simulated dairy herd population was 79%, while the assumed animal prevalence in the total simulated dairy population was 22%.

Assumptions in JohneSSim model for present study

In contrast to the previous studies, all herds in the present study were assumed to be closed and no new introduction of *Map* into any herd could occur during the simulations. For the present study, assumptions were made on the characteristics of tests (Table 1) and costs of the certification programme (Table 2). For each stage of infection, the sensitivity of different tests was assessed by an expert panel. The sensitivity of serial testing with the intradermal test (Johnin skin test) and the gamma interferon (γ-IFN) ELISA was calculated assuming that these tests were independent. In the JohneSSim model, the minimal age at which low infectious or high infectious animals could contribute to the transmission of *Map* is two years. Nevertheless, in the present study, it was assumed that low or high infectious animals between one and two years of age could be detected by faecal culture. Discounted costs of the certification programme were calculated assuming a real interest rate (approximated by interest rate minus inflation rate) of 5% per year.

Table 2. Variable costs (€) of participation in the '*Map* free' certification programme. Annual subscription costs of the programme were €88.49 per year. Costs do not include VAT (VAT for subscription and laboratory tests = 6%; VAT on other costs 19%.).

Test / action	Veterinary Costs	Transport costs per submission	Laboratory costs per submission	Laboratory costs per test
Veterinarians' visit	18.15			
Pooled faecal culture	2.72 per animal	7.26	6.81	34.49 per pool (max 5 animals)
Individual faecal culture	2.27 per animal	7.26	6.81	28.13 per animal
ELISA	2.27 per animal	7.26	6.81	5.67 per animal
Intradermal test*	3.18 per animal			
γ-IFN ELISA	2.27 per animal	7.26	6.81	11.34 per animal

*Two veterinary visits are required for an Intradermal test.

Validation

Results of a simulation of a large population closed dairy herds were compared with the results of a field experiment on 86 *Map* unsuspected closed dairy farms in the north of the Netherlands. In both the simulation and the field experiment, herds were selected in which a first herd examination of all cattle ≥ 2 years of age with the pooled faecal culture did not reveal any *Map* infections. The selected herds, in both the simulation and the field experiment, were subsequently examined seven times at half-yearly intervals by pooled faecal culture of all cattle ≥ 2 years of age. After each herd examination, the proportion of unsuspected herds in the simulation was compared with the proportion of unsuspected herds in the field experiment.

Table 3. Simulated test schemes for the certification of '*Map* free' herds. A positive result of the ELISA or pooled faecal culture was always confirmed by individual faecal culture of the ELISA positive individual or the faecal culture positive pools.

Scheme	1st herd examination		2nd – 5th herd examination			Year '*Map* free' status achieved
	Test	Animals	Test	Frequency	Animals	
St [1]	ELISA	All, ≥ 3 yr.	PF [2]	Once / yr.	All, ≥ 2 yr.	5
A[3]	ELISA	All, ≥ 3 yr.	IDT / γIFN	Once / yr.	All, 1 – 3 yr.	5
B[4]	ELISA	All, ≥ 3 yr.	PF / ELISA	Once / yr.	All, ≥2 / ≥3 yr.	8
C[5]	--	--	PF	Once / yr.	All, ≥ 2 yr.	4
D	ELISA	All, ≥ 3 yr.	PF	Once / 2 yrs.	All, ≥ 2 yr.	8
E	ELISA	All, ≥ 3 yr.	PF	Twice / yr.	All, ≥ 2 yr.	3
F	ELISA	All, ≥ 3 yr.	PF	Once / yr.	30 youngest, ≥ 2 yr.	5
H	ELISA	All, ≥ 3 yr.	PF	Once / yr.	All, ≥ 1 yr.	5
CD[5]	--	--	PF	Once / 2 yrs.	All, ≥ 2 yr.	7
DH	ELISA	All, ≥ 3 yr.	PF	Once / 2 yrs.	All, ≥ 1 yr.	8

[1] St = standard;

[2] PF = pooled faecal culture (Kalis et al. 2000);

[3] Scheme A includes testing of all cattle between 1 and 3 years of age with the Intradermal test (Johnin skin test). If any animal tested positive with the Intradermal test, then this animal was tested with the γIFN ELISA. If one or more animals in a herd were tested positive with the γIFN ELISA, then all cattle ≥ 2 years of age in the herd were tested with the pooled faecal culture;

[4] Scheme B includes an annual examination of all herds with alternating a pooled faecal culture of all cattle ≥ 2 years of age and a serological examination (ELISA) of all cattle ≥ 3 years of age. The '*Map* free status' was obtained after eight herd examinations (four serological and four faecal examinations);

[5] In schemes C and CD the first (serological) herd examination is skipped, and the status '*Map* free' can be obtained after four herd examinations by faecal culture

Certification of '*Map* free' herds

The current scheme for certification of '*Map* free' herds and nine alternative certification schemes were simulated (Table 3). For each of these certification schemes, the prevalence over time of pre-existing *Map* infections in closed dairy herds and the costs from the start of the programme until reaching the '*Map* free' status, were determined. As the time from the start of the programme to achieving '*Map* free' status differed between the various certification schemes, both the total discounted costs and annual discounted costs (annuity costs) were calculated. The animal prevalence on reaching the '*Map* free' status and the total and annual discounted costs until achieving this status were compared for the different certification schemes.

Sensitivity analysis

The influence of several parameters in the model was studied by sensitivity analysis. The input of these parameters was changed one at the time. Some of the parameters that were studied included:

1) The default herd size at the start of the simulations was 50 adult cattle and 46 young stock. Alternatively, a herd size of 100 adult cattle and 92 young stock was simulated.
2) The default sensitivity of the pooled faecal culture was 36% for low infectious cattle, 95% for high infectious cattle and 90% for clinical diseased cattle (Table 1). Alternatively, the sensitivity of the pooled faecal culture was decreased by 25% and tested.

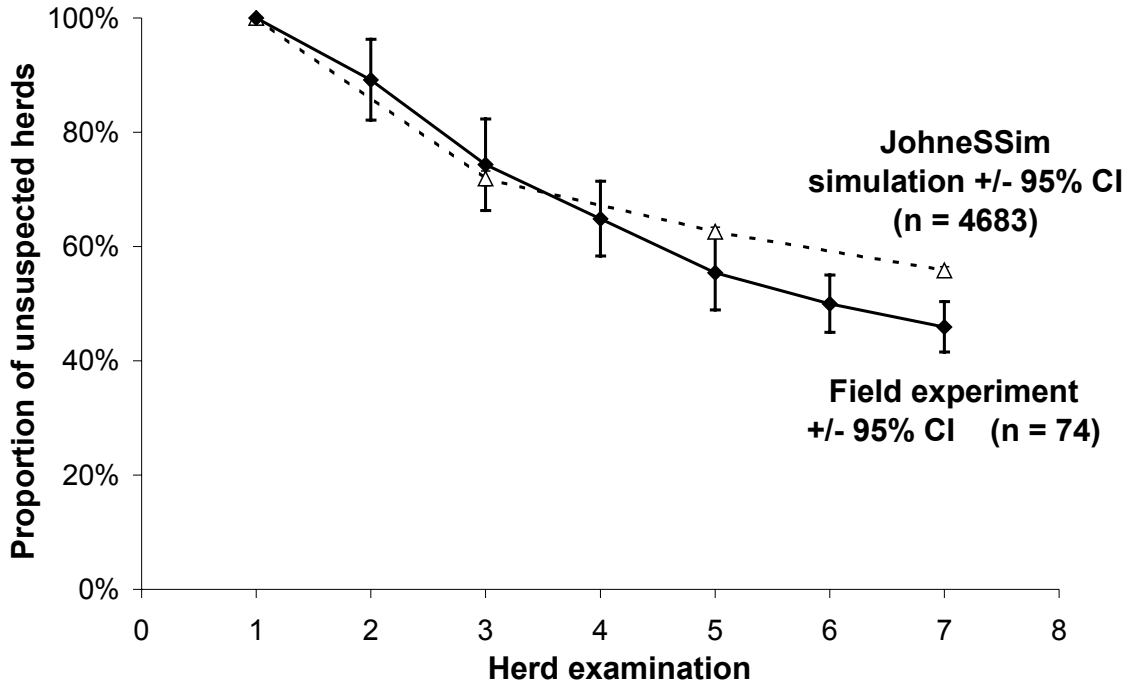

Fig. 1 Comparison of results of a simulation with the JohneSSim model and results of a field experiment. In both the simulation and the field experiment, herds were examined at half-yearly intervals by a pooled faecal culture of cattle ≥ 2 years of age. The number of herds that were persistently culture negative is expressed as a percentage of the number of herds that were culture negative at the first herd examination (in the field experiment 100% = 74 herds; in the JohneSSim simulation 100% = 4683 herds).

RESULTS

Validation

Of 86 unsuspected herds in the field experiment, 74 were faecal culture negative at the first herd examination. Of these 74 herds, only 46% (34 herds) was consistently culture negative at the following six herd examinations (Fig. 1). Of the simulated herds which were faecal culture negative at the first herd examination in the JohneSSim simulation, 56% were consistently culture negative at the following six herd examinations.

Certification of '*Map* free' herds

Using the standard certification scheme, 26.2% of the simulated herds reached the '*Map* free' status (Table 4). For these herds, the average total discounted costs for achieving '*Map* free' status was €3,392 (Table 4). On reaching '*Map* free' status, 11% of these herds and 0.6% of the animals in all '*Map* free' herds were still infected, whereas in infected '*Map* free' herds 5.7% of animals was infected (Fig. 2). At the same point in time, 34% of animals in all simulated herds (both '*Map* free' herds and known infected herds) were infected.

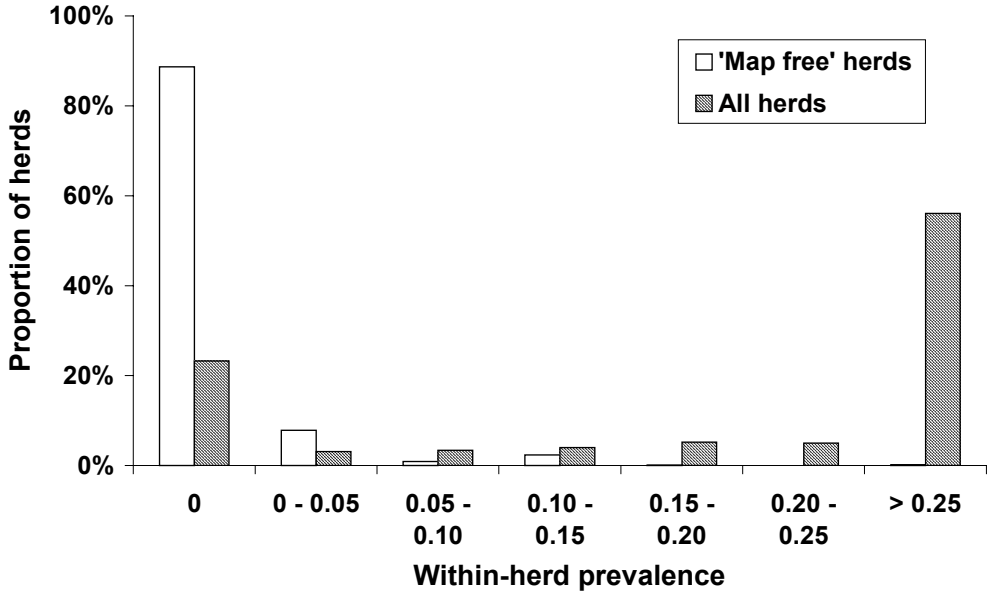

Fig. 2 Distribution of within-herd prevalence (i.e. number of infected animals / total number of animals in a herd) after simulation for five years for all herds and for herds that recently achieved '*Map* free' status with the standard certification scheme.

