SYSTEMS FOR THE PREVENTION AND CONTROL OF INFECTIOUS DISEASES IN PIGS

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The results of science remain hypotheses that may have been well tested, but not established: not shown to be true. Of course, they may be true. But even if they fail to be true, they are splendid hypotheses, opening the way to still better ones.

Karl R. Popper, A World of Propensities, 1990

Abstract

An expert system (RestiMATE) was designed that assists veterinary practitioners in assessing the respiratory health status of a pig farm. RestiMATE uses classification rules to identify patterns of environmental risk factors for respiratory diseases and to select optimal management interventions to control and prevent respiratory diseases. The classification rules are based on expert interviews and on empirical data collected in New Zealand. Recursive partitioning and neural network techniques have been applied for rule induction. These methods were compared with logistic regression and appeared to be similarly efficient in terms of classification while providing additional insight into the structure of a data set. Non-parametric analytical methods appear to be particularly suitable when analysing complex data sets and for exploratory data analysis.

EpiMAN-SF is an advanced decision-support system designed to manage and analyse data accumulated during an African swine fever or classical swine fever emergency. EpiMAN-SF offers state-of-the-art technology for managing data related to a swine fever epidemic, including laboratory results. An expert system was developed to support rapid classification of contacts between pig farms in terms of the risk of virus transmission. These classifications are used to set priorities in visiting farms for laboratory investigations. The validation of the expert system showed that its evaluation was more consistent and generally more risk-averse than that of human experts. A stochastic simulation model was developed to investigate the spread of swine fever infection within a farm and a second model (INTERSPREAD-SF) was designed to forecast the dynamics of the epidemic within a region and to evaluate control strategies. INTERSPREAD-SF has been validated using real outbreak data from Germany and was shown to be capable of realistically replicating the behaviour of classical swine fever. However, more research is needed to complete our knowledge about the detailed epidemiological processes during a swine fever epidemic.

A prerequisite for efficient disease control in pig populations is reliable animal identification. A series of trials was conducted in order to compare electronic ear tags and implantable identification chips with visual ear tags. It was shown that the difficulties with respect to implants are loss rates of up to 18.1% within 4 weeks after implantation while electronic ear tags were lost or damaged by processing at the abattoir in up to 23.4% of pigs.

Infectious aerosols were reviewed as an additional aspect of the causative network of infectious diseases in pigs. An air sampling system based on air filtration was developed and applied in combination with polymerase chain reaction assays. Using this technique, *Mycoplasma hyopneumoniae*, the major causative agent of enzootic pneumonia was isolated from air samples for the first time. However, the attempt to isolate classical swine fever virus from the air was unsuccessful, probably due to technical difficulties.

Acknowledgements

Doing a PhD is all about learning. I certainly learnt a lot during these last three years in the Epidemiology Group (now EpiCentre) at the Department of Veterinary Clinical Sciences (now Institute of Veterinary, Animal and Biomedical Sciences). Probably the experience of the most lasting value to me was to realise that no matter how good or bad a situation, I can always learn something: either how to do something or how not to do it. Therefore, first of all, I would like to thank everyone who has helped me learn.

In this context, I am particularly grateful to my chief supervisor Prof. Roger Morris, who provided me with the ideas and vision, which are the strong foundations of this thesis. The help of my second supervisor Dr. Dirk Pfeiffer was indispensable in successfully realising many aspects of my work. His 'you can do it' attitude helped me tackle many problems and allowed me to put analytical issues into perspective. I also worked with Mr. Mark Stern on software design issues and the INTERSPREAD model. Many other people have contributed significantly to the content of this thesis, and their help is acknowledged at the end of each chapter.

I would probably never have come to New Zealand if it had not been for the motivation and enthusiasm of my mentor and friend Prof. Ueli Kihm. He taught me the importance of integrity and modesty. I have also not forgotten the repeated advice given by my first boss and teacher in animal health, Prof. Hermann Keller. He showed me that research needs to serve the purpose of solving problems, a principle that I believe is very much present in this thesis.

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The preparation of a PhD inevitably impacts on family life. I would therefore like to acknowledge the support of my husband Marcus and of my family in Europe.

Katharina D.C. Stärk
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INTRODUCTION

Under intensive production systems pigs are kept at high density and slaughtered at a young age. Consequently, infectious diseases are more important than degenerative or proliferative problems. In a morbidity and mortality survey of swine conducted in the United States in 1990/1991 scours alone accounted for 58% of all observed conditions and illnesses (*Anonymous*, 1992). The course of infectious diseases is particularly severe in young piglets where most of the mortality is due to infectious pathogens (Wegmann, 1990; Zimmer *et al.*, 1997). Infectious diseases cause great losses to the producer and are important also from the animal welfare perspective. Additionally, drugs used to treat infectious diseases in pigs, if not properly applied, have the potential to remain as residues in meat destined for human consumption. For these reasons the prevention of infectious diseases is of great interest not only to the pig producer but also to the consumer. In the United States according to a major study, 57.2 % of farmers vaccinate all piglets against at least one infectious pathogen and 77.5 % vaccinated all sows (*Anonymous*, 1992).

If prevention is not possible or is not successfully achieved, infectious diseases have to be treated using medication. In the above cited US survey, 18.8% of farmers used oral antibiotics and 32.7% injected antibiotics to prevent and treat infectious diseases in piglets (*Anonymous*, 1992). Additional interventions addressing changes in housing or management may also be effective. As such interventions are expensive, they need to be based on sound epidemiological knowledge. A detailed understanding of the risk factors involved and their interaction is required. However, the number of relevant risk factors may be large and the most appropriate intervention difficult to select.

For each country, two categories of infectious diseases can be distinguished in relation to pig health: endemic and exotic diseases. An endemic disease is defined as a disease that is constantly present with only relatively minor fluctuations in its frequency within a defined geographic area or population (Last, 1988; Smith, 1991). Typically, there is no official control programme for endemic diseases, and it is up to the individual farmer and his/her veterinary consultant to plan therapeutic or preventive interventions or both.

If an infectious pathogen is not normally present in a region or a country, it is called exotic and any outbreak of the disease will qualify as an epidemic, i.e. provoke an occurrence exceeding the expected frequency (Smith, 1991). Most countries will adopt an eradication strategy for exotic diseases, particularly if the disease is harmful to humans (zoonosis) or will cause significant production losses. If a disease will prompt trade restrictions from trade partners who are free from the disease, eradication is also the strategy of choice. New occurrences of highly contagious diseases that are easily transmitted between animals and between farms are considered particularly serious. Because pigs are social animals, which are typically housed in groups, disease spread can be rapid and hard to prevent. Therefore, highly contagious exotic diseases require fast and powerful response coordinated by the veterinary services at national or regional level. Particularly in areas with high pig densities, a delayed response can have devastating consequences and disrupt production for several months (Davies, 1995). Obviously, an epidemic of such size will have severe direct and indirect economic consequences, out of which the actual costs of measures for the eradication of the disease will probably be a relatively small part (Vantemsche, 1995). Drastic measures such as the compulsory stamping-out strategy, where the entire stock of an infected farm is destroyed, may therefore be justified. Losing all stock, particularly breeding stock, is a tragedy for the individual farmer. The media and the public in general will keep a close watch over how the epidemic is

addressed and whether the 'right' decisions are being made. However, making the right decision under pressure is difficult, particularly if a new disease has been introduced or if a disease has not occurred for a long time, and there is a lack of knowledge and relevant expertise in the veterinary services.

With both endemic and exotic diseases, effective decision making is a crucial component of disease control. At the same time, decision making is potentially difficult, because the consequences of a particular decision are not known with certainty. The objective is to choose the best option amongst a number of possible decisions given the current circumstances, either on a farm or during an epidemic. Decisions have to be justifiable in terms of their efficacy and cost-effectiveness. In order to be able to make an informed choice, all information that is available should be considered and used as the basis. Because the amount of information may be large and complex, computer-aided decision support is likely to accelerate and improve the decision process by making information accessible. Today, computers are used to assist human experts in a vast number of areas. They are used to collect, record, store, retrieve and display data (Teichroew, 1993), but also to perform more demanding tasks such as data processing and data interpretation for reasoning. Such advanced information systems have also been developed in the veterinary field (Morris, 1991). A good overview is provided in an edition of the *Revue scientifique et technique de l'Office international des Epizooties* dedicated to epidemiological information systems (1991, issue no. 1).

In this thesis, the term 'information system' is used in the sense of being a structured approach to the definition and solution of a problem as defined by Morris (1991). Information systems are designed with the objective to support decision-makers. The user will work with a computer in order to provide data or models to "recognise, understand and formulate a problem and make use of analytical aids to evaluate alternatives" (Klein and Methlie, 1995).

In this thesis, the case of endemic and exotic infectious diseases in pigs is being considered. In each area, one disease was selected as an example. Respiratory diseases are used as a typical endemic disease problem complex (PART 1 of the thesis) and classical and African swine fever are used to illustrate the case of exotic diseases (PART 2 of the thesis). Respiratory diseases, more specifically enzootic pneumonia and pleuropneumonia were selected because they are equally important in all intensive pig-producing countries worldwide (Christensen and Mousing, 1992). Also, the causal web of factors related to respiratory diseases is complex, so that choosing the right strategy for an intervention is not straightforward. African and particularly classical swine fever (CSF) were chosen because swine fever is currently the single most devastating exotic pig disease in Europe. Huge outbreaks of CSF have destroyed significant parts of the national pig populations in Belgium, Germany and the Netherlands. The control of CSF is difficult because the disease can remain unnoticed for considerable time periods resulting in large numbers of secondary outbreaks due to unrestricted movements. The amount of data related to these outbreaks is likely to quickly become unmanageable without the help of computerised information technology.

The literature related to both diseases is reviewed in CHAPTERS 1.1 and 2.1. Because respiratory diseases are also airborne diseases, the literature on this additional aspect is also covered in CHAPTER 1.3. After having reviewed the available knowledge on the epidemiology of the example diseases, additional studies were conducted to complete the information. Two levels were considered: within-farm spread and between-farm spread.

With respect to between-farm spread, a key aspect of disease transmission is the movement of people, animals and goods between farms. These can be numerous (see CHAPTER 2.5) and when a disease outbreak is investigated, the farmer will probably not be able to remember all contacts. The movement of susceptible animals is of greatest risk. In order to be able to trace these movements, animals need to be individually identifiable. Without accurate animal identification, it is impossible to verify movements of animals between farms. For this reason compulsory identification of pigs has been introduced in many countries. Part of the current discussion on this topic involves issues related to the practicality and reliability of the current identification system for pigs. This question has been specifically addressed in a series of field trials described in CHAPTER 2.4. Although the chapters on contacts between farms and animal identification are in PART 2 of the thesis dedicated to exotic diseases, they are equally relevant and applicable to endemic diseases. There is some overlap between the areas related to endemic and exotic diseases.

In addition to observational field studies and questionnaire surveys, the relatively new technique of expert knowledge elicitation was used to obtain data on the epidemiology of infectious diseases (CHAPTER 2.3). Experiments to investigate the possibility of aerosol transmission were conducted for both diseases (CHAPTERS 1.4 and 2.2). Where the conduct of studies was not possible, simulation models were developed to identify critical points where our understanding of disease transmission is still incomplete (CHAPTER 2.8). State-of-the art techniques for data analysis were used to make the results available for use in an information system. A series of non-parametric techniques were also applied to investigate their potential in the analysis of complex data sets (CHAPTER 1.5). Finally, two decision-support systems were developed.

RestiMATE is a diagnostic guide to assist decision-makers in the control of respiratory diseases on individual farms. It uses data from a field survey (CHAPTER 1.2) and expert knowledge to assess the respiratory health status of a farm and to provide advice on effective interventions (CHAPTER 1.6). EpiMAN-SF (CHAPTER 2.6) is a more complex system designed to support veterinary services in containing and eradicating swine fever epidemics. Its expert system components are described in detail in CHAPTER 2.7. The use of EpiMAN-SF as an analytical tool is illustrated using data from a German classical swine fever outbreak in CHAPTER 2.9.

References

Anonymous (1992). National swine survey: Morbidity/mortality and health management of swine in the United States. National Animal Health Monitoring System, USDA:APHIS:VS, Fort Collins, CO.

Christensen, G. and Mousing, J. (1992). Respiratory System. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, and D.J. Taylor (eds.) Diseases of Swine, 7th Edition. Iowa State University Press, Iowa, 128-162.

Davies, G. (1995). An overview of recent epidemics of animal diseases. In: Diijkuizen, A.A., and Davies, G. (eds.) Animal health and related problems in densely populated livestock areas of the Community. Office for Official Publications of the European Communities, Luxembourg, EUR 16609, 35-40.

Klein, M.R., and Methlie, L.B. (1995). Knowledge-based decision support systems with applications in business. John Wiley & Sons, Chichester.