With alternative certification schemes, 25.3% - 27.4% of the herds reached '*Map* free' status (Table 4). The average discounted costs until the '*Map* free' status was reached varied between €1,905 and €4,753 for different certification schemes (Table 4). In comparison with the standard certification scheme, only scheme CD resulted in lower average total discounted costs than the standard scheme. The other alternative certification schemes resulted in either a higher animal prevalence at reaching the '*Map* free' status, or higher total discounted costs up to that point (Fig. 3A). In comparison with the standard certification scheme, scheme CD, and three

other alternative certification schemes (D, B, and DH), resulted in both lower annual costs until the '*Map* free' status was reached and a lower animal prevalence on achieving '*Map* free' status (Fig. 3B). In these four schemes, the pooled faecal culture was employed once every two years and hence the period until the '*Map* free' status was reached was prolonged (Table 3).

Table 4. Estimated probability and average total discounted costs (€) for reaching '*Map* free' status with various certification schemes. The certification schemes are defined in Table 3.

	Certification Schemes									
	St	A	B	C	D	E	F	H	CD	DH
Probability	26 %	26 %	25 %	27 %	27 %	27 %	27 %	26 %	26 %	27 %
Costs (€)	3,392	3,483	4,753	2,843	3,551	3,229	1,905	4,369	3,097	4,562

Sensitivity analysis

The animal prevalence on reaching '*Map* free' status was lower in herds with 100 adult cattle than in herds with 50 adult cattle. The animal prevalence on achieving '*Map* free' status at least doubled when the sensitivity of the pooled faecal culture was decreased by 25%.

DISCUSSION

Simulations with the JohneSSim model were considered to be in general agreement with field observations from 86 *Map* unsuspected herds. This supported the validity of using the model for evaluation of alternative certification and monitoring schemes.

The JohneSSim model is a stochastic simulation model, therefore, the outcomes are probability distributions, as shown for within herd prevalence in Fig. 2. However, an individual farmer who buys cattle from a '*Map* free' herd may only be interested in eliminating the risk of buying infected cattle. Since this farmer lacks information about the true infection status of the '*Map* free' herd of origin, the only relevant parameter to him is the overall animal prevalence in the population of '*Map* free' herds. Therefore, in the present study, this overall animal prevalence of the population of '*Map* free' herds was used to discriminate between alternative test schemes. In order to estimate this overall animal prevalence, the total animal population of '*Map* free' herds was considered to be based on every iteration of a JohneSSim simulation. The resulting proportion (prevalence) is therefore, a single value and not a distribution. However, it is important to realise that infected cattle are clustered on a small proportion of '*Map* free' herds and most herds are truly negative. The risk for the buyer is thus not evenly spread over all '*Map* free' herds, as might be suggested from our animal prevalence results.

The results show that an estimated 11% of the herds were not *Map* free on reaching '*Map* free' status. With the standard monitoring scheme, it took some eight more years before all pre-existing infections were either extinct or detected. Therefore, the name of the '*Map* free' status should be changed into, for instance, '*Map* unsuspected' or 'low risk *Map*'.

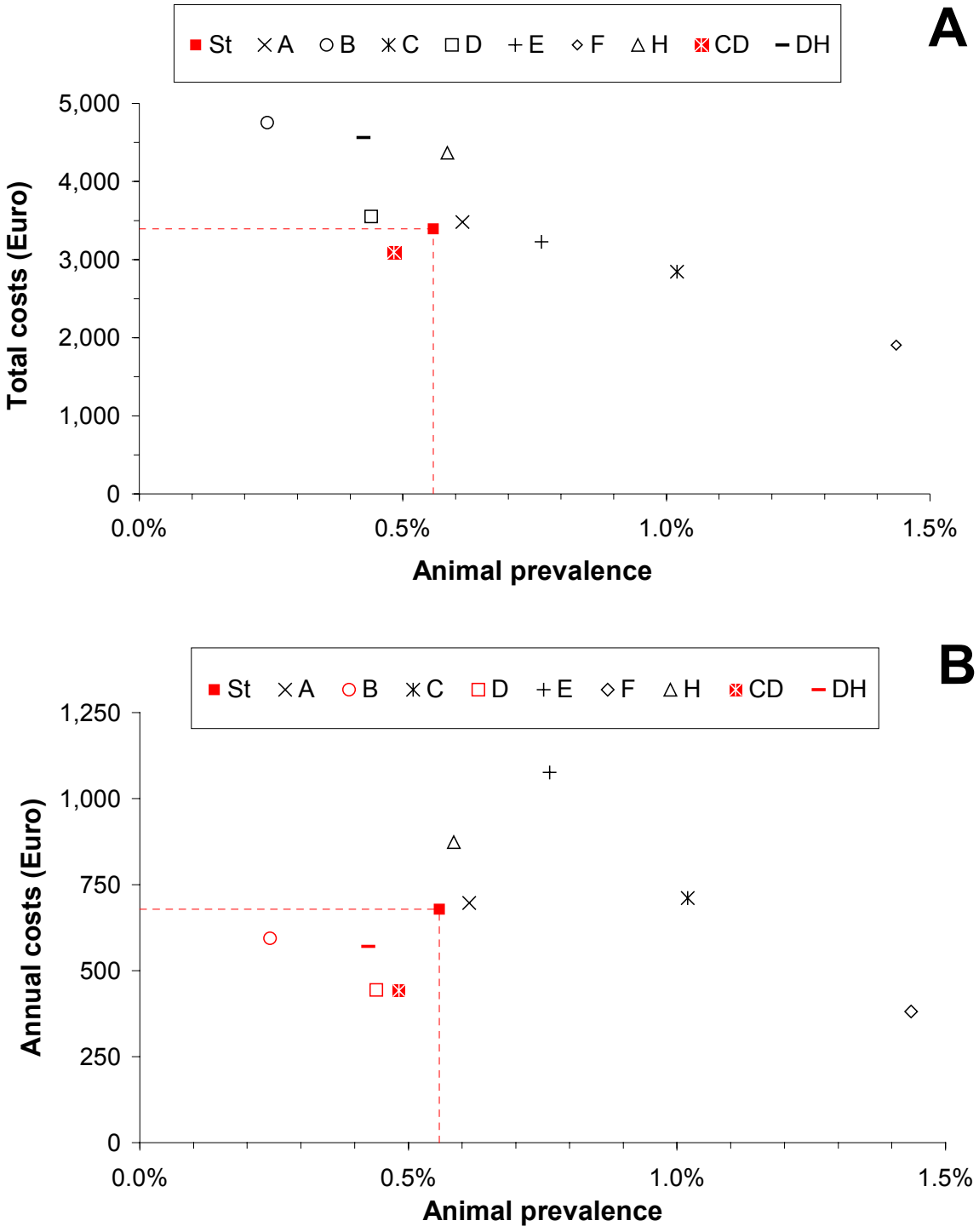

Fig. 3 Animal prevalence on reaching '*Map* free' status (i.e. number of infected animals / total number of animals in all herds that recently achieved the '*Map* free' status) and average total (A) and annual (B) discounted costs until the '*Map* free' status is reached for various certification schemes. The different certification schemes are defined in Table 3.

Alternative certification schemes, in which the interval between herd examinations is two years, lengthened the certification process by 3 years (schemes D, B, CD and DH; see Table 3). However, such alternative schemes resulted in both lower estimated annual discounted costs and a lower estimated *Map* prevalence on reaching '*Map* free' status. This lower prevalence is probably due to the fact that more animals were tested for *Map* infection over the longer time period and thus infected herds were more likely detected. The time from the start of the programme to achieving '*Map* free' status differed between the various certification schemes. Therefore, annual as well as total discounted costs were estimated. Only certification scheme CD resulted in lower estimated annual discounted costs, lower estimated total discounted costs and a lower estimated *Map* prevalence at reaching the '*Map* free' status, compared to the standard scheme. Thus, this scheme, in which the serological herd examination was skipped and the '*Map* free' status was reached after four pooled faecal cultures of all cattle ≥ 2 year of age at two-year intervals, seemed to be the most attractive alternative. Obviously, this assumes no new introduction of the infection.

In the present study, important assumptions were made on the sensitivity of tests for the various stages of infection as published data are generally based on studies using high faecal *Map* shedders. The results of the JohneSSim model were very sensitive to the assumed sensitivity of the faecal culture. In agreement with previous studies using the JohneSSim model, it was assumed that young stock between one and two years of age did not contribute to the transmission of *Map.* However, in the present study it was assumed that faecal *Map* shedders between one and two years of age could be detected by faecal culture. The efficacy of inclusion of this age group in herd examinations is expected to depend strongly on the sensitivity of faecal culture of this age group. In herds with clinical cases of Johne's disease, 2.1% of young stock between one and two years of age was found to be culture positive (Kalis 1999), but it is unknown whether this is similar in low prevalence herds.

Model studies may assist decision makers in selecting suitable alternatives for a control programme, such as a certification and monitoring programme for *Map* infections. It is concluded from the results of the present study that the current Dutch certification scheme for '*Map* free' herds may be optimised by: (1) certification of '*Map* free' herds after four herd examinations at two year intervals consisting of pooled faecal culture of all cattle ≥ 2 years of age and (2) renaming the status '*Map* free' into '*Map* unsuspected'.

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FOOT AND MOUTH DISEASE EPIDEMIOLOGY

EFFECT OF CONTROL MEASURES ON THE COURSE OF SIMULATED FOOT AND MOUTH DISEASE EPIDEMICS THAT STARTED ON DIFFERENT FARM TYPES IN VARIOUS DUTCH AREAS

M.C.M. MOURITS[1], M. NIELEN AND C.D. LÉON

SUMMARY

The influence of area characteristics on the consequences of various foot and mouth disease control strategies was studied for the Dutch situation using an adapted version of the spatial, stochastic simulation model, InterFMD. The regular EU control measures served as the base scenario for comparative evaluations. The alternative scenarios included additional measures such as a complete national restriction on animal movements at the start of the epidemic, pre-emptive slaughter and destruction of contiguous herds and suppressive vaccination of contiguous herds. The epidemiological impact of the additional control measures was studied for epidemics starting in a densely populated area as well as for epidemics commencing in a sparsely populated area.

INTRODUCTION

The usual measures adopted by the EU member states to control outbreaks of foot and mouth disease (FMD) are based on the strategy of destruction of infected herds with appropriate disposal of potentially infective material (stamping out) and control of the movements of live animals, animal products, persons, vehicles and any other substance liable to transmit the virus. It has been postulated that such a strategy may not be sufficient to eradicate the virus, especially when the epidemic takes place in an area with a high density of susceptible animals.