Last, J.M. (1988). A dictionary of epidemiology. Oxford University Press, Oxford.

Morris, R.S. (1991). Information systems for animal health: objectives and components. Revue scientifique et

technique de l'Office International des Epizooties 10:13-23.

Smith, R.D. (1991). Veterinary clinical epidemiology. Butterworth-Heinemann, Boston.

Teichroew, D. (1993). Information system. In: Ralston, A., and Reilly, E.D. The Encyclopedia of Computer Science 3rd Edition, Van Nostrand Reinhold Co., New York, 666-669.

Vantemsche, P. (1995). Classical swine fever 1993-1994 Belgium. In: Diijkuizen, A.A., and Davies, G. (eds.) Animal health and related problems in densely populated livestock areas of the Community. Office for Official Publications of the European Communities, Luxembourg, EUR 16609, 69-79.

Wegmann, P. (1990). Pathology of swine – a portrait of economic loss in pig production in Switzerland. Proceedings of the 11th IPVS Congress, Lausanne, 295.

Zimmer, K., Zimmermann, Th., and Heß, R.G. (1997). Todesursachen bei Schweinen. Der praktische Tierarzt 78:772-780.

PART I

Endemic infectious diseases

Example: Respiratory diseases

CHAPTER 1.1

EPIDEMIOLOGICAL INVESTIGATION OF THE INFLUENCE OF ENVIRONMENTAL RISK FACTORS ON RESPIRATORY DISEASES IN SWINE – A LITERATURE REVIEW

1. Summary

In this chapter, the influence of environmental factors on respiratory diseases in pigs is reviewed from an epidemiological point of view. The suitability of methods for the investigation of risk factors is discussed including aspects of study design, case definition, exposure assessment and data analysis. The results of published studies suggest a causal web of factor interaction, the analysis of which provides considerable challenge for current epidemiological techniques. New approaches to the problem, such as knowledge-based data interpretation systems, should be further explored in the future in order to provide reliable advice to decision makers.

2. Introduction

Respiratory diseases are a common problem in swine populations world-wide (Blaha, 1992; Christensen and Mousing, 1992). Most prevalent are pneumonia, pleuropneumonia and pleurisy commonly with multiple infectious agents contributing (Goiš *et al.,* 1980; Morrison *et al.,* 1985; Ciprian *et al.,* 1988; Falk *et al.,* 1991; Amass *et al.,* 1994). Generally, the morbidity of these diseases is high, while mortality is variable depending on the causative agents involved. There are two major subsets of lung disease within the respiratory disease complex: a high proportion of farms with respiratory problems are infected with *Mycoplasma (M.) hyopneumoniae* plus secondary invaders, while a somewhat lower proportion have disease due to *Actinobacillus (A.) pleuropneumoniae.* For this reason, these two agents deserve special attention and will be treated accordingly in this review.

The economic impact of respiratory diseases is considerable, due mainly to reduced growth and feed efficiency (Huhn, 1970; Goodwin, 1971; Braude and Plonka, 1975; Hoy *et al.*, 1987; Pointon *et al.*, 1985; Straw *et al.*, 1989; Straw *et al.*, 1990) and possibly reduced fertility (Hoy, 1994). In fact, respiratory diseases are still among the most devastating diseases in intensive swine production (Guerrero, 1990). The economic impact has been found to be more severe if respiratory disease occurs early in life (Wallgren *et al.,* 1990, 1993a; Morris *et al.,* 1995a) or is aggravated by other diseases (Bernardo *et al.*, 1990) or an adverse environment (Done, 1990a; Straw, 1991).

Besides the economic aspect, the animal welfare side of the problem has been addressed (Blaha, 1993) and public health concerns with regard to the syndrome have been raised. It has been shown that the probability of pigs being treated is higher among animals with respiratory disease (Willeberg *et al.*, 1978; Madsen, 1980; Elbers *et al.*, 1992a; Singer, 1993, Blaha *et al.*, 1994), particularly in the finishing period. Blaha *et al.* (1994) consequently postulated that the detection rate of antimicrobial residues is also expected to be higher in carcasses with lung lesions, as compared with others without pathological abnormalities.

Finally, the environmental factors causally related to respiratory problems in swine may also have a negative effect on the health of people working on pig farms (Larsson *et al.,* 1992). Increased incidences of respiratory conditions in swine confinement building workers (Cormier *et al.*, 1991) and veterinarians (Tielen *et al.*, 1996) have been reported.

Numerous attempts have been made to control respiratory problems by different methods (MacInnes and Rosendal, 1988; Zimmermann, 1990; Straw, 1992; Wallgren *et al.*, 1993b; Plonait and Gindele, 1995). The development of specific pathogen free (SPF) herds has been the most effective (Christensen and Mousing, 1992; Kuiper *et al.*, 1994; Bækbo *et al.*, 1996), although many of these herds have become re-infected at a later stage due to airborne disease transmission (Goodwin, 1985; Jorsal and Thomsen, 1988; Stärk *et al.*, 1992a). Besides eradication, various control measures designed to reduce infection pressure within the herd, e.g. management changes, medication and vaccination, have been applied (Christensen and Mousing, 1992).

Since the early seventies, it has been suggested that respiratory diseases in swine may be influenced not just by the presence of specific organisms but by a rather complex interaction between a number of factors related to the agent, the host and the environment (Kalich, 1970a, 1970b). Knowledge of these factors is essential for disease control and prevention (Hoy *et al.*, 1987). Epidemiological methods have helped to identify the most important risk factors and to investigate their interaction. The methods applied and the results of these studies are reviewed in this article.

3. Methods

The causal relationship between environmental risk factors and respiratory diseases is complex. One of the reasons for this is the possibility of direct and indirect effects of such factors on the respiratory system of the pig (FIGURE 1). A factor could have one or both effects. Using the concept of causal inference described by Rothman (1986) there are a number of so-called component causes involved in any disease process. A set of such components constitutes the minimal conditions required to start the disease and represents a 'sufficient cause'. For a given outcome, there may exist several sets of sufficient causes and consequently a large set of possible factor constellations having a similar effect, in this case influencing respiratory disease in swine. Within a sufficient cause interaction of factors may occur, thus changing the impact of a given factor depending on the level of another factor.

The objectives of epidemiological studies in this context can be: 1) identification of risk factors, 2) quantification of risk factor influence and 3) quantification of risk factor interaction and assessment of possible confounding.

In order to be able to achieve these goals, epidemiological studies have to be planned carefully. A number of requirements have to be met for the results to be valid.

3.1 Study design and sample size

Done (1991) lists alternative study designs for the investigation of the relationships between risk factors and respiratory diseases in swine. Basically, these are:

1) Assessment of the health status of animals and of the environmental status of the farm of origin, either subjectively or based on physical measurements at one point in time to investigate associations (cross-sectional approach, case-control studies).

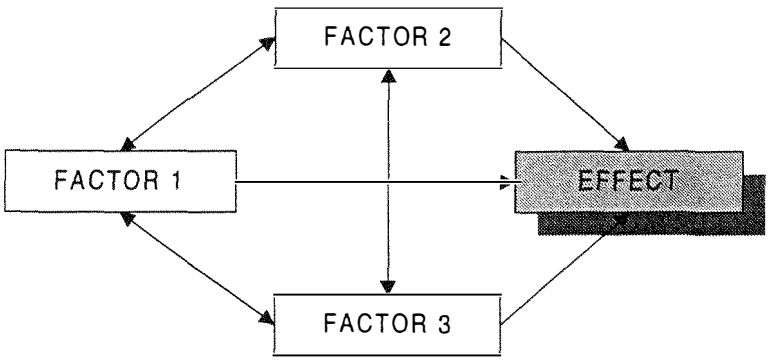

FIGURE 1. Causal web based on relationships between several risk factors

2) Monitoring of the situation on a farm(s) over a longer time period, collecting data on a number of putative risk factors and assessing their relationship with health measures (longitudinal approach).

3) Experimental exposure of animals in a controlled environment (experimental approach).

The largest group of the studies reviewed in this chapter used the first approach because it allows rapid identification and quantification of risk factors under field conditions. The longitudinal approach is not often used as it is more time- and resource-consuming. Results from experimental studies are hard to translate to field conditions and are therefore not well suited for this particular problem.

The second question when planning a study is: How many farms or animals are needed to detect a significant effect of a risk factor? The necessary sample size in observational studies basically depends on the following considerations:

- what difference between groups is relevant to be detected
– what is the prevalence of exposure
– what are the test characteristics
– what power requirements (1- β error) are needed
– what level of confidence (α error) is desired

If these figures can be estimated, the necessary sample size can be calculated using the formulas described in sampling text books (for example, Cannon and Roe, 1982).

FIGURE 2 shows the number of study units used in the publications reviewed in this article. More than 50% of the studies used less than 50 epidemiological units (farms or animals). The consequence of this is a limited power to detect differences between groups. This has to be acknowledged when interpreting results. Particularly negative results are difficult to interpret with low-powered studies. This problem has been long recognised and widely discussed in the literature, yet, logistical and financial limitations sometimes prevent obtaining larger samples.

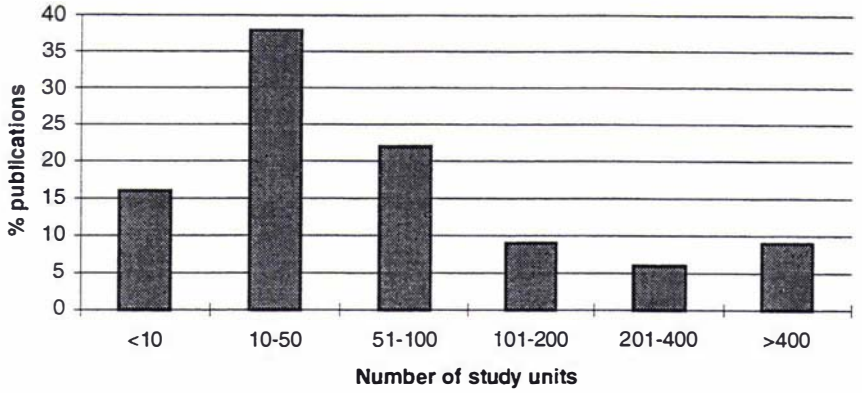

FIGURE 2. Distribution of number of study units in reviewed articles on respiratory diseases in pigs (n=32)

3.2 Case definition

When dealing with respiratory diseases, a case can either be an infected farm or an individual animal. In order to identify a case or a non-case, the disease needs to be diagnosed. It is relevant whether the outcome variable will be the occurrence of disease only (diseased vs. non-diseased, incidence, prevalence) or whether the severity of cases should also be measured. Not all of the following diagnostic approaches are good indicators of the latter.

Basically, four diagnostic approaches can be used to define a case of respiratory disease: definition by clinical signs, by serological analyses, by microbiological investigation or by lung scoring at slaughter or during *post mortem* investigations.

3.2.1 Diagnosis based on clinical signs

Respiratory diseases are commonly accompanied by the typical clinical signs of coughing, which can be used to estimate disease prevalence by defining a 'cough index' (Straw *et al.*, 1986a; Bahnson *et al.*, 1994). This system used on its own seems likely to miss cases because under good environmental conditions subclinical disease may develop (Keller, 1976). Applied at a herd level, clinical inspection failed to detect 30% of infected herds (Sørensen *et al.*, 1993). Morris *et al.* (1995a) reported a sensitivity of clinical cough of 37.7 % in market pigs when compared with gross lesions at slaughter but a comparatively high specificity (76.3 %). Coughing is also not considered to be a good indicator of severity (Straw *et al.*, 1990), although the inclusion of clinical parameters in other measurement systems has proved to be helpful. Bahnson *et al.* (1994) for example, were able to predict optical density measurements of an *M. hyopneumoniae* ELISA system at slaughter by using among others a cough index and the time of onset of coughing as explanatory variables. The use of veterinary treatment has been used as an indirect measure of clinical disease (Elbers *et al.*, 1992a), but is likely to be biased due to the influence of the farmer and the veterinarian.

3.2.2 Diagnosis based on antibody detection

Detection of antibodies is suitable both for clinical and subclinical cases. However, the dynamics of the disease as well as the test parameters (sensitivity, specificity, predictive values) have to be considered when interpreting test results.

Blood antibodies against *M. hyopneumoniae* and *A. pleuropneumoniae* rise 2-4 weeks after infection, peak at around 11-14 weeks *post inoculationem (p.i.)* and disappear about 6 weeks later (Bachmann, 1972; Strasser *et al.*, 1992; Yagihashi *et al.,* 1993; Kobisch *et al.*, 1993; Le Potier *et al.,* 1994; Sitjar *et al.*, 1994; Morris *et al.*, 1995b; Sørensen *et al.*, 1997). Under field conditions antibody levels against *A. pleuropneumoniae* rise from the age of 12 weeks with a peak at 23 weeks (Willson *et al.*, 1987; Gardner *et al.,* 1991; Sitjar *et al.,* 1994). The situation is similar for *M. hyopneumoniae* with a peak at 12-14 weeks of age and about 4 weeks after peak of clinical signs (Sitjar *et al.,* 1994). Seroconversion seems to coincide with the onset of coughing, but only moderate agreement between serology and occurrence of gross lesions at slaughter were reported (Falk and Lium, 1991; Morris *et al.,* 1995a; 1995b).

The tests of choice for detecting antibodies against respiratory diseases are indirect or blocking ELISA kits. With respect to *M. hyopneumoniae* the specificity of the ELISA can be reduced due to cross-reactions with *M. flocculare* or *M. hyorhinis* (Strasser *et al.*, 1992). Under field conditions, using a monoclonal blocking ELISA, the herd sensitivity and specificity were found to be 93-100% and 85.1-96%, respectively, if a farm was to be classified as infected with one out of 20 blood samples positive (Sørensen *et al.*, 1992, 1993). If the criterion was changed to two or more positive samples for an infected herd, sensitivity and specificity were 69% and 98%, respectively. The test characteristics for an *A. pleuropneumoniae* ELISA on a herd level were 89% for both sensitivity and specificity and 97% and 96% respectively on an individual animal level (Willson *et al.,* 1988).

A non-invasive method for antibody detection is the serology of colostrum samples (Zimmermann *et al.*, 1986; Volmer, 1994; Nielsen, 1995), but samples have to be collected immediately after farrowing, which may be unpractical because it requires constant monitoring of the sow. If colostrum samples are collected immediately after farrowing, positive ELISA test results are observed earlier and at a higher frequency than in serum among naturally infected pigs (Sørensen *et al.*, 1993) indicating a higher herd sensitivity of this technique. However, at least 30 colostrum samples per herd are required to obtain an accurate picture (Rautiainen *et al.,* 1996).

Another means for antibody detection is the analysis of saliva and fluid obtained by bronchoalveolar lavage for mucosal IgA which can be detected at an earlier stage of infection than humoral antibodies in blood serum (Loftager *et al.*, 1993).

Recently new techniques for serological detection of past infections have been developed. Frey *et al.* (1994) showed that antibodies against the species-specific L-lactate dehydrogenase of *M. hyopneumoniae* first occurred at 5 to 10 weeks *p.i.* when clinical signs and lung lesions were present. High titers persisted until 21 weeks *p.i.* which is much longer than antibodies against membrane proteins which are commonly used for diagnosis.

3.2.3 Diagnosis based on antigen detection

Respiratory disease agents can be cultured from nasal cavities of grower pigs based on samples collected using nasal swabs (Goodwin, 1972; Kume *et al.,* 1986; Willson *et al.*, 1987; Sørensen *et al.,* 1997) or from tracheobronchial lavages (Abiven and Pommier, 1993; Ganter *et al.*, 1993; Ganter, 1996) and cultured by microbiological techniques as used for culturing lung samples. Positive cultures have also been obtained in cases where no gross lung lesions were present (Goodwin, 1972). Culture is being used as the 'gold standard' in most comparative test evaluation studies and it is also the most sensitive method of detecting the infection at a late stage of the disease (Sørensen *et al.,* 1997).

As an indirect antigen detection method, immunofluorescence can be used to detect mycoplasmal infection with a specificity of 100% and a sensitivity of 59-100% until day 57 *p.i.* and 11-38% at 85 days *p.i.* (Sørensen *et al.*, 1994). Antigen-based ELISA tests are another alternative. Test characteristics for *M. hyopneumoniae* ELISA have been reported by Sørensen *et al.* (1994). In their experiment, the test was highly specific (93-100%). Sensitivity was also high between 14 and 57 days *p.i.* (66-100%) but dropped to 13-41% at 85 days *p.i.* It was concluded that positive predictive values were consistently high at comparable prevalence rates while negative predictive values of the assay decrease with infection stage.

Due to the fact that mycoplasma are very sensitive to culture media and slow-growing, polymerase chain reaction (PCR) will probably be used more frequently as a fast, sensitive and specific alternative for antigen detection (Sirois *et al.*, 1991; Mattson *et al.,* 1995). As little as the equivalent of the DNA of 1,000 organisms was required for a positive PCR result (Stemke *et al.*, 1994). PCR technique is also capable of rapid serotyping of *A. pleuropneumoniae* strains (Henessy *et al.*, 1993). Additionally, the examination of the genetic diversity of bacteria populations is becoming an important tool for epidemiological analyses. Frey *et al.* (1992) used molecular-biological techniques to analyse mycoplasmal field strains and found chromosomal diversity in different geographic regions. This technique has the potential to support outbreak investigations. Another research group in Denmark (Møller *et al.,* 1992) applied multilocus enzyme electrophoresis to divide *A. pleuropneumoniae* strains into electrophoretic types (ETs). This classification system could be used to investigate the epidemiology of different actinobacillus clones.

3.2.4 Diagnosis based on gross pathological lung lesions (slaughter checks)

As a final means of case identification, respiratory diseases cause distinct gross pathological lesions detectable at slaughter (Christensen and Mousing, 1992). In most cases, *M. hyopneumoniae* and *A. pleuropneumoniae* may be cultured from these lesions (Christensen, 1981; Morrison *et al.*, 1985; Til *et al.*, 1991; Awad-Masalmeh and Köfer, 1993; Grest, 1995). Lesions of greater severity may be observed when both agents are involved (Yagishi *et al.*, 1984). Scoring systems for lung lesions have been described by various authors (Morrison *et al.*, 1985; Straw *et al.*, 1986b; Pointon *et al.*, 1990). The technique basically consists of visual estimation of the proportion of lung tissue affected with lesions and characterising the type of lesion observed. Possible errors using this rapid subjective method have been described to be related to approximations of the relative proportions of lung lobes involved. This results in overestimating the affected lung proportion by approximately 20% and the median by 2.4

percentage points (Davies *et al.,* 1995). In order to derive a herd diagnosis from a group of pigs, Morrison *et al.* (1985) recommended that assessing the percentage of lung involved and calculating a mean was more informative than allocating lungs to severity categories. However, calculating the prevalence of lungs affected without scoring them individually or just scoring the most severely affected lung was considered less time consuming and equally informative as detailed scoring. Similarly, Davies *et al.* (1995) suggested the use of prevalence and median or mean calculations while they found the maximal lung score to be biased by sample size. In order to identify a farm as having respiratory disease problems, several threshold values for the prevalence of gross lesions have been used in the literature. A case farm was defined as having more than 5% of pigs affected (Aalund *et al.,* 1976) or more than 10% (Hurnik *et al.,* 1994b). As such a clear cut limit may not be realistic Tielen *et al.* (1978) defined herds with respiratory disease problems as having a prevalence of >25% of gross lesions while farms without problems should have <10% of animals affected. The best option however, is not to use artificial grouping of farms but to employ analytical methods that allow the use of the exact prevalence for each farm.

When compared with histology and bacteriology as 'gold standards', Hurnik *et al.* (1993) found that the scoring of gross lesions had a sensitivity of 76% and 77%, respectively, and a specificity of 71% and 51%. A diagnostic system with such test characteristics is considered to be adequate for estimating pneumonia in research of naturally acquired disease (Davies *et al.,* 1995). Additionally, the repeatability of the procedure when applied by lay inspectors reached Kappa values of 95% when compared with the principal investigator (Hurnik *et al.*, 1993).

However, only 1/3 of the lesions from early infection will still be active at the time of slaughter (Fellström and Wallgren, 1990; Wallgren *et al.*, 1994) and a large proportion will have completely resolved (Wallgren *et al.*, 1990; Sitjar *et al.*, 1994). *M. hyopneumoniae* has also been isolated from grossly normal lungs (Goiš *et al.,* 1980). Armstrong *et al.* (1984) therefore recommended that final diagnosis should be based on a laboratory follow-up.

Sitjar *et al.* (1994) concluded that slaughter checks are a poor indicator of lifetime pneumonia. Some authors consider it necessary to complement such data with other measurements such as growth rate and serological results in order to be a good instrument for assessing respiratory status over the entire rearing period (Fellström and Wallgren, 1990).

The minimal sample size needed when inspecting lungs from batches of pigs at slaughter has been discussed by several authors. There is a consensus that at least 30 lungs need to be inspected in order to reliably estimate prevalence and severity of pneumonia on a herd level (Straw *et al.,* 1986b; Pointon *et al.,* 1990; Davies *et al.,* 1995).

In conclusion, choosing a reliable yet practical 'gold standard' for case definition is not straightforward. The stage of infection and the characteristics of the diagnostic method (sensitivity, specificity) have to be taken into account. A combination of different techniques is suggested in order to provide meaningful results (Fellström and Wallgren, 1990; Morris *et al.*, 1995a). The use of different diagnostic techniques make the comparison of results between studies more complex.

3.3 Exposure definition and measurement

Potential risk factors for respiratory disease occurrence include environmental and managerial variables. Information characterising management factors are most readily collected using questionnaires, which can be completed by the investigator during a farm visit (Lindquist, 1974; Bäckström and Bremer, 1978; DiFranco *et al.*, 1989). A second possibility is the use of mailed questionnaires completed without supervision by the farm manager (Aalund *et al.*, 1976; Rosendal and Mitchell, 1983; Mousing *et al.*, 1990; Hurnik *et al.*, 1994a). For both alternatives, general guidelines for questionnaire design should be followed (see for example, Oppenheim, 1992). Environmental factors can be recorded using a similar methodology.

Careful interpretation of questionnaire data is particularly necessary if factors cannot be physically measured but are recorded by subjective judgement. Examples of such factors are hygiene, stress or air quality which are difficult to describe objectively.

Whenever possible, environmental factors should be measured using instruments. Some factors such as air temperature and relative humidity are relatively simple to assess, while others are more difficult, because high variability may occur over time, even within the same location (Hartung, 1994). In some studies air quality monitoring has been conducted using data loggers (Bauck *et al.*, 1990; Robertson *et al.*, 1990; Elbers *et al.*, 1992b). The monitoring of airborne bacteria is technically difficult and therefore has been used rarely. Nevertheless, different techniques for air sampling of bacteria have been described (Bauck *et al.*, 1990; see CHAPTER 1.3).

3.4 Data analysis

Given the fact that data collection is the most expensive part of any observational study, data analysis should be planned such that best possible use is made of the data. Unfortunately, the full potential of statistical techniques are being understood and utilised by the minority of authors.

Most published studies are based on the comparison of two or more groups of farms or animals. Where no comparison with a control group was reported, the observations become difficult if not impossible to interpret for the reader. As a first step of the analysis, the association between individual risk factors and the outcome variable is a good screening strategy. Results can be reported descriptively but should also include statistical tests for significant differences between groups. In order to quantify the influence of individual risk factors, the calculation of risk ratios (relative risk or odds ratio) is a basic yet not widely used technique. Out of 32 reviewed studies only 28% provided quantitative results in terms of either odds ratio or relative risk measures. Techniques for calculating attributable risk and therefore preventable risks were seldom used.

A more comprehensive approach is the use of multivariate techniques. They allow to distinguish different degrees of importance between multiple factors as well as to control for possible confounding. Given the complex structure of interaction between risk factors of respiratory diseases the use of multivariate techniques is even more important as the resulting models contribute significantly to the understanding of the epidemiology of these diseases. Multivariate causative models were developed in 45% of the papers included in this evaluation.

Recently, further techniques such as survival analysis (Vraa-Andersen, 1991; Thomsen *et al.*, 1992) and factor analysis (Hurnik *et al.*, 1994a) have been used. Nevertheless, quantitative information on risk factors is still scarce despite the relatively large number of published papers.

4. Results and discussion

Results of epidemiological investigations of respiratory disease risk factors have been reviewed in the literature by other authors (Whittlestone, 1976; Done 1991). An overview of the factors included in the publications reviewed in this article are shown in TABLE 1.