During the FMD outbreak of 2001 in the Netherlands, a number of extra control measures were taken in addition to the regular EU control policy. These measures consisted of a complete national restriction on animal movements at the start of the epidemic, pre-emptive slaughter and destruction of contiguous herds (1 to 2 km around an infected and detected farm) and suppressive vaccination of contiguous herds to gain time with respect to pre-emptive culling. This epidemic caused infection in 26 herds and resulted in the destruction of 267,992 animals on 2,763 farms (Bouma et al., 2001).

In general, FMD outbreaks generate considerable economic loss due to the costs of disease control, productivity losses and constraints on international meat and livestock trade. Implementation of additional control measures is only economically viable if the additional losses are offset by the gains of reducing the duration of the FMD epidemic. For instance, to

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regain the OIE recognised FMD disease free status, the country should be incident free for a period of three months after the last case or, where suppressive vaccination is applied, after the slaughter of the last vaccinated animal. Implementation of suppressive vaccination could therefore have a significant economic impact due to the additional export losses associated with the delay to slaughter the vaccinated animals.

Selection of an inadequate control strategy could cause large additional economic losses (Mahul and Gohin, 1999), while delayed implementation of additional control measures could cause extensive spread of the disease (Howard and Donnelly, 2000; Ferguson et al., 2001). In the case of an FMD outbreak, animal health authorities have to make disease control decisions under intense time pressures. A priori analyses of various disease control options by means of computer simulations can provide decision makers with some supporting guidelines.

In this simulation study, the possibility of defining 'area specific rules of thumb' was evaluated for the Dutch situation. In other words, do some Dutch areas generally require more rigorous control measures? To answer this question, the influence of area characteristics on the consequences of various control strategies was studied using an adapted version of the spatial, stochastic simulation model, InterFMD (Jalvingh et al., 1998).

MATERIALS AND METHODS

General framework of the simulation model

In this study, the spatial and stochastic simulation model, InterFMD, was used to simulate the spread and control of a FMD epidemic on a day-by-day basis. The conceptual model of InterFMD, InterSpread, was developed by Sanson (1993) as part of EPIMAN, a decision support system for the control of FMD outbreaks. Jalvingh et al. (1998) developed and modified InterSpread to match Dutch conditions.

Within the general framework of InterSpread/InterFMD, the stochastic and spatial simulation of the spread and control of FMD starts with an initialisation phase, in which the farm specific data (e.g. geographic location, number of animals) are loaded into the model and the spread and control mechanisms are assigned their parametric value. All spread and control mechanisms act spatially by using the geographic location of farms and contain variation and uncertainty (mostly reflected by empirical probability functions). As a result of this Monte Carlo simulation, several replications, each representing a possible course of an epidemic, are necessary to get insight into the possible range of outcomes.

At the start of each replication, the primary infected farm is initialised. The disease spread from the infected farm is simulated through three different spread mechanisms: 1) contacts by animals (= high risk), vehicles (= medium risk) or professional persons (= low risk), 2) local/neighbourhood spread, and 3) airborne spread. When the disease spread results in the infection of another farm, this farm is assigned relevant dates (e.g. moment of infectiousness) and will become one of the list of infected farms. In the situation where detection of an infected farm takes place, control measures are initialised. Control measures apply to the infected farm (e.g. stamping out), all farms within a certain radius around the infected farm (e.g. pre-emptive slaughter, movement control, suppressive vaccination) and contact farms that have been traced (e.g. pre-emptive slaughter, movement control).

The output of InterSpread/InterFMD consists of descriptive epidemiological characteristics of the simulated epidemic, such as the number of infected farms, the number of pre-emptively slaughtered farms, the number of farms in control zones and the duration of the epidemic.

For more details on InterSpread, refer to Sanson (1993) and Jalvingh et al. (1998).

Main adaptations

The main model modifications made during the current research project were related to the introduction of:

1) Individual farm information representing the complete Dutch population of farms with FMD susceptible animals (cattle, pigs, sheep and goats);
2) Species specific characteristics for the spread mechanisms;
3) The introduction of limited destruction capacities to slaughter and render infected animals and pre-emptively slaughtered animals.

Individual farm data: The individual farm information was available from the 2000 census data as recorded by the National Animal Health Service. Census data for each farm included a unique identifier, location co-ordinates, number of animals present specified by species and applied type of production system. The input data set for the model contained information on 78,405 farms with FMD susceptible animals of which 51,618 of these farms contained cattle, 17,170 farms had pigs and 29,532 farms had small ruminants (sheep and/or goats).

Species specific characteristics: In the original Interspread model (Sanson, 1993; Jalvingh et al., 1998), spread of FMD was simulated based on the characteristics of an 'average species'. Given the wide variation between different species in quantity of virus excretion, susceptibility to infection and the likely infection routes, using an average species is an oversimplification and therefore, in certain circumstances, could generate inaccurate forecasts and decision support. In this adapted model, species-specific contact structures were included to define species-specific spread mechanisms. The contact structure of farms with cattle and/or pig units was based on the Dutch Identification and Registration (I&R) data of 1999 (Vonk Noordegraaf et al., 2000; Mourits et al., 2001). The contact structure of small ruminants was estimated due to the absence of a comparable I&R data system.

To simulate spread between species on mixed farms (= more than one FMD susceptible species present), a so-called interspecies transmission procedure was included.

Destruction capacity: Implementation of control measures was restricted by the finite amount of available resources to kill and destroy animals. In the model, the slaughter and rendering capacity was limited to a maximum of 5 farms a day during the first week of the epidemic, rising to 15 farms a day from the third week onwards.

Control strategies evaluated

In total, 8 control strategies were evaluated to study the impact of the various additional control measures. The regular EU control measures of culling infected herds, implementation of the protection (3 km) and surveillance zone (10 km), and preventive slaughter of dangerous contact herds served as the base scenario for comparative evaluations. The simulated

alternatives consisted of the regular EU measures and one or more of the following additional control measures:

72 MSS = national movement standstill (MSS) for 72 hours after the first detection.
PRE = pre-emptive slaughter of neighbouring farms within a radius of 1 km around a detected farm.
VAC = suppressive vaccination within a radius of 2 km around a detected farm, resulting in full immunity 10 days after vaccination.

Organisation of calculations

Livestock areas in the Netherlands are rather heterogeneous. Density of susceptible herds varies considerably as depicted by Fig. 1. Moreover, livestock areas are characterised by specialised production systems. The south of the Netherlands can be defined as a high density pig production area while the north is predominately dairy cattle farming. By varying the spatial and herd characteristics of the index farm (= first infected farm), the simulation results provide insight in the consequences of the simulated control strategies within the different Dutch livestock areas. The calculations in this study were, therefore, organised by the selection of the index farm.

Firstly, calculations were performed to evaluate the impact of the various control strategies. The index farm for these calculations was situated in a densely populated livestock area (DPLA) and resembled the index farm of the real outbreak of 2001 (Fig. 1 and Table 1). This index case was a dairy goat farm in Oene.

Secondly, the influence of farm density was studied by performing the same calculations with a comparable goat index farm situated in a sparsely populated livestock area (SPLA) (Fig. 1 and Table 1).

Table 1. Density characteristics of index farms within the selected DPLA and SPLA areas.

	Index DPLA	Index SPLA
No. of farms within 1 km	20	19
No. of farms within 3 km	160	81
No. of farms within 10 km	1198	498

In the final part of the analysis, the impact of the type of index farm on the course of the epidemic was examined. In the Netherlands, farm production types are highly specialised, resulting in specific contact patterns per production type. For instance, pig breeding farms will have more (high risk) contacts than fattening farms. Therefore, are the consequences of the introduction of the disease through a pig breeding farm more severe than when the disease started at a fattening farm? At the time of writing these index type specific simulation runs have not been completed. However, these findings will be presented and discussed during the oral presentation.

RESULTS

For all scenarios, 100 replications were carried out to obtain insight into the possible range of outcomes. Since the results were skewed, all results show the 5th, 50th and 95th percentiles

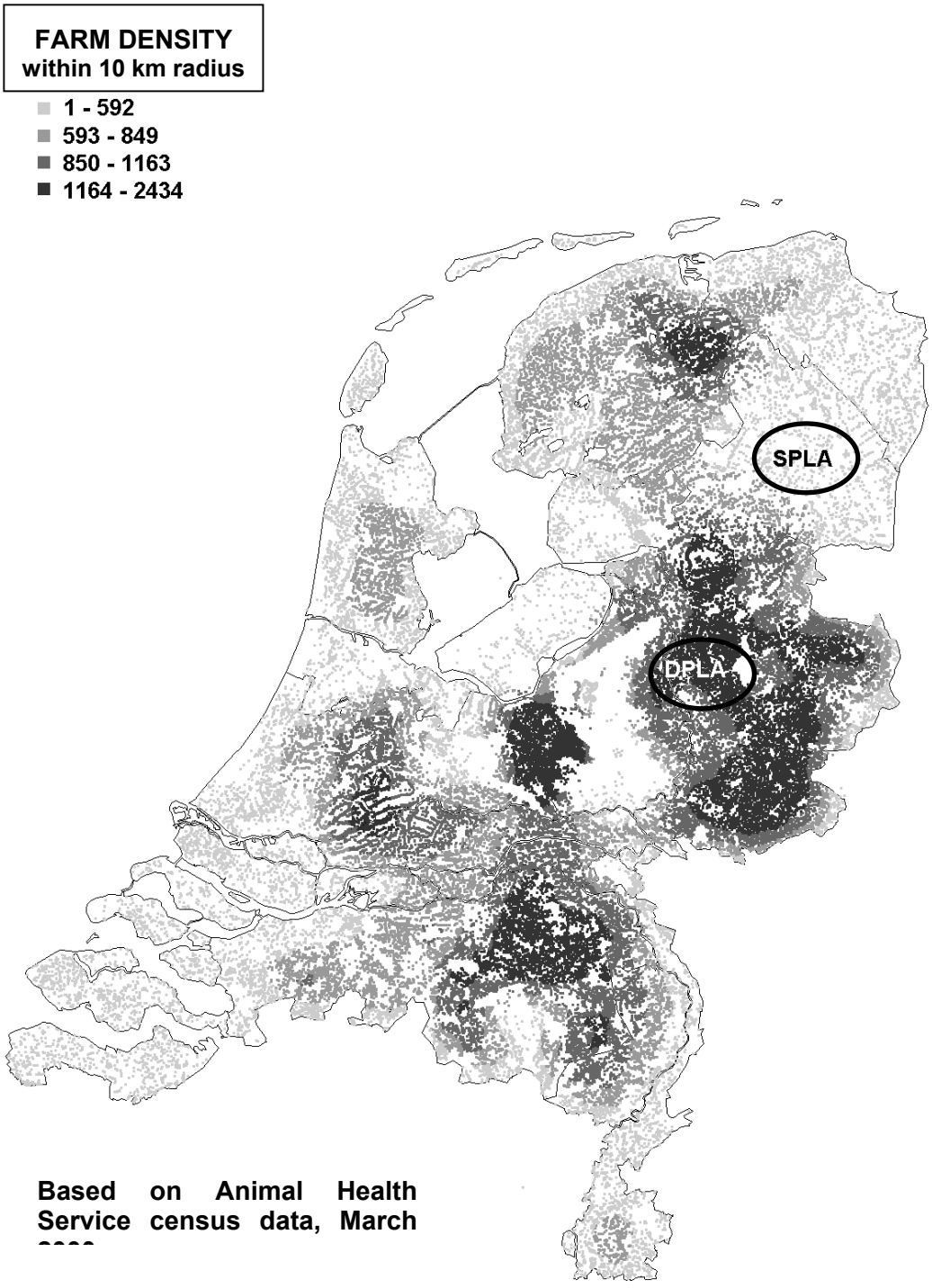

Figure 1. Density of farms with FMD susceptible animals based on the number of farms within a radius of 10 km around each farm. The circled terms DPLA and SPLA reflect the areas in which the two selected index farms are situated.

for several output parameters. It should be noted that these percentiles are based on the ranking of the individual parameters, that is, results within a column do not represent one particular replication. Simulation time within the replications was set at a maximum of 400 days. The course of the epidemic was simulated until this predefined number of days or until the epidemic was over and control measures were no longer present.