TABLE 1. Factors influencing respiratory disease occurrence or the incidence of re-infection of respiratory disease-free herds

FACTOR GROUP	DESCRIPTION	REFERENCES
Herd characteristics	Herd size	Aalund *et al.*, 1976; Bäckström and Bremer, 1978; Tielen *et al.*, 1978; Flesjå and Solberg, 1981; Bahnson *et al.*, 1990; Mousing *et al.*, 1990; Elbers, 1991; Hurnik *et al.*, 1994a, 1994b; Mousing, 1991; Hofer 1993; Tuovinen *et al.*, 1997
	Air volume, shared airspace	Lindquist, 1974; Bäckström and Bremer, 1978; Tielen *et al.*, 1978; Tuovinen *et al.*, 1990; Stärk *et al.*, 1992a; Hurnik *et al.*, 1994a, 1994b; Skirrow and Nicholls, 1994; Skirrow *et al.*, 1995; Cargill *et al.*, 1996
	Stocking density	Lindquist, 1974; Bäckström and Bremer, 1978; Tuovinen *et al.*, 1990; Cargill *et al.*, 1996; Jensen and Blaha, 1997
	Diarrhoea	Aalund *et al.*, 1976; Willeberg *et al.*, 1978
	Sow characteristics	Hoy *et al.*, 1987; Vraa-Andersen 1991
	Herd type (breeding vs. fattening)	Mousing, 1991; Stärk *et al.*, 1992a; Hofer, 1993
Management	Purchase policy	Aalund *et al.*, 1976; Bäckström and Bremer, 1978; Tielen *et al.*, 1978; Flesjå and Solberg, 1981; Rosendal and Mitchell, 1983; DiFranco *et al.*, 1989; Thomsen *et al.*, 1992; Elbers, 1991; Hurnik *et al.*, 1994a, 1994b; Maes, 1997
	Production system (all-in/all-out vs. batch vs. continuous)	Lindquist, 1974; Bäckström and Bremer, 1978; Tielen *et al.*, 1978; Flesjå and Solberg, 1981; Scheidt *et al.*, 1990; Clark *et al.*, 1991; Hurnik *et al.*, 1994a, 1994b
	Construction of building/pen	Tielen *et al.*, 1978;; Flesjå *et al.*, 1982; Elbers, 1991; Elbers *et al.*, 1992b; Hurnik *et al.*, 1994a, 1994b; Morris *et al.*, 1995b
	Manure handling	Lindquist, 1974; Bäckström and Bremer, 1978; Flesjå *et al.*, 1982; Tuovinen *et al.*, 1997
	Feeding technique	Hurnik *et al.*, 1994a, 1994b
	Access to water	Bäckström and Bremer, 1978; Flesjå *et al.*, 1982; Tuovinen *et al.*, 1990; Hurnik *et al.*, 1994a, 1994b; Tuovinen *et al.*, 1997

FACTOR GROUP	DESCRIPTION	REFERENCES
	Ventilation	Aalund *et al.*, 1976; Bäckström and Bremer, 1978; Flesjå *et al.*, 1982
	Draught	Scheepens *et al.*, 1991
	Bedding, floor	Tielen *et al.*, 1978; Tuovinen *et al.* 1990; Flesjå *et al.*, 1982; Hurnik *et al.*, 1994a, 1994b; Jensen and Blaha, 1997; Tuovinen *et al.*, 1997
	Light	Tuovinen *et al.*, 1990
	Heating	Elbers, 1991; Jensen and Blaha, 1997
	Hygiene	Bäckström and Bremer, 1978; Hoy *et al.*, 1987; Tuovinen *et al.*, 1990; Elbers, 1991
	Characteristics of manager	Bäckström and Bremer, 1978; Tuovinen *et al.*, 1990; Elbers, 1991
	Time dependent management factors (weaning, moving)	Gardner and Hird, 1991; Vraa-Andersen 1991
	Movement of animals	Tielen *et al.*, 1978; Hurnik *et al.*, 1994a, 1994b
	Veterinary consultation	Elbers, 1991; Hurnik *et al.*, 1994a, 1994b
Air parameters	Temperature	Gordon, 1963; Geers *et al.*, 1989; Done, 1990b; Stärk *et al.*, 1992b; Goodall *et al.*, 1993; Tuovinen *et al.*, 1997
	Humidity	Gordon, 1963; Done, 1990b; Stärk *et al.*, 1992b
	Gases	Donham, 1991; Tuovinen *et al.*, 1990
	Bioaerosols	Donham, 1991; Awad-Masalmeh and Köfer, 1993; Skirrow *et al.*, 1995
	Dust	Donham, 1991; Skirrow *et al.*, 1995
	Season	Christensen, 1981; Cowart *et al.*, 1991; Goodwin, 1985, Awad-Masalmeh and Köfer, 1993; Goodall *et al.*, 1993; Jensen and Blaha, 1997; Maes, 1997
Neighbourhood	Distance to possibly infected farm	Goodwin, 1985; Thomsen *et al.*, 1992; Stärk *et al.*, 1992; Hurnik *et al.*, 1994a, 1994b
	Size of neighbouring farm	Goodwin, 1985; Thomsen *et al.*, 1992; Stärk *et al.*, 1992a
	Swine density in region	Goodwin, 1985; Stärk *et al.*, 1992a

The factors involved can be categorised as characteristics of the herd, the management, the air and the neighbourhood. As already explained, the abundance of factors influencing respiratory diseases and their interaction make it difficult to understand the epidemiology of the disease. In order to allow a more structured and general approach, in the following discussion the factors will be divided into different groups. It has been suggested that factors affecting the presence of a causative agent (infection pressure) can be distinguished from a range of other factors probably influencing the susceptibility of the individual animal and consequently the establishment of the infection (Christensen and Mousing, 1992; FIGURE 3). This approach has been adopted in the following paragraphs, however, factors can be involved in both areas at the same time, which complicates the situation.

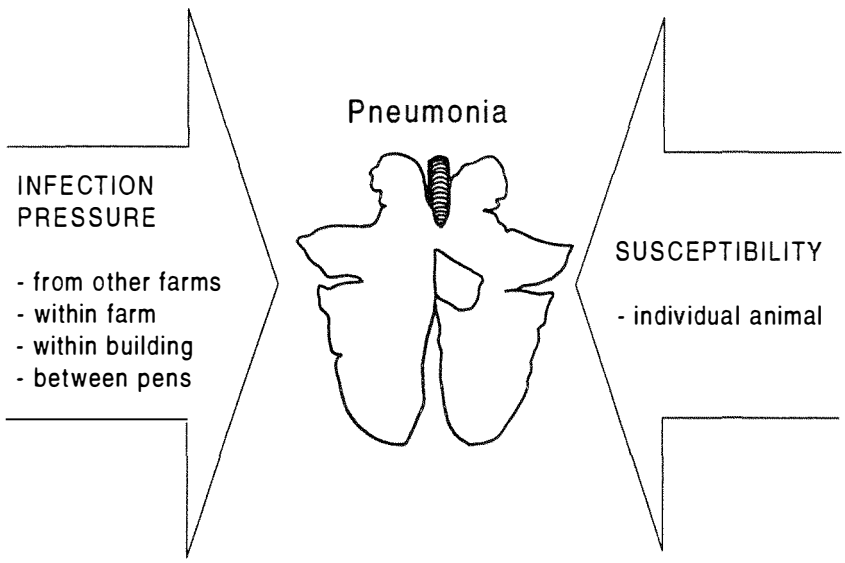

FIGURE 3. Alternative pathways for respiratory disease to be influenced by environmental risk factors

4.1 Infection pressure

The transmission of respiratory disease agents has been investigated using infection experiments. Nose-to-nose contact and the airborne pathway are the most common routes of natural transmission and will therefore be considered in the following reasoning.

Basically, three requirements have to be met for respiratory disease agents to be transmitted between animals: 1) excretion by infected animals, 2) critical concentration, and 3) contact between susceptible animals and causative agent.

As the causative agents of respiratory diseases are easily introduced into a farm with live animals, the purchase policy of a farm is one of the most important risk factors. Many authors have documented this association (TABLE 1). The general consensus of the results is that closed farms are at the lowest risk while exclusively fattening farms purchasing all pigs are at highest risk, particularly if they buy in from several sources or a market. A crude relative risk of 13.54 for farms purchasing all weaners as compared with farms with their own weaner production was reported by Aalund *et al.* (1976). Another study (Hurnik *et al.*, 1994b) identified a farm type with multiple source purchase policy and a low emphasis on disease entry to have an odds ratio of 2.38 for a respiratory diseases prevalence >10%. The number of pigs purchased at a time also seems to be important (Rosendal and Mitchell, 1983) as the propability of buying in one infected animal increases with the number of animals bought. Compared with farms with no purchases the odds ratio of *A. pleuropneumoniae* infection when purchasing 1-10, 10-100 or >100 pigs at a time was 0.197, 2.865, and 6.889, respectively in this study. The same authors also reported a negative influence of high frequency purchases (odds ratio weekly purchases vs. no purchases = 10.870) and purchasing from a salesbarn (odds ratio salesbarn vs. other sources = 4.691). As an alternative approach the protective ef-

fect of particular purchase policies were also calculated in the same paper. The odds ratios for a closed herd, quarantining new stock and purchasing health examined stock were 0.439, 0.830, and 0.700, respectively.

Different purchase policies are also reflected in different herd types, which may explain an indirect influence of this factor on respiratory health. Exclusively fattening farms have to buy-in all stock while breeding farms are less likely to buy large numbers of animals and from different sources. Consequently, fattening farms are at higher risk of respiratory diseases than exclusively breeding or mixed breeding-fattening farms. Hofer (1993) for example reported in a survey of antibodies against *A. pleuropneumoniae* 36.6% sero-positive fattening farms, 25.1% sero-positive mixed farms and 13.0% sero-positive breeding farms. A similar result was found by Mousing (1991) where fattening units had a risk of being actinobacillus-infected that was 1.57 times as high as for sow herds. Different herd types also present different demographic structures. This aspect will be discussed in the following section.

Besides herd type, herd size was commonly found to be an influential risk factor for respiratory diseases in swine. An increasing herd size was consistently described to increase the risk of respiratory disease which was quantified as relative risk or odds ratio in a number of publications. Aalund *et al.* (1976) calculated crude and adjusted relative risk measures with respect to the number of pigs produced during three consecutive years. They found a gradual increase of the crude risk with increasing number of pigs produced. When adjusting for other management factors the initial crude measures were generally reduced yet still distinct (TABLE 2). Because of the nature of this analysis only one factor could be controlled at a time.

TABLE 2. Influence of herd size on the frequency of respiratory lesions at slaughter expressed as odds ratios (Aalund *et al.*, 1976)

	Number of pigs produced 1969, 1970 and 1971		
	< 500	500-800	800-1,200
Unadjusted	1	10.50	13.50
Adjusted for:			
Weaner recruitment system	1	10.13	6.76
Ventilation system	1	11.87	10.24
Other diseases	1	8.20	10.67
Infectious diarrhoea	1	9.62	10.56

A less pronounced influence of herd size in mixed breeding-fattening farms was published by Bahnson *et al.* (1990; odds ratio = 1.28 per increase in herd size by 200 sows). Similar results were reported by Flesjå and Solberg (1981). They described an increasing prevalence for both pneumonia and pleurisy with increasing number of pigs slaughtered per year. However, they concluded that this effect was less important as an over-all risk factor than the type of rearing system (purchase of weaner pigs, all-in/all-out system) used on the farm. In a recent publication several particular farm types were identified as being at higher risk of respiratory diseases (Hurnik *et al.*, 1994a). One type is an integrated, larger-than-average farm that is also in close proximity of other farms. Such farms had a higher risk of having more than 10% pneumonia prevalence (odds ratio = 2.31, Hurnik *et al.*, 1994b).

One possible reason for the large effect of herd size is its influence on different areas such as disease dynamics (influence on disease introduction through purchase of infected animals or airborne infection; spread and maintenance within herd) and management (large herds being managed differently from small herds; Bäckström and Bremer, 1978; Willeberg *et al.*, 1994).

When considering airborne transmission not only infected animals on-farm act as infection sources but also nearby herds with respiratory problems. In searching for possible risk factors for re-infection of SPF farms with respiratory diseases the distance to the closest possibly infected farm, its size and the pig density in the area were found to be important factors. Increasing risk measures for increasing herd size and decreasing distance of the neighbour were reported (Stärk *et al.*, 1992a; Thomsen *et al.*, 1992). When looking at airborne particle dispersion models, it can be shown that factors such as source strength (herd size) and distance are important variables (Pasquill, 1961). Goodwin (1985) calculated a maximal distance for mycoplasma transmission of 3.2 km. These factors together with the size of the herd at risk have been successfully used to calculate risk indices to predict the risk of re-infection for a particular farm (Goodwin, 1985; Stärk *et al.*, 1992a; Thomsen *et al.*, 1992).

After release, infectious agents can be found as airborne or non-airborne particles. A number of management procedures are available in order to reduce their concentration. This is important as the chance of a contact between a susceptible animal and an agent increases when more particles are available in the environment. With respect to airborne particles, the number of pigs sharing the same air space in a building, the stocking density and consequently the air volume available per pig are factors influencing aerosol levels. One of the first authors to describe this in detail was Lindquist (1974). He stratified the farms in the study according to their herd size (<500 pigs produced per year vs. ≥500 pigs produced per year), production type (continuous vs. batches) and feeding system (floor vs. trough) and compared the frequency of gross lesions in pigs from these farms at slaughter. The results indicated that respiratory diseases were more prevalent in farms where there were ≥ 500 pigs in one section, < 3 m^3 air space per pig, and < 0.7 m^2 pen area per pig. The latter factor can be interpreted as an indirect indicator of stocking density. This effect was also quantified in a somewhat different study by Tuovinen *et al.* (1990). They estimated an odds ratio of partial carcass condemnation of 4.2 for a decrease of the total pen area per pig by 0.1 m^2. More than 3.5 m^3 of air volume per pig seemed to be preventive with respect to pleurisy (Flesjå *et al.*, 1982). The influence of the number of pigs per barn was confirmed by Tielen *et al.* (1978) as well as Elbers (1991), who observed a negative effect when there were >100 pigs in one compartment. Tuovinen *et al.* (1990) calculated an increase of the risk of partial carcass condemnation by 2.4 for every 50 additional pigs in the barn.