Table 2 gives an overview of the major results of the alternative control strategies based on the index farm situated in the selected DPLA area (Fig. 1). Under the regular EU policy, the number of infected farms was 54 in 50% of the simulated outbreaks. Most of the infections were the result of local spread (n = 47). However, the epidemics were accelerated by the spread through contacts, resulting in the introduction of the disease into neighbouring areas. The duration of the epidemic was defined as the period between the day the first farm was confirmed as positive until the day the last restriction zone was removed. In the selected DPLA area, an epidemic controlled by the regular EU measurements lasted at least 116 days, in 50% of the cases. The maximum distance parameter reflects the maximum distance the disease spreads from the index farm. So, in 50% of the cases the epidemic remained within a radius of 44 km around the index farm.

Addition of the 72MSS measure did not result in significantly different results; the median number of infected farms is a little lower compared to the basic EU strategy. However, due to the stochastic variation, the extreme values within this strategy were even higher. On the other hand, application of PRE or VAC as an additional measure resulted in a considerable reduction in the number of infected farms and the duration of the epidemic. For example, the risk of an extreme number of outbreaks was strongly decreased (95% percentile = 60 and 58 infected farms, respectively). Application of combinations of additional measures resulted in a further reduction of the extreme values. The impact of combinations of 72 MSS with PRE and/or VAC on the maximum distance the epidemic spread from the index farm was the most striking. In 50% of the cases, this was less than 9 km.

Table 3 reflects the major results of the alternative control strategies based on the index farm situated in the selected SPLA area (Fig. 1). In general, the median epidemics based on EU policy were smaller in the SPLA than in the DPLA. However, extremely large outbreaks were also present in the SPLA variant. In contrast to the DPLA results, addition of the 72MSS measure to the EU policy resulted in a dramatically reduced risk of a large number of outbreaks, in the SPLA. On the other hand, the spatial spread of the epidemic could not be blocked by the combination of 72 MSS with PRE and VAC measures to the same extent as was observed for the DPLA variant.

DISCUSSION

In this simulation study, only the epidemiological effects of the control strategies were considered. In general, the economic loss of a FMD epidemic is strongly correlated with the duration of the epidemic and, for exporting countries, the duration of the imposed export bans (Tomassen et al., 2002). In a subsequent phase, the simulated epidemiological results will be used to evaluate the economic consequences of the various control strategies.

Based on the simulated number of large epidemics that could develop despite the application of EU control measures in DPLA as well as SPLA areas, it can be concluded that for the Dutch situation, the regular EU policy alone was a rather risky policy for controlling a FMD epidemic.

Table 2. Simulation results of alternative control strategies based on a goat index farm within the selected DPLA. EU = regular EU control, 72 MSS = national stand still for 72 hours after the first detection, PRE = pre-emptive slaughter within a radius of 1 km, VAC = suppressive vaccination within a radius of 2 km. Percentiles are based on the ranking of the individual parameters; results within a column do not represent one particular replication.

	EU			+ 72 MSS			+ PRE			+VAC		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
No. farms infected [a]	3	54	1467	3	44	1552	3	15	60	3	18	58
No. farms pre-emptive	7	56	828	6	45	763	19	70	328	6	23	73
No. farms vaccinated	-	-	-	-	-	-	-	-	-	98	285	1044
Route of infection												
local	1	47	1375	1	39	1470	1	10	41	1	14	43
contacts	0	6	68	0	5	60	0	3	18	0	3	16
airborne	0	0	21	0	0	26	0	0	1	0	0	1
Duration (days)	37	116	381	37	108	> 400	37	51	83	37	55	69
Max. distance (km)	<1	44	140	<1	37	125	<1	40	140	<1	41	139

	+ 72 MSS + PRE			+ 72 MSS + VAC			+ PRE + VAC			+ 72 MSS + PRE + VAC		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
No. farms infected	2	12	34	3	15	37	3	14	52	2	12	30
No. farms pre-emptive	17	59	173	3	14	31	19	68	316	17	57	164
No. farms vaccinated	-	-	-	85	222	597	89	263	1195	75	205	567
Route of infection												
local	1	8	25	1	11	30	1	10	35	1	8	23
contacts	0	1	9	0	1	9	0	3	17	0	1	9
airborne	0	0	1	0	0	1	0	0	1	0	0	1
Duration (days)	37	49	68	37	53	66	37	50	70	37	48	61
Max. distance (km)	<1	9	109	<1	9	114	<1	40	140	<1	9	114

[a] Index farm included

Table 3. Simulation results of alternative control strategies based on a goat index farm within the selected SPLA. EU = regular EU control, 72 MSS = national stand still for 72 hours after the first detection, PRE = pre-emptive slaughter within a radius of 1 km, VAC = suppressive vaccination within a radius of 2 km. Percentiles are based on the ranking of the individual parameters; results within a column do not represent one particular replication.

	EU			+ 72 MSS			+ PRE			+ VAC		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
No. farms infected [a]	2	18	1366	2	17	251	2	11	42	2	13	53
No. farms pre-emptive	5	25	464	4	21	186	18	54	212	4	18	56
No. farms vaccinated	-	-	-	-	-	-	-	-	-	38	159	760
Route of infection												
local	1	14	1284	1	12	237	1	7	28	1	10	39
contacts	0	4	60	0	2	35	0	3	15	0	3	15
airborne	0	0	19	0	0	2	0	0	0	0	0	0
Duration (days)	39	69	>400	39	66	238	35	50	81	39	53	81
Max. distance (km)	<1	45	183	<1	29	171	<1	57	172	<1	33	173

	+ 72 MSS + PRE			+ 72 MSS + VAC			+ PRE + VAC			+ 72 MSS + PRE + VAC		
	5%	50%	95%	5%	50%	95%	5%	50%	95%	5%	50%	95%
No. farms infected	2	10	26	2	13	33	2	11	36	2	10	26
No. farms pre-emptive	18	50	135	3	16	44	18	55	193	18	50	129
No. farms vaccinated	-	-	-	38	155	483	38	154	700	38	149	464
Route of infection												
local	1	7	18	1	9	26	1	7	24	1	7	18
contacts	0	1	11	0	1	10	0	3	14	0	1	11
airborne	0	0	0	0	0	0	0	0	0	0	0	0
Duration (days)	35	47	73	39	52	75	35	50	75	35	47	71
Max. distance (km)	<1	32	163	<1	29	173	<1	57	171	<1	32	163

[a] Index farm included

Addition of the 72MSS measure in the SPLA resulted in a considerably reduced risk of a large number of outbreaks. The 72 hours of MSS after the first detection gives the opportunity to trace the outbreak to the source (back tracing) and to secondary outbreaks (forward tracing), resulting in a reduction in the risk of spread. The prevention of infectious contacts to other (dense) livestock areas will concentrate the epidemic in one particular area and will reduce the size of the epidemic. Within the DPLA, addition of 72MSS did not reduce the risk of large outbreaks. At the moment of detection of the first infected farm (= start of 72MSS), the number of infected farms is expected to be higher in the DPLA than in the SPLA. Prevention of spread by tracing the source or secondary outbreaks from the infected farm will therefore be less effective, compared to the SPLA situation. Additional research will be carried out to study this aspect in more detail.

Independent of herd density, the EU policy in combination with PRE turned out to be a very effective control strategy. Pre-emptive slaughter of neighbouring farms reduced local spread considerably. However, arguments against the application of PRE are the prohibitive costs to destroy healthy animals and to compensate the farmers along with the debatable ethics of destroying healthy animals. Application of the VAC measure was at least as effective as the PRE addition. However, presence of vaccinated animals can result in considerable economic losses due to increased constraints on international meat and livestock trade (Tomassen et al., 2002). From an economic point of view, suppressive vaccination is effective when it shortens the duration of the epidemic, reduces its total costs and facilitates the return to the disease-free status (Mahul and Gohin, 1999). In the real epidemic of 2001, the Dutch authority decided to slaughter the vaccinated animals (approximately 190,000 animals) on economic grounds. In this study, the choice between slaughtering vaccinated animals (to regain the FMD disease-free status as soon as possible) or letting them live and slaughter them on their normal age (resulting in increased export restrictions) is not considered. The reflected duration of the epidemics does not account for the additional time required to slaughter vaccinated animals or the extended time of movement restrictions due to the presence of vaccinated animals.

Addition of VAC to a policy of EU + PRE resulted in a small reduction in the number of large epidemics. The simulated destruction capacity was sufficient to cope with the destruction on detected farms as well as the timely pre-emptive slaughter on neighbouring farms. In reality, the destruction capacity can be more restricted, especially when the infection is spread over different areas. Sensitivity analyses will be performed to determine the impact of a reduced capacity and therefore, the significance of applying VAC within the control strategy.

The control strategy applied during the Dutch epidemic of 2001 is reflected by the EU + 72MSS + PRE + VAC control alternative. According to the DPLA simulation results, application of the suppressive vaccination resulted only in a small contribution in the control of the epidemic.

Simulated epidemics starting in the SPLA tended to spread over a larger distance than the epidemics starting in the DPLA. This difference can be attributed to two effects, a model effect and an effect due to differences in contact structures. With respect to the model effect, InterFMD only simulates contacts within the Netherlands. Due to difference in location, contacts can be simulated over larger distances from the selected SPLA than from the selected DPLA. On the other hand, differences in composition of farm types within the selected regions also contribute to the distance the disease may spread from the index farm. Farm production types within the selected SPLA were predominately dairy cattle farms. Dairy cattle farms

maintain contacts over large distances. According to the I&R data of 1999, 11% of the cattle movements occurred over a distance of more than 100 km. Only 42% of the cattle movements remained within a radius of 20 km (Vonk Noordegraaf et al., 2000). In the selected DPLA, cattle as well as pig farm types were present. Due to the highly structured pig industry, transport distances from a pig farm are much shorter than from a cattle farm; 73% of the I&R registered pig movements in 1999 remained within a radius of 20 km (Mourits et al., 2001). The contact structures within the model are derived from these I&R data and therefore, take into account these differences in transportation distances per production type. Additional simulations will be carried out to determine the impact of the separate effects (model versus contact effect).