Ventilation has also a direct influence on aerosol levels and has been found to be related to respiratory diseases. Aalund *et al.* (1976) reported an unadjusted relative risk of 3.52 for use of ventilation shaft with fan and 3.60 for no ventilation shaft when compared with a ventilation shaft without fan. In another study it was observed that an air exchange rate of >60 m^3 per hour per pig had a protective effect on pneumonia (Flesjå *et al.*, 1982).

With respect to non-airborne infectious particles other management factors are important. Production systems where pigs are moved through the different production stages in batches (all-in/all-out) as opposed to continuous flow systems generally include cleaning and disinfecting steps which reduce micro-organism concentrations. In most reviewed papers the all-in/all-out system was highly advantageous in terms of respiratory disease reduction. Some

authors considered its influence to be even more important than herd size (Flesjå and Solberg, 1981).

Finally, a set of variables may influence the possibility of agents getting in contact with susceptible pigs within and between pens. These factors are mainly related to building characteristics. It was shown that >12 pigs per pen can have a negative effect on respiratory health (Flesjå *et al.*, 1982), which can again be interpreted as an indicator of stocking density. Solid pen walls that prevent contact between pens are preferable (Flesjå *et al.*, 1982; Hurnik *et al.*, 1994b). Morris *et al.* (1995b) showed that pigs in pens adjacent to *M. hyopneumoniae*-infected pigs and in nose-to-nose contact with them were 7 times as likely to sero-convert as other pigs. Also, compartments within a unit should be completely separated (Elbers *et al.*, 1992b).

4.2 Susceptibility

Within the susceptibility complex there are mainly two groups of factors to be considered: 1) air parameters, and 2) other diseases and stress in general.

The relevance of air characteristics such as temperature, humidity, dust, gases and airborne bacteria with respect to respiratory diseases has been less frequently investigated than over environmental risk factors. Together these variables characterise the microclimate of a building. If the climatic conditions in a pig building are unfavourable, the animals are exposed to climatic stress which influences their health (Scheepens, 1996). Temperature and draught are the main sources of climatic stress. However, quantitative data on the association between certain conditions and respiratory health in pigs is quite limited.

A number of studies describe the influence of cold air draughts on the immune system of pigs. It was shown that the immune response of pigs challenged with *A. pleuropneumoniae* was significantly influenced by draughts and fluctuating temperatures (Noyes *et al.*, 1986, 1990; Kreukniet *et al.*, 1990). However, the results were not conclusive as some indicators of the immune response appeared to be reduced while others were increased. This was interpreted as being the result of the different types of climatic stressors or differences in acclimation. Similar results were obtained by Scheepens *et al.* (1994) who interpreted climatic stress as a disturbance of homeostasis of the pig's immune system. Scheepens (1996) demonstrated that climatic stress also influences the behaviour of pigs. During periods of draught the activity level was higher than both before and after the intervention. An increased activity level is associated with higher dust levels, which increases the risk of aerosol transmission. Thus, climatic stress is likely to influence the occurrence of respiratory disease by both increasing the risk of exposure and by increasing the susceptibility.

Air temperature and humidity are closely linked variables. Gordon (1963) was one of the first authors to investigate the influence of temperature and humidity on the incidence of pneumonia. He compared two farms, farm A with a warm (25-30°C), extremely humid climate (>90%), and farm B with what he called a 'normal' environment. Farm A was a finishing unit, farm B a mixed breeding-fattening unit. The farms also differed in other areas such as ventilation and feeding practice. The results of this study showed a lower incidence and less severe lesions in the hot-humid environment. This finding led to the postulation of the 'sweat-house' climate as being beneficial for respiratory health in pigs. In a more standardised set-

ting, Geers *et al.* (1989) similarly observed a negative relationship between coughing and air temperature, i.e. the higher the temperature the less coughing occurred. Also, the number of necessary treatments for pneumonia was reported to be higher for low and highly variable temperatures (Done, 1990b). Relative humidity also correlated with treatment requirements but with a lag of 2 weeks. In the same study it was also found that the number of treatments was closely linked with stocking density, and as the microclimate partially reflects stocking density as well, it was difficult to disentangle the two factor groups. Earlier reviews on the influence of the thermal environment on pig respiratory diseases concluded that the effect of humidity and probably also temperature is mostly an indirect one (Whittlestone, 1976; Harrison, 1986) as these factors influence a number of other variables such as micro-organism survival and airborne particle concentration. The survival of airborne respiratory pathogens depends on air humidity and the number of respirable particles is also related to air humidity (see CHAPTER 1.3). Fišer and Král (1969) investigated the influence of air humidity on bacteria sedimentation rates. They found a significant difference between winter and summer with higher rates in the wetter season. They also showed the influence of feeding practice on particle concentration.

Another aspect of the microclimate is the occurrence of airborne gases, dust and micro-organisms. These factors have also been studied with respect to respiratory health in pigs. Donham (1991) found dust, ammonia, carbon dioxide and microbes correlated with the occurrence of pneumonia and pleurisy at slaughter. In this study, ammonia concentrations were highest in the fattening units. Ammonia reduces the ciliary activity in the respiratory tract (Straw and Wassom, cited by Bollwahn, 1989) which enhances the attachment of respiratory pathogens (Narita *et al.*, 1995). Donham subsequently calculated maximal recommended concentrations for air contaminants (TABLE 3). Smith *et al.* (1996) showed in a behavioural experiment that pigs showed distinct preference for fresh air when compared with ammionated air (100 p.p.m.). The conduct of environmental monitoring is therefore recommended to ensure an optimal climate for pig production and a healthy environment for swine confinement building workers (Bossow, 1995).

TABLE 3. Recommended maximal values for air contaminants in swine buildings (Donham, 1991)

Air contaminant	Recommended maximal level
Dust	2.4 mg/m^3
Ammonia	7 ppm[a]
Endotoxin	0.08 mg/m^3
Total microbes	105 colony-forming units/m^3
Carbon dioxide	1,540 ppm

[a]parts per million

Management factors influencing airborne particle concentrations are stocking density, ventilation, use of bedding, insulation, drinking water access, manure disposal method and feeding system (Bresk and Stolpe, 1975; Wathes *et al.*, 1983; Heber *et al.*, 1988; Bækbo, 1990; Hartung, 1994). Slatted floors and poor floor insulation (no bedding) were also described to have a negative impact on respiratory health (TABLE 1). However, the influence of these factors has not yet conclusively been quantified. Likewise, the magnitude of the adverse influence of

floor feeding, limited access to and delivery type of drinking water as well as the lack of heating and insufficient light intensity have not yet been conclusively established. The latter factor was only described by one author (Tuovinen *et al.*, 1990). Better documented is the importance of good hygiene. But again, quantitative evaluation of this risk factor is scarce, because objective measurements are difficult.

As indoor air parameters such as temperature and humidity are strongly influenced by outdoor conditions, it could be expected that respiratory diseases are also influenced by season, which can thus have an indirect impact on animal health (Webster, 1981; Heber *et al.*, 1988). The influence of weather factors on enzootic pneumonia as an example of a respiratory disease has been reviewed in detail by Whittlestone (1976). On a herd level, the risk of onset of clinical signs was described to be higher during the cold, wet season (Stärk *et al.*, 1992b). This finding is supported by high frequencies of pleurisy and atrophic rhinitis in slaughter pigs in summer that would have been infected during the colder season (Christensen, 1981; Cowart *et al.*, 1991). However, the contrary was found for pneumonic lesions (Awad-Masalmeh and Köfer, 1993). The latter is explained by the fact that pneumonic lesions may heal rapidly and only the latest infections are still visible at slaughter.

The susceptibility of pigs to respiratory diseases is also likely to depend on the general health and immune status of the animal. The influence of other diseases on the occurrence of respiratory diseases or vice versa may also be tentatively explained by changed immune status. Diarrhoea was identified as a risk factor for respiratory diseases (TABLE 1). However, this might be related to a common third factor influencing both respiratory and other diseases and therefore be caused by confounding (Willeberg *et al.*, 1978). General immunesuppressive factors are for example, selenium and vitamin E deficiency and mycotoxin- or heavy metal-contaminated feed (Bollwahn, 1989).

Gardner and Hird (1990) found that the age of pigs at weaning and birth weight were influential host factors, as well as possibly breed, all influencing the extent of pneumonic lesions at slaughter. Another management factor of influence is the number of shifts during the fattening period (Tielen *et al.*, 1978; Hurnik, 1994a). It was shown that the risk of getting infected with *M. hyopneumoniae* and *A. pleuropneumonia* is related to time dependent variables such as the age at weaning and the age at first and second moving (Vraa-Andersen, 1991). As the time since the last shift elapsed the risk for infection decreased. Whether this risk is due to stress during re-establishing the social groups or due to exposure to infectious agents in the new compartments cannot be specified. Hessing *et al.* (1994) demonstrated an association between social status of a pig and its immune response. Dominant pigs had significantly higher values in a lymphocyte proliferation assay than subdominant and subordinate pigs. Furthermore it was demonstrated that regrouping of pigs after weaning is an acute stressor (Blecha *et al.*, 1985). A somewhat similar hypothesis was proposed by Brumm (1997) with respect to the significance of pen group size. He reported that if a group is larger than 20-25 pigs, no stable social ranking among the pigs will occur resulting in increased fighting and probably social stress. This may explain the influence of pen group size on the occurrence of respiratory diseases.

4.3 Path model hypothesis

A different approach to the analysis of factor influence and interaction is the development of a path model hypothesis. The analysis of this structure reveals different types of relationships. Attempts to identify groups of interrelated factors are not new. Bäckström and Bremer (1978) suggested the term 'environmental syndromes' to describe clusters of risk factors. A possible outline of factor interactions is given in FIGURE 4. Certain factors are influenced by few other factors while having themselves an effect on a large number of other factors. Such factors are for example HERD SIZE and STOCKING DENSITY. On the other hand there are factors that are influenced by a large number of components but propagate their effect into a limited number of risk pathways. In this group are for example measures of airborne particle concentrations. Given the fact that the first group of factors is influenced by few other factors only, these parameters may be easier to alter and therefore more suitable for preventive interventions.

At this stage analysis of this web is only provisional. More rigorous techniques are necessary to provide more specific hypotheses which can then form the basis for further epidemiological investigations.

5. Conclusion

In a study setting, the number of farms that can be included is usually limited as compared with the number of factors that merit assessment. Lindquist (1974) observed 150 factor variants among 155 pig houses. Therefore, Jericho *et al.* (1975) concluded that any calculations made are applicable only to the particular conditions of the study and that transposing results to other farms is not appropriate. While this appears to be too pessimistic, the generalisation of results certainly needs to be applied with care. Negative results in particular always should be interpreted in the context of the study's power to detect differences. This is even more important because some parameters can only be subjectively assessed, causing additional bias of the estimated effects. The comparison of studies is difficult due to the large number of necessary decisions with respect to study design as well as exposure and outcome assessment. Quantitative techniques for literature review (meta anlysis) are therefore not applicable. Unfortunately, the traditional narrative literature review as applied in this chapter can be rightly criticised for its lack of objectivity (Peipert *et al.*, 1997). However, there does not seem to be an alternative available at this stage.

The abundance and broad range of different factors influencing the occurrence of respiratory diseases complicate decision-making on problem farms. Bollwahn (1989) developed a list of problem areas that should be examined when dealing with a multifactorial problem. He considered the following areas to be most influential:

Hygiene: cleaning, disinfecting, fly and rodent control, animal and person traffic, vehicles

Husbandry: climate, feeding, installations (pens, effluent system, troughs, ventilation etc.)