InterFMD is a useful tool for comparing the effects of different control strategies on FMD epidemics starting in various livestock areas of the Netherlands. Despite the recent epidemics in the Netherlands and the UK, data on FMD epidemics is rather scarce. As a result, many model parameters concerning spread and control are based on a paucity of data and/or expert opinions. Therefore, sensitivity analyses are important in determining those input parameters that have a large impact on the results. The calculations presented in this paper were based on a fixed set of parameter settings to provide general insight in the influence of area characteristics on the control of FMD epidemics. To define 'area specific rules of thumb' with respect to the selection of FMD control measures, additional calculations will require to be carried out for a range of area characteristics (variation in density of farms and distribution of production types) and sets of spread and control parameters.

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RISK MANAGEMENT OF FOOT AND MOUTH DISEASE TO PREVENT ITS INTRODUCTION AND DISSEMINATION IN CHILE FROM NEIGHBOURING COUNTRIES

H. ROJAS[1], J. NARANJO, J. PINTO AND J. ROSERO

SUMMARY

Recent foot and mouth disease (FMD) events in the world have compelled countries to strengthen their preventive measures against introduction of the disease. Chile has been free of FMD since 1981 with only two outbreaks occurring in 1984 and 1987. In recent years, neighbouring countries to Chile have had active outbreaks and they will probably maintain this status for the near future. To maintain the FMD free status of Chile, a risk management process has been implemented by the use of HACCP principles. This included the determination of critical control points, critical limits, monitoring, corrective actions, verification procedures, and schedule and record keeping procedures. A risk analysis has been carried out and a strict preventive programme has been implemented. This included prevention of introduction along with early detection and a rapid, effective response to actual disease outbreaks. The measures introduced include creating depopulated areas, increased police co-operation at national borders and active clinical and serological surveillance. Economic evaluation of these measures has been completed and has justified these control measures against the impact of an outbreak in Chile.

INTRODUCTION

Recent events in the world have forced countries to strengthen preventive measures against risk from countries infected with Foot and mouth disease (FMD). This is the case in Chile, which is the only country in South America that has remained free of FMD without the use of vaccination. Chile has been free of the disease since 1981 with only two outbreaks (1984 and 1987) since that date (OIE, 2001). Both outbreaks were controlled by stamping-out procedures. Since 1981, Chile has applied a preventive policy for FMD based on the global situation, with particular focus on the occurrence of the disease within South America.

Chile has a risk of introduction of FMD from different sources. However, the most important FMD threat comes from its neighbouring countries (NC), Peru, Bolivia and Argentina. These NC are not FMD free but outbreaks in provinces close to Chile are rare and sporadic. Therefore, in certain zones, there is a probability that Chilean livestock will come into contact with potentially infected animals from these NC and that the disease may then spread to the rest of the country.

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In border areas, Chile has permanently applied measures to prevent FMD risk coming from NC. Decisions on what measures and where they have to be applied, are the result of a process of informal risk analysis. This is based on expert meetings and official records collected by the Chilean veterinary service (Servicio Agricola y Ganadero [SAG]) over many years. Additionally, a useful source of information has been the contact with NC veterinary service authorities and contacts established at other international meetings.

Although the FMD preventive policy has yielded good results (no FMD outbreaks in 15 years), there is a need to initial a formal risk analysis, especially risk management, as the preventive policy. The problem is that there is no formal risk analysis established in the Chilean veterinary service, where decisions have had to be taken quickly using limited information where and the available methods are not being optimally used.

Formal risk analysis methods are easier to perform than they were in the past. In addition, there are techniques available, such as Hazard Analysis and Critical Control Point (HACCP), that can facilitate risk management analysis. The simplicity (or some characteristics) of HACCP for risk management could be applied to a stricter national preventive programme for an exotic disease such as FMD. This paper proposes a framework for a risk analysis on the introduction of FMD in Chile from NC through the movement of livestock.

MATERIAL AND METHODS

Neighbouring countries

Chile shares a border with three countries: Peru (165 km), Bolivia (726 km) and Argentina (4,000 km). All of the 13 Chilean regional veterinary services, 40 of 63 (64%) local veterinary services and 66 of 339 (19%) municipalities have borders with NC. Although most of them have the Andes Mountains as a natural barrier, borders have different geographical characteristics (mountains, lakes, plains, *etc*.).

Approach

The risk assessment approach has been taken as a basis for the FMD Preventive Plan (Rojas, 2001) which is currently being implemented by the Chilean veterinary service. Therefore, this is an on-going research and development project. The framework for the risk assessment (release assessment, exposure assessment and consequence assessment) is based on recommendations suggested by OIE (2000), in relation to veterinary services, animal health surveillance and risk analysis.

In the case of risk management, the principles of HACCP were used as described in European Union (1997). This technique is a scientifically based, systematic approach that has been used for prevention of food safety problems. The prerequisite five preliminary tasks and the seven principles of HACCP have been explored for the risk management of FMD.

RESULTS

Hazard identification

The pathogenic agent of concern is the List A disease, FMD. Details of the disease are described in OIE (2000).

Risk assessment

Flow chart: FMD can be spread from the endemic areas of NC to different areas of Chile. The disease has to pass through different geographical territories for it to become established in Chile (Fig. 1). In each step, there are susceptible animals that have a probability of becoming infected by contact with infected animals originating from the 'previous' geographical territory. The expected movement of the disease is from the endemic areas of NC through to the valley in Chile, passing over the national border that divides both countries. The disease is endemic in the valley regions of the NC, whereas it is sporadic in the rest of the NC, including the provinces bordering with Chile. If the disease passes into Chile, it would be from the NC border regions and the exposure would be in the Chilean border provinces. From here, the disease would spread to the rest of Chile. Each specific geographical sector of Chile (regions, local veterinary services or municipalities) has a particular flow chart with a similar pathway.

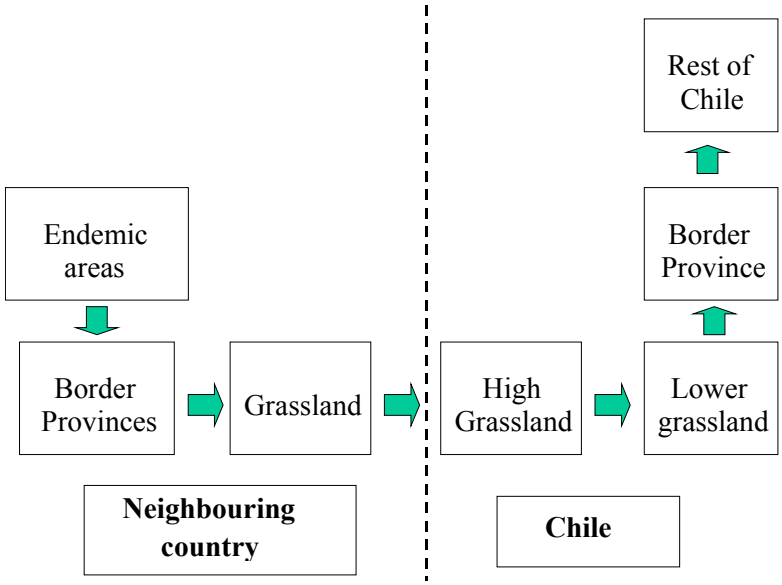

Fig. 1 Flow chart for the introduction of FMD to Chile from neighbouring countries

Source of infection from NC: The potential FMD spread from NC to Chile would certainly be through illegal movement of various animals (contrabandos) or through animals that accidentally cross the border. Firstly, the illegal entrance of animals from NC is carried out by people that live near the border, facilitated by several factors such as geographical features, weather conditions, a difficult economic situation, plentiful supply of animals from the NC, competitive prices and no effective border police action in any of the countries. The main problem is that these illegal animals may be infected by FMD and may come into contact with susceptible animals in Chile before they can be detected. Secondly, the animals that accidentally cross the border are those that graze near the boundary and try to feed in Chile. Fig. 2 shows both models of introduction FMD to Chile. Since 1992, there have been 115 illegal movements of animals from NC with 1,886 animals detected. All of these animals were slaughtered and none of them were positive for FMD (SAG, 2001).

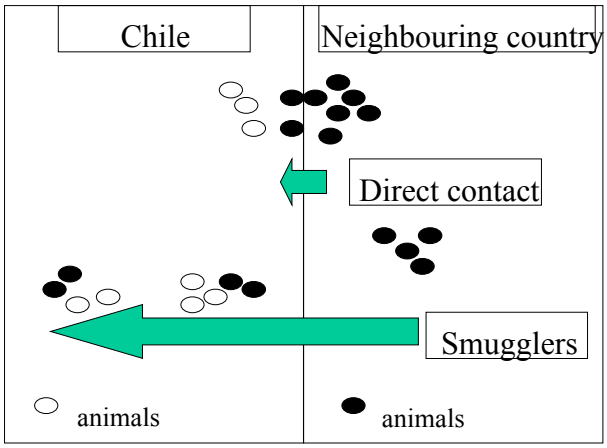

Fig. 2 Routes of possible entrance of animals from NC to Chile

FMD situation in neighbouring countries: The FMD status in NC with the outbreak location is shown in Fig. 3. In year 2001, there were 2,557 outbreaks in Argentina, 144 in Bolivia and 0 in Peru (SAG, 2001). The number of outbreaks has increased in Argentina and Bolivia in recent years but the incidence has decreased in Peru.

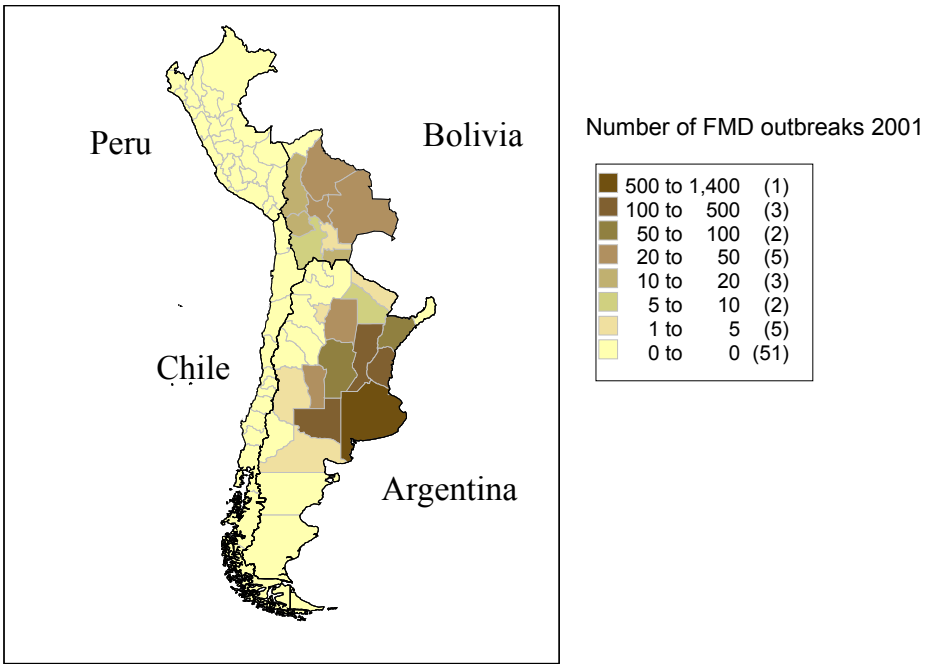

Fig. 3 FMD outbreaks in Chile and neighbouring countries in 2001

Release assessment: This represents the probability that infected animals from a NC will be within the border of Chile. The variables considered for the risk assessment are the number of animals, density of animals, FMD status, immunity of animals, ability of the veterinary service to control the disease, the geographical characteristics of the border and the intensity of illegal

movement. These variables were considered from the endemic areas to the border province in the NC and, from there, onto the grassland in the border with Chile.

Exposure assessment: This represents the probability that FMD infected animals from NC come into effective contact with one or more susceptible animals in Chile. This risk of exposure also depends on the animals' characteristics, such as type of animals (cattle, sheep and goat), their density and movement dynamics. A particular situation occurs in spring and summer, where animals are moved to graze on high grasslands. These sectors are called 'veranadas' and exist from the IV to the X region of Chile. There are people who own the veranadas that use or rent these places to one or more herds. During the 2000-2001 season, there were 250,000 animals of all susceptible species recorded as grazing the veranadas (Rojas, 2001).

The control measures considered in the FMD Prevention Plan for NC and Chilean geographical zones are listed in the risk management section (Table 1).

Consequence assessment: This represents the direct and indirect consequences of the entry of FMD into Chile. The consequences depend on the following issues:

- The location of the index case;
- The places to which FMD spreads;
- Number of animals exposed during the outbreak;
- Methods used to control the outbreak (e.g. stamping-out and/or vaccination);
- Time spent in controlling the last outbreak;
- Export products and markets and the reaction of importing countries.

These variables depend on the ability of the veterinary service to ensure early detection of the disease, to effect a rapid response to such an outbreak and the ability to negotiate the timely removal of market restrictions with livestock and meat importing countries.

In each geographical sector, there is a probability of a different scenario and each of them will suffer a direct and indirect impact in the case of an FMD event. Each scenario also has an estimated probability of occurrence.

Risk assessment: Based on the release, exposure and consequences, the total risk of introduction of FMD to Chile can be estimated for each geographical sector. Estimation of an individual risk index provided a preliminary risk assessment for each municipality. For each of them, the following variables were considered: In NC: The number of animal units, the animal density, the presence of veranadas, the degree of illegal movement and the geography of the border. In Chile: The number of animal units and the animal density.

Fig. 4 shows the risk for each of the Chilean municipalities. These values are from 0 to 71. Results demonstrated that the risk is different depending on the geographical zone. The highest risk areas are the VII and VIII regions of southern central Chile.

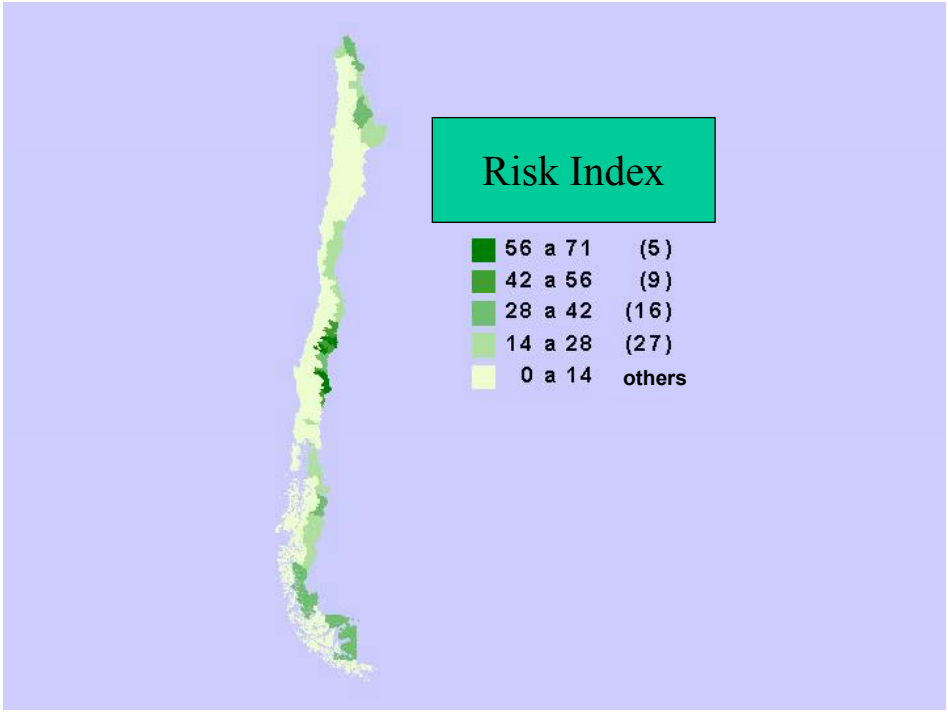

Fig. 4 Risk index for the introduction of FMD to Chile from neighbouring countries

Risk management (HACCP approach)

Every geographical sector (national, regional and local veterinary services) is a unit of interest. Therefore, there needs to be a HACCP plan for each of them.

Prerequisites: The selected prerequisites were a formal relationship with veterinary services, a quality assurance for the diagnostic laboratory, the police structure in the border, telecommunications, a process of passive surveillance, training of official personnel as well as others not mentioned within this paper. The following are examples of these prerequisites in the FMD preventive plan. An audit from PANAFTOSA was performed on the veterinary service diagnostic laboratory. Also, there was an agreement made between the veterinary service and the police, and training for official and private veterinarians as well as farmers was provided.

Five preliminary tasks: Two of these tasks have been considered. Firstly, the constitution of the HACCP team at three different levels of the official veterinary service (national, regional and local) was agreed. Secondly, the flow diagram of the process and its verification have already been included in the risk assessment**.** The remaining preliminary HACCP tasks do not appear to have any relevance to the FMD situation.

HACCP STEPS

Step 1: Conduct a hazard analysis: The identification of hazards and their risk assessment have been completed using the risk analysis approach. Based on this analysis, appropriate control measures have been defined (Table 1). They have to be focused on the elimination of the risk or its reduction to an acceptable level. This has been completed based on the epidemiology

Table 1. Control measures by each geographical sector (step)

Step	Country	Control measures
Endemic areas	NC	Animal and product legislation
Endemic areas	NC	Active surveillance
Endemic areas	NC	Outbreak control measures
Endemic areas	NC	Massive vaccination
Endemic areas	NC	Transparency and opportunity of information
Border provinces	NC	Animal and product legislation
Border provinces	NC	Internal official barriers
Border provinces	NC	Active surveillance
Border provinces	NC	Outbreak control measures
Border provinces	NC	Massive vaccination
Border provinces	NC	Restriction to approach animals to Chilean zone
Border provinces	NC	Transparency and opportunity of information
Grassland	NC	Internal official barriers
Grassland	NC	Active surveillance
Grassland	NC	Outbreak control measures
Grassland	NC	Massive vaccination
Grassland	NC	Police surveillance
Grassland	NC	Depopulated (animals) areas close to borders
Grassland	NC	Transparency and opportunity of information
High grassland	Chile	Fences in border farms
High grassland	Chile	Depopulated (animals) area in veranadas
High grassland	Chile	Police stations
High grassland	Chile	Actions against illegal animal movements
High grassland	Chile	Surveillance by helicopter
High grassland	Chile	Internal official barriers
Lower grassland	Chile	Control zone and sub-zones definition
Lower grassland	Chile	Herd identification
Lower grassland	Chile	Animal marking
Lower grassland	Chile	Quarantine before returning
Lower grassland	Chile	Movement control and restrictions
Lower grassland	Chile	Clinical surveillance
Lower grassland	Chile	Serological surveillance
Lower grassland	Chile	Passive surveillance ([a])
Lower grassland	Chile	Free telephone line for notification ([a])
Lower grassland	Chile	Preparedness of contingency plan ([a])
Rest of Chile	Chile	Transport requirements
Rest of Chile	Chile	Quarantine in destiny
Rest of Chile	Chile	Livestock market surveillance
Rest of Chile	Chile	Slaughterhouse surveillance
Rest of Chile	Chile	Rubbish dumps surveillance
Rest of Chile	Chile	Risk herds surveillance
Rest of Chile	Chile	Passive surveillance
Rest of Chile	Chile	Control of movement and restrictions

[a] The same for the rest of Chile.

of the disease and current expert opinion on its control. The control measures are part of a comprehensive preventive approach that has four components. Namely, early warning, prevention of entry, early detection and rapid, effective response. These measures have been focused to minimise both the release of FMD from NC and exposure to the disease within Chile.

Control measures in NC are those recommended by the OIE for infected countries. These include vaccination and general restrictions. Control measures in border areas are less generalised and vary according to the country and the region within each country. Moreover, they vary depending on the budget and the importance of livestock.

Control measures to prevent the entrance of FMD from NC to Chile are those that avoid the contact between possibly infected and susceptible animals. Most of them are concentrated in the central region of Chile, where animals tend to go to veranadas. There, animals are most likely to have contact with smuggled animals and also with the ones that cross the border accidentally. Depopulated areas help to avoid direct contact and tend to be in areas which are difficult for animal smugglers to access. A legal body can create these depopulated areas. Both the owners of the veranadas, who stop receiving a rent, and the small farmers who cannot use the grassland areas, obtain compensation from the government. Police help with general surveillance to prevent animal smuggling and, together with national veterinary service staff, they perform surveillance by helicopter. In the case of detection of an animal smuggling operation, there is a common procedure that is instigated by the police and the national veterinary service. Dealers are penalised and animals are tested and slaughtered. Additionally, in Patagonia, due to its particular geographical features, fences and stricter surveillance by the national veterinary service are performed.

Control measures for early detection are implemented in the high and lower grasslands as well as in the rest of the country. Lower grassland areas are set up as control areas. Herds and animals are marked, and have frequent visits from veterinary brigades (veterinarians and veterinary technicians), which use horses or vehicles for formal gathering of animals. They carry out active clinical and serological surveillance during these round ups. Samples are sent to the official veterinary service diagnostic laboratory. In 2001, there were 12,920 samples taken in veranadas as well as 4,356 taken in other high risk areas. In addition, 1,767 samples were taken in Tierra del Fuego in conjunction with the veterinary service of Argentina to declare the territory free of FMD (SAG, 2001). Animals that go to the control area have to pass through a national veterinary service barrier where they are marked and registered. Only negative animals to FMD can return to their origin. Animals are also subjected to a compulsory quarantine at their destination farm and permission is required in order from them to be taken to a livestock market. Frequent visits to livestock markets, rubbish dumps and slaughterhouses are also included as part of the surveillance procedures.

The measures required for a rapid and effective response are considered within a contingency plan and these measures are available for different scenarios. Statistics from the 2001-2002 FMD Prevention Plan are shown in Table 2.

The communication programme is an important measure that helps both components of the control strategy. One part is directed at the general public involving with radio, TV, written media and schools. The second component is targeted at more specific sectors of the public (farmers, veterinarians, border users and community traders). There are translations into local languages to assist in promoting compliance in various regional areas, particularly for the latter component.