Management: stocking density, purchase policy, animal movements within a farm

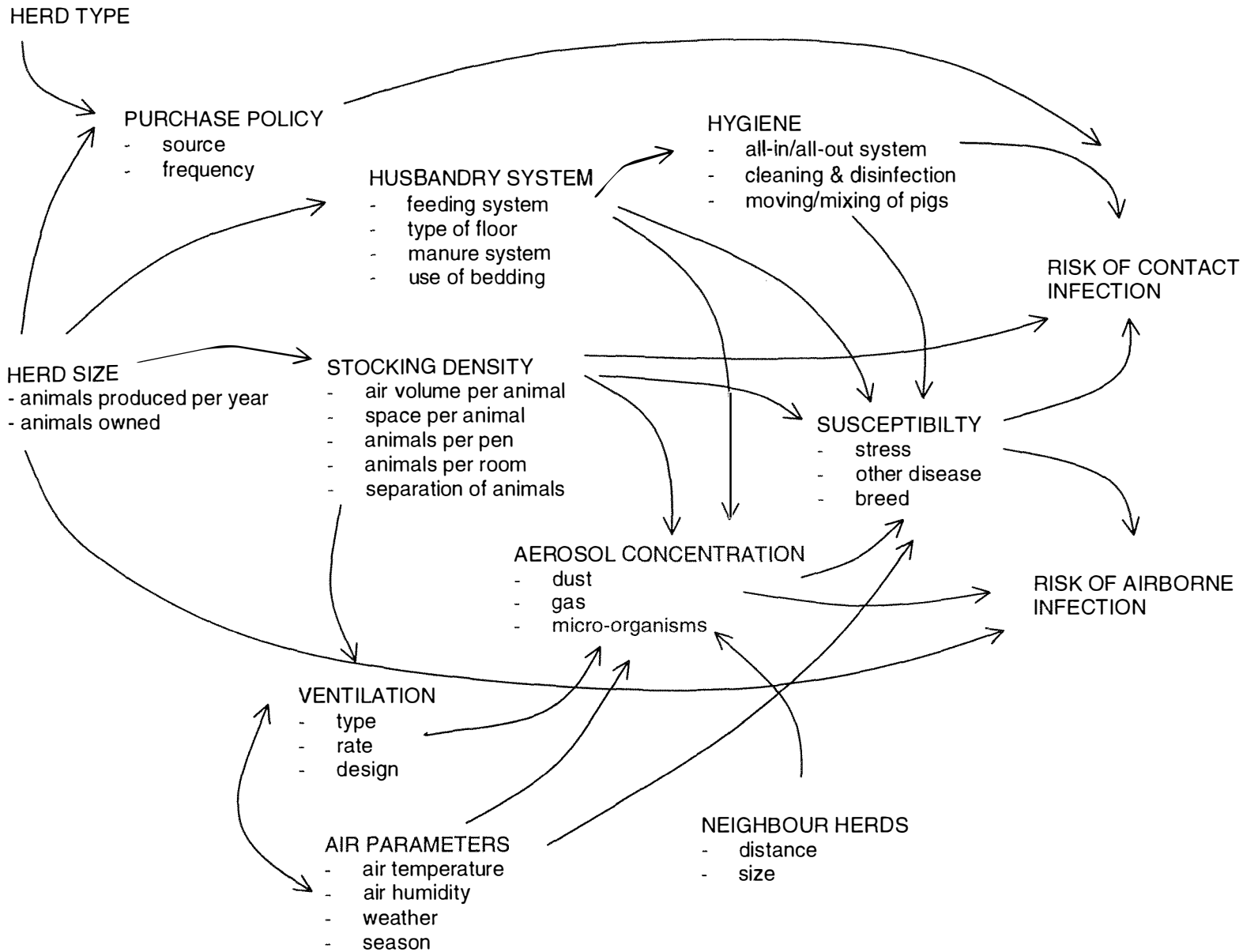

FIGURE 4. **Path model hypothesis for the associations between risk factors for respiratory diseases in pigs**

Only limited information is available as to the quantitative importance of single parameters. Because not all of the problem areas may be easily addressed, a careful evaluation of alternatives is required in order to provide helpful and realistic advice to farmers. Methods allowing quantification of the importance of a variable in comparison with others and discrimination between interaction and confounding should therefore be used whenever possible both in research and in the veterinary practice.

Christensen and Mousing (1992) have listed factors of potential influence and weighted their contribution to the overall risk. They considered the following to be the most important ones:

Large herd size, high stocking density, non-SPF production system, introduction of pigs of unknown health status, continuous flow of pigs through facilities, badly insulated or poorly ventilated facilities with improper regulation of temperature and drafts, lack of partitions and pigs of different ages sharing same air space, pen dividers without solid partitions, insufficient regulation of climate.

Morrison and Morris (1985) have developed a prediction guide for estimating the prevalence of lung lesions in slaughter swine originating from a particular farm. In their spreadsheet model, the authors included the following parameters:

more than 500 pigs in the same room, continuous flow type farm, introduction of pigs from outside herd, temperature fluctuation ratio, room is 12 m or more wide, nose to nose contact between pens, less than 1.25 m2 per 45 kg body weight, diarrhoea as clinical problem, liquid manure in pit beneath pigs, evidence of endoparasites, presence of active Aujeszky's disease virus.

Although this system was not designed to produce statistically valid predictions, it was considered to be a valid tool for education and illustration of the multi-factorial nature of the problem and a guide for on-farm problem solving.

More recently, Turner *et al.* (1993) developed a program called RESPIG which simulates and predicts respiratory disease prevalence and severity as well as the economic effects in relative to environmental factors. RESPIG is based on a state transition model where the probability of a transition is stochastically determined by Monte Carlo sampling from uniform random distributions. First the air quality on a farm is classified into one of 6 categories, then the disease process is simulated along with its effect on feed intake, feed conversion and growth. The authors suggest that this model may be useful in gaining insight into the epidemiology of respiratory diseases and in identifying gaps in knowledge.

An important factor that has not yet been looked at very intensively is the pig farmer. For example, some investigators collected data on a farmer's interest in disease prevention, education and similar characteristics. Bäckström and Bremer (1978) observed a higher interest in preventive measures in farmers with less respiratory problems in their herds. This type of farmer was also better educated (Tuovinen *et al.*, 1990) and more likely to be involved in continued education (Elbers *et al.*, 1992a). The consultation of veterinarians also seems to be relevant (Elbers, 1991; Hurnik *et al.*, 1994a)

Although a large number of studies has been conducted, many of the details of the epidemiology of respiratory diseases and the involvement of environmental factors in this process are still not fully understood. The results of some of the studies reviewed in this article have provided only limited insight into the causal relationships between single factors due to limited

sample size and inadequate design and/or analysis. However, with the objective of taking preventive measures and improving respiratory health and performance, the final proof of causality does not seem to be an absolute necessity. And it has been suggested that probably joint effects of factors are the most important ones (Lindquist, 1974).

In the future, new tools such as knowledge-based data interpretation systems could provide new approaches to more effective decision making and eventually to the prevention of respiratory diseases in swine herds.

References

Aalund, O., Willeberg, P., Mandrup, M., and Riemann, H. (1976). Lung lesions at slaughter: Associations to factors in the pig herd. Nordic Veterinary Medicine 28:487-495.

Abiven, P. and Pommier, P. (1993). Tracheobronchial lavage by the intranasal route for the detection of *Mycoplasma hyopneumoniae* in living, non anaesthetized pigs. Veterinary Research 24:515-522.

Amass, S.F., Clark, L.K., Alstine, *et al.* (1994). Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. Journal of the American Veterinary Medical Association 204:102-107.

Armstrong, C.H., Scheidt, A.B., Thacker, H.L., Runnels, L.J., and Freeman, M.J. (1984). Evaluation of criteria for the postmortem diagnosis of mycoplasmal pneumonia of swine. Canadian Journal of Comparative Medicine 48:278-281.

Awad-Masalmeh, M. and Köfer, J. (1993). Environmental factors, prevalence of pneumonia and lung lesions of slaughter swine suffering from chronic respiratory disease. Livestock Environment IV. University of Warwick, Coventry, England 6-7 July, 1993, 915-921.

Bachmann, Ph. (1972). Beitrag zur Epidemiologie der kontagiösen Pleuropneumonie beim Schwein. Schweizer Archiv für Tierheilkunde 114:362-382.

Bäckström, L., and Bremer H. (1978). The relationship between disease incidences of fatteners registered at slaughter and environmental factors in herds. Nordic Veterinary Medicine 30:526-533.

Bahnson, P.B., Pointon, A.M., Dial, D.G., and Marsh, W.E. (1990). Prevalence of lesions at slaughter in Minnesota swine herds. Proceedings of the 11th IPVS Congress, Lausanne, 564.

Bahnson, P.B., Marsh, W.E., and Dial, G.D. (1994). The associations among serology to *M. hyopneumoniae*, lesions at slaughter, and a clinical index of cough in groups of growing pigs. Proceedings of the 13th IPVS Congress, Bangkok, 131.

Bækbo, P. (1990). Air quality in Danish pig herds. Proceedings of the 11th IPVS Congress, Lausanne, 395.

Bækbo, P., Kooij, D., Mortensen, S., Barfod, K., and Mousing, J. (1996). Economic evaluation of national eradication and control strategies for *Mycoplasma hyopneumoniae* in Denmark. Proceedings of the 14th IPVS Congress, Bologna, 230.

Bauck, S.W., Rhodes, C.S., and Barber, E.M. (1990). Environment, respiratory disease, and performance of pigs in three Saskatchewan grower finisher barns. Canadian Veterinary Journal 31:506-513.

Bernardo, T.M., Dohoo, I.R., and Donald, A. (1990). Effect of ascariasis and respiratory disease on growth rate in swine. Canadian Journal of Veterinary Research 54:278-284.

Blaha, Th. (1992). Zur Prävalenz der respiratorischen Erkrankungen des Schweines in den wichtigen schweinefleischproduzierenden Ländern. Collegium Veterinarium XXIII:64-67.

Blaha, Th. (1993). Erfassung pathologisch-anatomischer Organbefunde am Schlachthof; 1. Ansatz zu neuen Wegen bei der Wahrnehmung der Verantwortung für Verbraucherschutz und Tiergesundheit. Fleischwirtschaft 73:877-881.

Blaha, Th., von Hammel, M.-L., and große Beilage, E. (1994). Slaughter checks - Integrated quality assurance systems, meat quality, antibiotics. Proceedings of the 13th IPVS Congress, Bangkok, 424.

Blecha, F., Pollmann, D.S., and Nichols, D.A. (1985). Immunologic reactions of pigs regrouped at or near weaning. American Journal of Veterinary Research 46:1934-1937.

Bollwahn, W. (1989). Infektiöse Faktorenkrankheiten beim Schwein - Pathogenese und Bekämpfung. Berliner und Münchener Tierärztliche Wochenschrift 102:410-412.

Bossow, H. (1995). Climatic control inside of pig houses - significance for practice, animal health and human health. Der praktische Tierarzt 76:780.

Braude, R. and Plonka, S. (1975). Effect of enzootic pneumonia on the performance of growing pigs. Veterinary Record 96:359-360.

Bresk, B. and Stolpe, J. (1975). Influence of dust in intensive pig units on animal performance and health. Monatshefte für Veterinärmedizin 30:572 576.

Brumm, M.C. (1997). Space allocation decisions. PIC Technical Update Series Vol. 1. PIC Inc., Franklin, Kentucky.

Cannon, R.M. and Roe, R.T. (1982). Livestock disease surveys: A field manual for veterinarians. Australian Bureau of Animal Health, Canberra.

Cargill, C.F., Skirrow, S.Z., and Banhazi, T. (1996). The relationship between pig population size, stocking density, air quality parameters and pleurisy in pig herds. Proceedings 14th IPVS Congress, Bologna, 521.

Christensen, G. (1981). Pleuropneumonia in swine due to *Haemophilus pleuropneumoniae s. parahaemolyticus*. II. studies on the epidemiology and the relation to chronic pleuritis (pleural scars) in baconers. Nordic Veterinary Medicine 33:239-249.

Christensen, G. and Mousing, J. (1992). Respiratory System. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, and D.J. Taylor (eds.) Diseases of Swine, 7th Edition. Iowa State University Press, Ames, Iowa, 128-162.

Ciprian, A., Pijoan, C., Cruz, T., *et al.* (1988). *Mycoplasma hyopneumoniae* increases the susceptibility of pigs to experimental *Pasteurella multocida* pneumonia. Canadian Journal of Veterinary Research 52:434-438.

Clark, L.K., Scheidt, A.B., Armstrong, C.H., Knox, K., and Mayrose, V.B. (1991). The effect of all in/all out management on pigs from a herd with enzootic pneumonia. Veterinary Medicine 86:946, 948-951.

Cormier, Y., Boulet, L.P., Bedard, G., and Tremblay, G. (1991). Respiratory health of workers exposed to swine confinement buildings only or to both swine confinement buildings and dairy barns. Scandinavian Journal of Work, Environment and Health 17:269-275.

Cowart, R.P., Boessen, C.R., and Kliebenstein, J.B. (1991). Patterns associated with season and facilities for atrophic rhinitis and pneumonia in slaughter swine. Journal of the American Veterinary Medical Association 200:190-193.