Table 2. Statistics from the FMD Prevention Plan season 2001-2002

Item	Figure
Grassland fields total (ha)	2,806,000
Grassland fields under restriction (numbers)	160
Grassland fields under restriction (ha)	446,000
Grassland under active surveillance (numbers)	716
Grassland under active surveillance (ha)	2,360,000
Farmers with permission	2,163
Number of cattle	48,415
Number of sheep	50,109
Number of goats	188,126
Number of horses	13,098
Number of internal barriers	34
Number of veterinary inspection teams	31
Number of police stations	30
Number of official veterinarians	67
Number of official veterinary technicians	41
Number of FMD veterinarians	28
Number of FMD veterinary technicians	74

Step 2: Determine the critical control points (CCPs): All the process steps (control measures) are defined as a CCP because they are located where hazards could be eliminated, reduced or controlled to an acceptable level. Therefore, there are CCPs located in NC, while others are in the Chilean mountains or in the valley regions.

Step 3: Establish critical limits: These are defined for each control measure in the CCP. However, in some cases, they have to be interpreted by considering other epidemiological information.

Step 4: Establish monitoring procedures: These have been defined to see if each CCP is under control and for use in future verification exercises. Each CPP should have a proper recording system which is based on records used by the FMD Prevention Plan. This plan is produced by the veterinary services, the police and the NC veterinary services. The monitoring of FMD is achieved by active and passive surveillance in each geographical step, both in NC and in Chile.

Step 5: Establish corrective actions: Corrective action will result as a consequence of a loss of control of the critical limits which will take into account the epidemiological interpretation. After the initial analysis, more precise corrective actions may have to be implemented.

Step 6: Establish procedures for verification or auditing to confirm that the HACCP system is working effectively: This may be undertaken by the national veterinary service along with the assistance of the regional veterinary service. An on-going and long-term verification procedure is required. Auditing is also a prerequisite. Both national and regional veterinary services have to be involved in the validation of the regional and local plans, respectively. Permanent international auditing is compatible with the proposed system.

Step 7: Establish documentation concerning all procedures and records appropriate to these principles and their determination: Each sector, regional and national level should keep the required documentation. This has to include the risk analysis and the HACCP Plan at the very least.

DISCUSSION

Risk analysis

Preliminary results from a formal a risk analysis appear to be satisfactory, especially with regard to risk management in the FMD Prevention Plan. However, there are some qualifications required.

Risk analysis is a qualitative and quantitative tool. Although it is easy to apply, for a quantitative approach, it is difficult to obtain an accurate probability for unknown events. For example, for illegal animal movement, it is possible to account for the smuggling that is identified, but impossible to account for the smuggling that goes on undetected.

A formal risk analysis with emphasis on risk management is important for veterinary services because:

a) It makes it easier for the participation of all experts and, their experience can be used in a systematic way;
b) It makes it easier to act immediately upon a changing situation;
c) It is easy to audit and improve the system;
d) It is helpful to assess the points that need more research;
e) It facilitates dialog with national authorities regarding preventive disease programmes, their progress and the need for budgetary adjustments. Additionally, it is helpful in encouraging dialog with NC authorities when a co-ordination policy has to be addressed;
f) It is useful for the export market, as the veterinary services can demonstrate that preventive actions have been properly implemented.

In the initial risk analysis, variables that affect the introduction of FMD from NC to Chile have been detected. Additionally, a preliminary model for risk assessment has been developed. This has allowed a preliminary prioritisation of the geographical sectors in relationship to the risk of introduction of FMD. The model is a combination of quantitative and qualitative data. There is a high priority being given to improving the model to include all the control measures, both in NC and in Chile, within a changing scenario. To this end, the priority is to receive reliable and timely information from NC and also the main non-structured problems, such as the illegal movement of animals, have to be addressed. Finally, a detailed analysis of consequences has to be performed.

Risk management (HACCP)

The HACCP methodology presents the possibility of providing a good risk management system. Prerequisite tasks and the five previous steps have to be performed and prioritisation of the components of a central project of exotic disease prevention has to take place. In general, all issues relating to personnel and systems have to be included in these sections.

There is the possibility to improve and change the steps and the CCPs definitions. HACCP literature advocates having as few CCPs as possible. However, in this disease prevention plan there have been many CCPs defined as it is impossible to say that the steps will be fully controlled, as may be possible within an industrial process. Therefore, it is not possible to say that any of the CCPs are unnecessary. Nevertheless, in this scenario having many CCPs should not be a problem as there are an adequate number of teams that can deal with all of them.

This HACCP is easy for the veterinary service to accept as a system of work as most veterinarians and farmers are familiar with this method. Therefore, it should not be difficult to implement.

The issue that is extremely important both in risk assessment and in risk management is the collaborative and co-ordinated work with NC. This includes the selection of control measures and the possibility of influencing their policies, considering both technical and financial issues. Confidence between NC's veterinary services is crucial in achieving an effective risk management system for exotic disease prevention.

The tools (epidemiology, risk analysis and economics) are available for this kind of prevention programme. Nevertheless, it is generally difficult to introduce their effective use by veterinary services, mainly because they have to be constantly dealing with alerts and emergency situations. However, it is precisely these situations, like those recently observed in Argentina and UK, that demonstrate the urgency of realising that the tools presented in this paper are more cost effective to implement than continuing with no formal analysis.

ACKNOWLEDGEMENTS

The authors are grateful for the co-operation of all the people involved in the FMD Preventive Plan for Chile.

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HERD LEVEL RISK FACTORS FOR FOOT AND MOUTH DISEASE IN THE ADAMAWA PROVINCE OF CAMEROON

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SUMMARY

Foot and mouth disease (FMD) is a highly contagious viral disease of even-toed ungulates (*Artiodactyla*) and is one of the most important economic diseases of livestock in the world. This paper describes the risk factors for FMD cattle herds in the Adamawa Province of Cameroon, as reported by farmers. These results form part of a larger study of the epidemiology of FMD in this region. A cross-sectional study design was used and a stratified, two-stage random cluster sample of cattle herds in Adamawa was selected. A standardised questionnaire was developed and then pre-tested at a local cattle market before a final version was translated into Foulfoulde (the local Fulani dialect). The multivariable models were developed by a forward selection process. Important risk factors include feeding cotton seed cake, buying in cattle from markets, seeing buffalo near the herd, going on transhumance and administrative Division.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious viral disease (*Picornaviridae,* genus *Aphthovirus*) of even-toed ungulates (*Artiodactyla*) and is one of the most important economic diseases of livestock in the world. In Cameroon, as in many other parts of Africa, cattle are an important form of financial security and banking. One of the main cattle rearing areas in Cameroon is the Adamawa Province. The cattle population is estimated to be between 2-3 million and livestock rearing is the single largest economic activity in the Province. The Fulani (known in Cameroon as the Fulbé and Mbororo), who raise cattle throughout sub-Saharan Africa, dominate cattle rearing. In recent years, an increasing number of people from other ethnic groups have begun using cattle for draught power. Additionally, a small dairy industry has been encouraged by the construction of a dairy processing plant in Ngaoundere, the Provincial capital. Many cattle owners also keep small numbers of sheep and goats, but there are few pigs in the Province and none were kept by any of the cattle owners (Bronsvoort et al., 2002). The exception is Ngaoundere where there is a lairage at the train station for pigs being transported from the north of Cameroon to the capital, Yaounde. There is currently no control programme for FMD and no licence has been issued for vaccine importation into Cameroon. Moreover, it is probable that the current vaccine would be too expensive for widespread use.

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These issues, highlight the need for an improved understanding of the epidemiology of the disease in an endemic area, in order to design ecologically and culturally appropriate control measures (Carlos, 1992).

This paper describes the risk factors for FMD cattle herds in the Adamawa Province, as reported by farmers. These results form part of a larger study of the epidemiology of FMD in the Adamawa Province of Cameroon.

MATERIALS AND METHODS

Study Design

A cross-sectional study design was used and a stratified, two-stage random cluster sample of cattle herds in the Adamawa Province was selected. The Adamawa Province lies between latitudes 6^0N and 8^0N and covers an area of 64,000km^2 at an elevation of 1000-2000m. The Province is divided into 5 administrative Divisions with a total of 88 Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) veterinary centres distributed across the region. A sampling frame was constructed using the rinderpest vaccination records from the MINEPIA veterinary centres. Vaccination records from 1999/1998 or the next most recent year were obtained and a total of 13,006 herds were entered into an 'Access'[1] database. The sample size was calculated using the program, 'Survey Toolbox'[2], and based on an assumed FMD herd level period prevalence of 50% for the previous 12 months, 88 veterinary centres, an average of 150 herds per centre, a 10:1 cost ratio of centre to herd and a 90% confidence interval. The probability of a centre being selected in any strata was proportional to the number of herds listed for that centre; that is, the probability proportional to size (PPS) option in the program was used. Centres were selected with replacement so that more than one independent sample could be drawn per centre. The program calculated a first-stage sample of 54 centres and 3 herds per centre were selected without replacement.

A standardised questionnaire was developed and pre-tested at a local cattle market before a final version was translated into Foulfoulde (the local Fulani dialect). It was also translated back into English in order to correct any misunderstandings in use of language (Lee et al., 1999). The questionnaire covered as many aspects of herd management as were culturally acceptable. It included aspects of the annual migration (transhumance), types of grazing, feeding, watering, the numbers of contacts between herds, other species of livestock kept and contacts with wildlife. Questions regarding herd size were not socially acceptable and were avoided. The questionnaire was administered by interview and took between 30 and 40 minutes to complete.

Between April and November 2000, the herds were contacted by mailing the relevant veterinary centre. Farmers were informed of the method of selection and of the date of the proposed visit. The herds were first examined and probang and serum samples collected from 10 animals per herd. The owner or his herdsman also completed the questionnaire. Detailed descriptive analyses of the data are presented in a separate paper on the livestock systems of the Adamawa Province (Bronsvoort et al., 2002). Additionally, the results of virus isolation from the probangs and serology will be presented elsewhere.

Statistical Analysis

[1] Microsoft Corp., Redmond, Seattle, USA.
[2] http://www.ausvet.com.au/surveillance/toolbox.htm

Statistical analysis was carried out using the 'STATA' software package[1]. Univariable analysis was carried out using the *tabodds* command. A threshold value of $p<0.3$ for the χ^2 test was used to screen variables for the multivariable model. Categorical variables with more than 2 levels were included using dummy variables. The remaining variables were checked for the number of observations and any with less than 130 were removed. These were mainly variables related to transhumance, which involved only 67 herds and these will be modelled separately.

The outcome variable was defined as 'Having had at least one outbreak of FMD in the herd in the previous 12 months' (**disltyr**) as reported by the herdsman. The multivariable model was developed using a forward selection process. The stratification design effect, Division (**div**) (Table 3), was included as a fixed effect with 5 levels with Vina Division being used as the comparison. Clustering and non-response design effects were not included in the analyses. The variables 'going on transhumance last year' (**trans1yr**) and 'seeing buffalo near the herd either grazing or while on transhumance' (**buffevr**) were included *a priori* in the model because of previous evidence that they are important risk factors (Dawe et al., 1994a; Dawe et al., 1994b; Macpherson, 1995).

Where more than one variable related to the same risk factor, such as 'Do your cows mix with other herds where they graze?' (**mixgraz**) and 'How many herds do they mix with while grazing?' (**mixgzct**) or 'Do your cows mix with other herds where they drink?' (**drkmix**), 'How many other herds do they mix with where they drink?' (**drkmixct**), 'How many other herds use this place for their herds to drink?' (**othdrkct**), alternative models were examined with one or other variable model in the model to prevent problems of collinearity.