Davies, P.R., Bahnson, P.B., Grass, J.J., Marsh, W.E., and Dial, G.D. (1995). Comparison of methods for measurement of enzootic pneumonia lesions in pigs. American Journal of Veterinary Research 56:709-714.

DiFranco, E., Marois, P., Descoteaux, J.P., Lacroix, M., and Flipot, P. (1989). Enzootic pneumonia in feeder pigs: observations on causal factors. Canadian Veterinary Journal 30:241-245.

Done, S.H. (1990a). Relationship between clinical respiratory disease and production parameters in pigs. Proceedings of the 11th IPVS Congress, Lausanne, 391.

Done, S.H. (1990b). The relationship between treatment factors for pneumonia and outdoor and indoor climatic factors in fattening pigs. Proceedings of the 11th IPVS Congress, Lausanne, 401.

Done, S.H. (1991). Environmental factors affecting the severity of pneumonia in pigs. Veterinary Record 128:582-586.

Donham, K.J. (1991). Association of environmental air contaminants with disease and productivity in swine. American Journal of Veterinary Research 52:1723-1730.

Elbers, A.R.W. (1991). The use of slaughterhouse information in monitoring systems for herd health control in pigs. PhD Thesis, Univeristy of Utrecht, Utrecht, The Netherlands.

Elbers, A.R.W., Tielen, M.J.M., Cromwijk, W.A.J., van der Voorst, P.H., Bais, J.T., Verhaegh, G., and de Bruyn, A.A. (1992a). Logbook recording on pig finishing farms in an integrated quality control (IQC) project. II. Use of veterinary drugs in relation to clinical observations, management, housing and climatic conditions and prevalence of pathological findings. Tijdschrift voor Diergeneeskunde 117:41-48.

Elbers, A.R.W., Tielen, M.J.M., Cromwijk, W.A., and Hunneman, W.A. (1992b). Variation in seropositivity for some respiratory disease agents in finishing pigs: epidemiological studies on some health parameters and farm and management conditions in the herds. Veterinary Quarterly 14:8-13.

Falk, K., Høie, S., and Lium, B.M. (1991). An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. II. Enzootic pneumonia of pigs: Microbiological findings and their relationship to pathomorphology. Acta Veterinaria Scandinavica 32:67-77.

Falk, K. and Lium, B.M. (1991). An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. III. Serological findings and their relationship to pathomorphological and microbiological findings. Acta Veterinaria Scandinavica 32:79-88.

Fišer, A. and Král, F. (1969). Air temperature and air humidity effect on number of air bacteria in piggeries with a different feed technology. Acta Veterinaria (Brno) 38:579-587.

Fellström, C. and Wallgren, P. (1990). The relationship between seroconversion to *Mycoplasma hyopneumoniae* and lung findings at slaughter. Proceedings of the 11th IPVS Congress, Lausanne, 308.

Flesjå, K.I. and Solberg, I. (1981). Pathological lesions in swine at slaughter. IV. Pathological lesions in relation to rearing system and herd size. Acta Veterinaria Scandinavica 22:272-282.

Flesjå, K.I., Forus, I.B., and Solberg, I. (1982). Pathological lesions in swine at slaughter. V. Pathological lesions in relation to some environmental factors in the herds. Acta Veterinaria Scandinavica 23:169-183.

Frey, J., Haldimann, A., and Nicolet, J. (1992). Chromosomal heterogeneity of various *Mycoplasma hyopneumoniae* field strains. International Journal of Systematic Bacteriology 42:275-280.

Frey, J., Haldimann, A., Kobisch, M., and Nicolet, J. (1994). Immune response against the L-lactate dehydrogenase of *Mycoplasma hyopneumoniae* in enzootic pneumonia of swine. Microbial Pathogenesis 17:313-322.

Ganter, M. (1996). Detection of *Actinobacillus pleuropneumoniae* serotype 2 by immunofluorescence test in bronchoaleolar lavage of live pigs. Proceedings of the 14th IPVS Congress, Bologna, 213.

Ganter, M., Kipper, S., Schottger Wegener, H., Beckmann, G., and Bunka, S. (1993). Diagnosis of pneumonia in pigs with bronchoalveolar lavage. Berliner und Münchener Tierärztliche Wochenschrift 106:330-333.

Gardner, I.A. and Hird, D.W. (1990). Host determinants of pneumonia in slaughter weight pigs. American Journal of Veterinary Research 51:1306-1311.

Gardner, I.J., Bosse, J.T., Sheldrake, R.F., Rosendal, S., and Johnson, R.P. (1991). Serological response to *Actinobacillus pleuropneumoniae* serovar 7 infection in a commercial pig herd. Australian Veterinary Journal 68:349-352.

Geers, R., Dellaert, B., Goedseels, V., Hoogerbrugge, A., Vranken, E., Maes, F., and Berckmans, D. (1989). An assessment of optimal air temperature in pig houses by the quantification of behavioral and health related problems. Animal Production 48:571-578.

Goiš, M., Kuksa, F. and Šišák, F. (1980). Microbiological findings in the lungs of slaughter pigs. Proceedings of the 6th IPVS Congress, Copenhagen, 214.

Goodall, E.A., Menzies, F.D., McLoughlin, E.M., and McIlroy, S.G. (1993). Prevalence of pleurisy and pneumonia in pigs in Northern Ireland (1969-1989). Veterinary Record 132:11-14.

Goodwin, R.F.W. (1971). The economics of enzootic pneumonia. Veterinary Record 89:77-81.

Goodwin, R.F.W. (1972). Isolation of *Mycoplasma suipneumoniae* from the nasal cavities and lungs of pigs affected with enzootic pneumonia or exposed to this infection. Research in Veterinary Science 13:262-267.

Goodwin, R.F.W. (1985). Apparent re-infection of enzootic pneumonia free pig herds: search for possible causes. Veterinary Record 116:690-694.

Gordon, W.A.M. (1963). Environmental studies in pig housing. V. The effects of housing on the degree and in-

cidence of pneumonia in bacon pigs. British Veterinary Journal 119:307-314.

Grest, P. (1995). Zur Prävalenz von Lungenläsionen bei Schlachtschweinen in der Schweiz. Doctoral Thesis, University of Zurich, Zurich, Switzerland.

Guerrero, R.J. (1990). Respiratory disease: an important global problem in swine industry. Proceedings of the 11th IPVS Congress, Lausanne, 98.

Hartung, J. (1994). The effect of airborne particulates on livestock health and production. In: I. Ap Dewi, R. F. E. Axford, I. F. M. Marai, and H. M. Omed (eds.) Pollution in livestock production systems CAB International, Oxford, 57-69.

Harrison, T.D. (1986). Aerial environment and pig respiratory disease: a literature survey. Pig Veterinary Society Proceedings 15:36-48.

Heber, A.J., Stroik, M., Nelssen, J.L., and Nichols, D.A. (1988). Influence of environmental factors on concentrations and inorganic content of aerial dust in swine finishing buildings. Transactions of the American Society of Agricultural Engineers 31:875-881.

Hennessy, K.J., Iandolo, J.J., and Fenwick, B.W. (1993). Serotype identification of *Actinobacillus pleuropneumoniae* by arbitrarily primed polymerase chain reaction. Journal of Clinical Microbiology 31:1155-1159.

Hessing, M.J.C., Scheepens, C.J.M., Schouten, W.G.P., Tielen, M.J.M., and Wiepkema, P.R. (1994). Social rank and disease susceptibility in pigs. Veterinary Immunology and Immunopathology 43:373-387.

Hofer, J. (1993). Serological studies on the prevalence of *Actinobacillus pleuropneumoniae* infection in Austria with special attention on farm specific affecting factors. Wiener Tierärztliche Monatsschrift 80:124-125 (Abstract of thesis, Veterinary University Vienna, 1992).

Hoy, S. (1994). Zu den Auswirkungen von Atemwegserkrankungen auf die Mast- und Fruchtbarkeits-leistungen der Schweine. Der praktische Tierarzt 75:121-127.

Hoy, S., Mehlhorn, G., Fuchs, W., Muller, K., and Mockel, H.G. (1987). Importance of complex environmental influences on the unweaned piglet for the occurrence of enzootic pneumonia and the fattening and slaughter performance of pigs. Tierzucht 41:192-195.

Huhn, R.G. (1970). Swine enzootic pneumonia: incidence and effect of body weight gain. American Journal of Veterinary Research 31:1097-1108.

Hurnik, D., Hanna, P.E., and Dohoo, I.R. (1993). Evaluation of rapid gross visual appraisal of swine lungs at slaughter as a diagnostic screen for enzootic pneumonia. Canadian Journal of Veterinary Research 57:37-41.

Hurnik, D., Dohoo, I.R., and Bate, L.A. (1994a). Factor analysis of swine farm management practices on Prince Edward Island. Preventive Veterinary Medicine 20:135-146.

Hurnik, D., Dohoo, I.R., and Bate, L.A. (1994b). Types of farm management as risk factors for swine respiratory disease. Preventive Veterinary Medicine 20:147-157.

Jensen, A. and Blaha, Th. (1997). Zum Zusammenhang zwischen Management- und Hygienefaktoren in Schweinemastbeständen und Organveränderungen am Schlachthof. Der praktische Tierarzt 78:494-504.

Jericho, K.W.F., Done, S.H., and Saunders, R.W. (1975). Pneumonia and efficiency of pig production. Canadian Veterinary Journal 16:44-49.

Jorsal, S.E. and Thomsen, B.L. (1988). A Cox regression analysis of risk factors related to *Mycoplasma suipneumoniae* re-infection in Danish SPF herds. Acta Veterinaria Scandinavica Suppl. 84:436-438.

Kalich, J. (1970a). Untersuchungen über die Beziehungen zwischen enzootischer Pneumonie (Ferkelgrippe) und Umwelt. Berliner Münchner Tierärztliche Wochenschriften 83:289-308.

Kalich, J. (1970b). Untersuchungen über die Beziehungen zwischen enzootischer Pneumonie (Ferkelgrippe) und Umwelt. Berliner Münchner Tierärztliche Wochenschriften 83:309-328.

Keller, H. (1976). Subclinical *M. suipneumoniae*-infections in SPF-herds. Proceedings of the 4th IPVS Congress, Ames, Iowa,11.

Kreukniet, M.B., Visser, W., Verhagen, J.M.F., and Verstegen, M.W.A. (1990). Influences of climatic treat-

ments on systemic immunological parameters in pigs. Livestock Production Science 24:249-258.

Kobisch, M., Blanchard, B., and Le Potier, M.F. (1993). *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and resistance to re-infection. Veterinary Research 24:67-77.

Kume, K., Nagano, I., and Nakai, T. (1986). Bacteriological, serological, and pathological examinations of *Haemophilus pleuropneumoniae* infection in 200 slaughtered pigs. Japanese Journal of Veterinary Science 48:965-970.

Kuiper, C.J., Martens, M.R.Th.M., and van Groenland, G.J. (1994). Specific pathogen-free health programs. Proceedings of the 13th IPVS Congress, Bangkok, 440.

Larsson, K., Eklund, A., Malmberg, P., and Belin, L. (1992). Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine confinement workers. Chest 101:767-774.

Le Potier, M.F., Abiven, P., and Kobisch, M. (1994). A blocking ELISA using a monoclonal antibody for the serological detection of *Mycoplasma hyopneumoniae*. Research in Veterinary Science 56:338-345.

Lindquist, J.-O. (1974). Animal health and environment in the production of fattening pigs. Acta Veterinaria Scandinavica Suppl. 51:1-78.

Loftager, M.K., Eriksen, L., and Nielsen, R. (1993). Antibodies against *Actinobacillus pleuropneumoniae* serotype 2 in mucosal secretions and sera of infected pigs as demonstrated by an enzyme linked immunosorbent assay. Research in Veterinary Science 54:57-62.

MacInnes, J.I. and Rosendal, S. (1988). Prevention and control of *Actinobacillus (Haemophilus) pleuropneumoniae* infection in swine: a review. Canadian Veterinary Journal 29:572-574.

Madsen, A. (1980). Environmental influence on health of bacon pigs. Proceedings of the 6th IPVS Congress, Copenhagen, 320.

Maes, D. (1997). Risk indicators for the seroprevalence of *Mycoplasma hyopneumoniae* in slaughter pigs from farrow-to-finish pig herds. Epidémiolgie et Santé Animale 31-32:05.29.1-05.29.3.

Mattson, J.G., Bergström, K., Wallgren, P., and Johansson, K.-E. (1995). Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. Journal of Clinical Microbiology 33:893-897.

Møller, K., Nielsen, R., Andersen, L.V., and Kilian, M. (1992). Clonal analysis of the *Actinobacillus pleuropneumoniae* population in a geographically restricted area by multilocus enzyme electrophoresis. Journal of Clinical Microbiology 30:623-627.