The variables were included starting with the most statistically significant from the univariable analysis. As each new variable was added, all the variables were reassessed for removal using the Wald statistic and for its effect on other variables. Effect modification was also assessed after each new variable was added. Variables that had entered and been removed were allowed to re-enter at each stage of the process. The final model fit was assessed using the Pearson's chi-square statistic and the residuals were plotted against the predicted probability to check for trend.

RESULTS

A total of 147 of the 162 herds originally selected were examined. Fifteen herds were not examined either because they refused (4 herds), had stopped keeping cattle (5 herds) or the centre failed to get in touch with the owner/herdsman (6 herds). Twenty-five of the 147 herds had to be replaced because the selected herd had moved away from the area (

Table 1). The descriptive statistics are presented in another paper (Bronsvoort et al., 2002).

Sixty-nine exposure variables were screened for association with the outcome variable, 'FMD in the previous 12 months' (**disltyr**). Twenty-one variables passed the initial screening for use in the multivariable model. These are listed in Table 2. The majority of variables related to contacts with other herds or potential contact with wildlife.

[1] STATA v6.0, Stata Corporation, 702 University Drive East, College Station, Texas 77840, USA.

Table 1. Breakdown of the number of herds listed as vaccinated, the target to sample and the number finally examined per Division

Division	No. of herds listed as vaccinated	Target herds	Actually examined
Vina	3804	48	48
Mbere	2334	30	25
Djerem	2671	33	28
Faro et Deo	1528	18	15
Mayo-Banyo	2669	33	31
Total	13006	162	147

The final model is given in Table 3 and includes the variables for Division, feeding cotton seed cake, going on transhumance the previous year, seeing buffalo near the herd, buying cattle from markets and mixing with other herds at the watering point. No significant effect modifications were observed at the $p<0.05$ level. The Pearson chi-square statistic was 56.78 ($p=0.302$) and a plot of the predicted probabilities against the residuals showed no trend so the model was accepted.

Table 3. Multivariable model for 'FMD in the previous year'

Disltyr	Odds Ratio	P>|z|	90% Confidence Interval	
Cotton	3.29	0.027	1.359	7.966
Buycow	2.22	0.048	1.142	4.331
Div_{Mbere}	0.49	0.241	0.180	1.332
Div_{Djerem}	0.24	0.018	0.090	0.647
$Div_{Mayo\text{-}Banyo}$	0.22	0.018	0.079	0.636
$Div_{Faro\ et\ Deo}$	0.69	0.646	0.186	2.581
Trans1yr	2.58	0.065	1.109	6.008
Drkmix	2.43	0.082	1.049	5.645
Buffevr	2.21	0.137	0.920	5.297

n =146; Log likelihood = -77.757433

Table 2 Univariable analysis of risk factors for FMD in the last 12 months, unadjusted for the stratification and clustering design effects.

Variable	Name	n	Code	Odds Ratio	90% CI	p-value
Do you see buffalo near your cattle at grazing or when on transhumance	buffevr	147	yes	2.8	1.46-5.46	0.007
			no			
Do you see warthogs near your cattle at grazing or when on transhumance	wartevr	147	yes	2.2	1.19-4.15	0.030
			no			
Do you see antelope near your cattle at grazing or when on transhumance	antevr	147	YES	2.6	1.33-4.96	0.015
			no			
Do you own sheep	ownshp	147	yes	1.4	0.81-2.57	0.293
			no			
Do you own goats	owngt	147	yes	1.7	0.81-3.52	0.233
			no			
Do your cows mix with other herds where they graze	mixgraz	147	yes	3.0	0.70-13.15	0.190
			no			
How many herds do they mix with while grazing	mixgzct	143	0			
			1-3	1.8	0.40-8.44	0.511
			4-6	3.9	0.80-18.60	0.127
			7-10	5.8	1.06-31.10	0.054
			>10	2.5	0.46-13.55	0.356
Did any of the herds your herd mixed with while grazing have FMD in the last 4 weeks	mixgrdis	139	yes	8.1	1.37-48.46	0.021
			no			
Do your cows mix with other herds where they drink	drkmix	146	yes	3.51	1.62-7.59	0.004
			no			
How many other herds do they mix with where they drink	drkmxct	143	0			
			1-3	2.4	1.03-5.52	0.080
			4-6	6.1	2.18-16.96	0.001
			7-10	2.1	0.74-5.96	0.232
			>10	13.2	1.55-112.54	0.010

How many other herds use this place for their herds to drink	othdrk	143	0			
			1-3	4.2	1.03-17.08	0.068
			4-6	13.1	2.62-65.48	0.001
			7-10	7.3	1.39-38.35	0.021
			>10	49.5	1.84-1245.51	0.001
Do you buy cattle	buycow	147	yes	2.1	1.21-3.80	0.026
			no			
Do you lend your bull to neighbours	lendbull	147	yes	2.1	0.97-4.41	0.110
			no			
Do you feed natron to your cows	natron	147	yes	1.8	1.04-3.25	0.074
			no			
Do you feed cotton seed cake to your cows	cotton	147	yes	3.8	1.85-7.87	0.001
			no			
How many years have you lived in this area	yrsliv	145	1-3			
			4-6	0.8	0.25-2.51	0.734
			7-10	1.2	0.42-3.57	0.752
			11-20	4.5	1.38-14.62	0.021
			>20	1.6	0.63-4.01	0.402
If there is a herd with FMD in your village would you know about it	disvilkn	147	yes	6.1	0.93-40.59	0.071
			no			
Do you know if sheep can get FMD	knshpfmd	147	yes	1.9	1.05-3.27	0.069
			no			
Do you graze your herd on a pasture that is fenced in	pastfen	147	yes	2.9	0.77-11.27	0.167
			no			
Went on transhumance last year	trans1yr	147	yes	1.85	1.04-3.28	0.073
			no			
Division	div	147	Vina			
			Mbere	0.4	0.15-0.9	0.064
			Djerem	0.2	0.08-0.46	0.001
			Mayo-Banyo	0.3	0.11-0.63	0.007
			Faro et Deo	0.8	0.24-2.52	0.768

DISCUSSION

The model suggested several important risk factors for FMD outbreaks for cattle herds in Adamawa Province. Feeding cotton seed cake (**cotton**) was one of the most influential variables. This may represent a direct risk due to contamination of cotton seed cake with FMD virus. However, given the chemical processing involved in the production process and the low humidity of the dry season when it is being fed, it is unlikely that the virus could survive on the feed or the sacks (Donaldson, 1972). Alternatively, it may be a proxy for some factor that has not been measured. Cotton seed cake is predominantly fed in the Vina and Mbere Divisions, but this spatial effect should be controlled by the inclusion in the model of the stratification variable, Division. One possible explanation may be that herds that feed cotton seed cake are owned by wealthier people who employ herdsmen to tend their herds. These herdsmen may not be as particular about avoiding the disease as they do not own the cattle. Unfortunately, the data on ownership of herds and the use of hired herdsmen is not available.

Buffalo are believed to be a major risk for FMD outbreaks in livestock, largely through their role as persistent virus carriers (Hedger, 1972; Hedger et al., 1973; Hedger and Condy, 1985; Dawe et al., 1994a; Vosloo et al., 1996). For this reason, it was included in the model *a priori*. Cameroon is home to the forest buffalo, which is the sub-species, *Syncerus caffer namus*. There has not been any similar study of the forest buffalo, but it would have been unwise to ignore their potential role as carriers. The variable, **buffevr**, in the model suggested that having seen buffalo near the herd doubled the odds of having FMD. The p-value for the Wald statistic to remove it from the model was not highly significant, but given the potential role as a virus carrier and the fact that it was confounding the variable for going on transhumance in the last dry season, it was decided that it should remain in the model.

Mixing at watering points appeared in the final model as the binary variable, **drkmix**. If the categorical variable, **othdrk,** was included, the estimates for some of the levels became unrealistically large, with large variances which were believed to be due to stratification, therefore, the binary variable was used. Another approach might have been to collapse some of the categories. However, the apparent increased risk with increased number of herds using the watering place, which was observed in the univariable analysis, adds weight to the importance of mixing at watering points. A similar association was found in Thailand (Cleland et al., 1996) where the odds ratio increased by 1.6 for every additional village that shared a water source (village equates with herd in this study). This may be due to either the increased mixing and hence increased potential for transmission, or may be due to a higher survival rate of the virus in the environment, due to more humid microclimate (Donaldson, 1972; Dawe, 1978) or a combination of both.

Transhumance (**trans1yr**) has been suggested as a risk factor for a number of diseases (Macpherson, 1995), due to the movement and mixing of stock at higher than usual densities, and contact with wildlife. However, no specific evidence has been presented for FMD. For this reason, it too was included *a priori* in the model. It was confounded by seeing buffalo near the herd. This is not unexpected given that transhumance herds migrate to remoter areas in search of grass and these are also the areas in which higher densities of buffalo are found.

Buying cattle from markets (**buycow**) and their introduction into a herd poses an obvious risk when there is no restriction on movement of sick animals. Markets create a lot of mixing of animals from a wide geographical area, they are stressful for the animals and they provide an

ideal situation for infection to spread from clinically affected or carrier animals. This is particularly a problem in Cameroon where small groups of animals will be walked to a market and only one or two animals will be sold and the rest walked back afterwards. This method is used in order to accommodate easier movement of the animals as herding one or two animals is very difficult. At the market, all animals sold are penned together prior to being walked to their new herd or another market. This creates plenty of opportunity for transmission.

Finally, there appears to be an important spatial component to the disease which also confounds the feeding cotton seed cake as a risk factor. Djerem and Mayo-Banyo Divisions have much lower odds of disease compared to Vina Division. There are clearly many variables relating to cattle density and management, the quality of veterinary services and other socio-economic factors, as well as possible ecological factors that vary across the Province which the questionnaire did not measure and will need further investigation.

This study identified several important risk factors for having had FMD in the herd in the previous 12 months, such as buying in cattle from markets, feeding cotton seed cake, mixing of herds at watering points, contact with buffalo, going on transhumance, and administrative Division. Though clearly more investigations are needed for some of these factors such as feeding cotton seed cake and going on transhumance, interventions can be suggested at various levels. For example, individuals can make their own decisions about cattle purchases and communities can influence control over access to watering points. The potential role of wildlife is again highlighted though it may not be as important as other factors.

Creating a sampling frame from which to select herds was extremely difficult. The vaccination lists were the most reliable information available but they were not complete, since anecdotal evidence suggested that some herds did not vaccinate every year. An attempt was made to estimate how reliable the lists were (Bronsvoort et al., 2002). It is also the intention to validate the outcome variable using the results of the knowledge of the herdsmen of FMD lesions and then to reanalyse the data. In addition, risk factors associated with transhumance herds need to be assessed, Furthermore, comparison of results with those of a multi-level model has to be completed as this would allow stratification and cluster design effects to be included as random effects. As well as the herd-level questionnaire, 10 animals per herd were blood sampled. These data will allow a more objective outcome variable, antibody titre, to be used and additional animal level risk factors to be included in the analyses.

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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