Morris, C.R., Gardner, I.A., Hietala, S.K., and Carpenter, T.E. (1995a). Enzootic pneumonia: comparison of cough and lung lesions as predictors of weight gain in swine. Canadian Journal of Veterinary Research 59:197-204.

Morris, C.R., Gardner, I.A., Hietala, S.K., Carpenter, T.E., Anderson, R.J., and Parker, K.M. (1995b). Seroepidemiologic study of natural transmission of *Mycoplasma hyopneumoniae* in a swine herd. Preventive Veterinary Medicine 21:323-337.

Morrison, R.B. and Morris, R.S. (1985). RESPITE - A computer-aided guide to the prevalence of pneumonia in pig herds. Veterinary Record 117:268-271.

Morrison, R.B., Pijoan, C., Hilley, H.D., and Rapp, V. (1985). Microorganisms associated with pneumonia in slaughter weight swine. Canadian Veterinary Journal 49:129-137.

Mousing, J., Lybye, H., Barfod, K., Meyling, A., Ronsholt, L., and Willeberg, P. (1990). Chronic pleuritis in pigs for slaughter: an epidemiological study of infectious and rearing system related risk factors. Preventive Veterinary Medicine 9:107-119.

Mousing, J. (1991). The relationship between serological reactions to swine respiratory infections and herd related factors. Proceedings of the 6th ISVEE Conference, Ottawa, Canada, 416-418.

Narita, M., Kawashima, K., Morozumi, T., and Takashima, H. (1995). Effect of physical defences of the respiratory tract on the development of pneumonia in pigs inoculated endobronchially with *Actinobacillus pleuropneumoniae*. Journal of Veterinary Medical Science 57:839-844.

Nielsen, R. (1995). Detection of antibodies against *Actinobacillus pleuropneumoniae*, serotype 2 in porcine colostrum using a blocking enzyme-linked immunosorbent assay specific for serotype 2. Veterinary Microbiology 43:277-281.

Noyes, E.P., Pijoan, C., Jacobson, L., and Boedicker, J. (1986). Effects of cold-air drafts on early-weaned pigs. Proceedings of the 9th IPVS Congress, Barcelona, 403.

Noyes, E.P., Jacobson, L., Mendez, A., and Pijoan, C. (1990). Study of immunological parameters of pigs housed with cold air drafts or fluctuating temperatures Proceedings of the 11th IPVS Congress, Lausanne, 25.

Oppenheim, A.N. (1992). Questionnaire design, interviewing and attitude measurement Pinter Publishers, London.

Pasquill, F. (1961). The estimation of the dispersal of wind-borne material. The Meteorological Magazine 90:33-53.

Peipert, J.F., Gifford, D.S., and Boardman, L.A. (1997). Research design and methods of quantitative synthesis of medical evidence. Obstetrics and Gynecology 90:473-478.

Plonait, H. and Gindele, H.R. (1995). Management and health aspects of specialised early weaning piglet production. Tierärztliche Umschau 50:316-321.

Pointon, A.M., Byrt, D., and Heap, P. (1985). Effect of enzootic pneumonia of pigs on growth performance. Australian Veterinary Journal 62:13-18.

Pointon, A.M., Morrison, R.B., Hill, G., Dargatz, D., and Dial, G. (1990). Monitoring pathology in slaughtered stock: Guidelines for selecting sample size and interpreting results. Proceedings of the 11th IPVS Congress, Lausanne, 393.

Rautiainen, E., Nyberg, M., and Levonen, K. (1996). The use of sow colostrum samples for regular herd health control of *Mycoplasma hyopneumoniae* infection. Proceedings of the 14th IPVS Congress, Bologna, 219.

Robertson, J.F., Wilson, D., and Smith, W.J. (1990). Atrophic rhinitis: The influence of the aerial environment. Animal Production 50:173-182.

Rosendal, S. and Mitchell, W.R. (1983). An epidemic of *Haemophilus pleuropneumoniae* infection in pigs: A survey of Ontario pork producers, 1981. Canadian Journal of Comparative Medicine 47:1-5.

Rothman, K.J. (1986). Modern epidemiology. Little, Brown and Company, Boston.

Scheepens, C.J.M. (1996). Climatic stress in swine: hazards for health. The Pig Journal 37: 130-136.

Scheepens, C.J.M., Tielen, M.J.M., and Hessing, M.J.C. (1991). Influence of daily intermittent draught on the health status of weaned pigs. Livestock Production Science 29:241-254.

Scheepens, C.J.M., Hessing, M.J.C., Hensen, E.J., and Henricks, P.A.J. (1994) Effect of climatic stress on the immunological reactivity of weaned pigs. The Veterinary Quarterly 16:137-143.

Scheidt, A., Clark, K., Mayrose, V., Cline, T., Jones, D., and Frants, S. (1990). All in, all out finishing as a means for improving growth in a swine herd affected by enzootic pneumonia. Proceedings of the 11th IPVS Congress, Lausanne, 92.

Singer, J. (1993). Serological studies on prevalence of *Actinobacillus pleuropneumoniae* infection in Austria in consideration of epidemiological factors. Wiener Tierärztliche Monatsschrift 80:153.

Sirois, M., Lemire, E.G., and Levesque, R.C. (1991). Construction of a DNA probe and detection of *Actinobacillus pleuropneumoniae* by using polymerase chain reaction. Journal of Clinical Microbiology 29:1183-1187.

Sitjar, M., Noyes, E., Moreso, J. M., Fernández de Aragón, J., and Pijoan, C. (1994). Relationship between respiratory pathogen seroconversion and lung lesions in pigs. Proceedings of the 13th IPVS Congress, Bangkok, 133.

Skirrow, S.Z. and Nicholls, R.R. (1994). Environmental factors affecting pleurisy in Western Australian pig herds. Proceedings of the 13th IPVS Congress, Bangkok, 445.

Skirrow, S.Z., Cargill, C., Mercy, A., Nicholls, R., Banhazi, T., and Masterman, N. (1995). Risk factors for pleu-

risy in pigs. Final Report DAW 28P, Agriculture Western Australia and South Australian Research and Development Institute.

Smith, J.H., Wathes, C.M., and Baldwin, B.A. (1996). The preference of pigs for fresh air over ammoniated air. Applied Animal Behaviour Science 49:417-424.

Sørensen V., Barfod, K., and Feld, N.C. (1992). Evaluation of a monoclonal blocking ELISA and IHA for antibodies to *Mycoplasma hyopneumoniae* in SPF-pig herds. Veterinary Record 130:488-490.

Sørensen V., Barfod, K., Feld, N.C., and Vraa-Andersen, L. (1993). Application of enzyme-linked immunosorbent assay for the surveillance of *Mycoplasma hyopneumoniae* infection in pigs. Revue scientifique et technique de l'Office International des Epizooties 12:593-604.

Sørensen, V., Barfod, K., Friis, N.F *et al.* (1994). Comparison of four different methods for demonstration of *Mycoplasma hyopneumoniae* in lungs of experimentally inoculated pigs. Proceedings of the 13th IPVS Congress, Bangkok, 188.

Sørensen V., Ahrens, P., Barfod, K., Feenstra, A.A., Feld, N.C., Friis, N.F., Bille-Hansen, V., Jensen, N.E., and Pedersen, M.W. (1997). *Mycoplasma hyopneumoniae* infection in pigs: Duration of the disease and evaluation of four diagnostic assays. Veterinary Microbiology 54:23-34.

Stärk, K.D.C., Keller, H., and Eggenberger, E. (1992a). Risk factors for the re-infection of specific pathogen free pig breeding herds with enzootic pneumonia. Veterinary Record 131:532-535.

Stärk, K.D.C., Keller, H., and Eggenberger, E. (1992b). Climatic conditions and airborne transmission of *Mycoplasma hyopneumoniae*. Proceedings of the 12th IPVS Congress, The Hague, 559.

Stemke, G.W., Phan, R., Young, T.F., and Ross, R.F. (1994). Differentiation of *Mycoplasma hyopneumoniae*, *M. flocculare*, and *M. hyorhinis* on the basis of amplification of a 16S rRNA gene sequence. American Journal of Veterinary Research 55:81-84.

Strasser, M., Abiven, P., Kobisch, M., and Nicolet, J. (1992). Immunological and pathological reactions in piglets experimentally infected with *Mycoplasma hyopneumoniae* and/or *Mycoplasma flocculare*. Veterinary Immunology and Immunopathology 31:141-153.

Straw, B.E. (1991). Performance measured in pigs with pneumonia and housed in different environments. Journal of the American Veterinary Medical Association 198:627-630.

Straw, B.E. (1992). Controlling pneumonia in swine herds. Veterinary Medicine 78-86.

Straw, B.E., Henry, S.C., Schultz, R.S., and Marsteller, T.A. (1986a). Clinical assessment of pneumonia levels in swine through measurements of the amount of coughing. Proveedings of the 9th IPVS Congress, Barcelona 275.

Straw, B.E., Backstrom, L., and Leman, A. D. (1986b). Examination of swine at slaughter. Part II. Findings at slaughter and their significance. Compendium on Continuing Education for the Practising Veterinarian 8:S106-SS111.

Straw, B.E., Tuovinen, V.K., and Bigras-Poulin, M. (1989). Estimation of the cost of pneumonia in swine herds. Journal of the American Veterinary Medical Association 195:1702-1706.

Straw, B.E., Shin, S.J., and Yeager, A.E. (1990). Effect of pneumonia on growth rate and feed efficiency of minimal disease pigs exposed to *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae*. Preventive Veterinary Medicine 9:287-294.

Thomsen, B.L., Jorsal, S.E., Andersen, S., and Willeberg, P. (1992). The Cox regression model applied to risk factor analysis of infections in the breeding and multiplying herds in the Danish SPF system. Preventive Veterinary Medicine 12:287-297.

Tielen, M.J.M., Trujen, W.T., van der Groes, C.A.M., Verstegen, M.A.W., de Bruin, J.J.M., and Conbey, R.A.P.H. (1978). Conditions of management and the construction of piggeries on pig-fattening farms as factors in the incidence of diseases of the lung and liver in slaughtered pigs. Tijdschrift voor Diergeneeskunde 103:1155-1165.

Tielen, J.M., Elbers, A.R.W., Snijdelaar, M., van Gulick, P.J.M.M., Preller, L., and Blaauw, P.J. (1996). Prevalence of self-reported respiratory disease symptoms among veterinarians in the southern Netherlands. Ameri-

can Journal of Industrial Medicine 29:201-207.

Til, L.D. van, Dohoo, I.R., Morley, R.S., and Van Til, L.D. (1991). Epidemiological associations between *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* antibody titres and lung lesions in Prince Edward Island swine herds. Canadian Journal of Veterinary Research 55:347-351.

Tuovinen, V.K., Gröhn, Y.T., Straw, B.E., and Boyd, R.D. (1990). Environmental factors in feeder pig finishing units associated with partial carcass condemnations in a slaughter house. Proceedings of the 11th IPVS Congress, Lausanne, 394.

Tuovinen, V.K., Gröhn, Y.T., and Straw, B.E. (1997). Farrowing unit housing and management factors associated with diseases and disease signs of importance for feeder pig quality. Acta Agriculturae Scandinavica Section A, Animal Science 47:117-125.

Turner, L.W., Wathes, C.M., and Audsley, E. (1993). Dynamic probabilistic modelling of respiratory disease in swine including production and economic effects. Livestock Environment IV. University of Warwick, Coventry, England 6-7 July, 1993, 89-97.

Volmer, R. (1994). Serological surveillance of sow colostrum. Tierärztliche Umschau 49:421-426.

Vraa-Andersen, L. (1991). Analysis of risk factors to infection with *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* in swine. Proceedings of the 6th ISVEE Conference, Ottawa, Canada.

Wallgren, P., Mattsson, S., Artursson, K., and Bölske, G. (1990). Time relationship between *Mycoplasma hyopneumoniae* infection, age at slaughter and lung lesions at slaughter. Proceedings of the 11th IPVS Congress, Lausanne, 82.

Wallgren, P., Artursson, K., Fossum, C., and Alm, G.V. (1993a). Incidence of infections in pig bred for slaughter revealed by elevated serum levels of interferon and development of antibodies to *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. Journal of Veterinary Medicine B 40:1-12.

Wallgren, P., Sahlander, P., Hassleback, G., and Heldmer, E. (1993b). Control of infections with *Mycoplasma hyopneumoniae* in swine herds by disrupting the chain of infection, disinfection of buildings and strategic medical treatment. Journal of Veterinary Medicine B 40:157-169.

Wallgren, P., Bewkow, P., Fellström, C., and Renström, L.H.M. (1994). Porcine lung lesions at slaughter and their correlation to the incidence of infections by *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* during the rearing period. Journal of Veterinary Medicine B 41:441-452.

Wathes, C.M., Jones, C.D.R., and Webster, A.J.F. (1983). Ventilation, air hygiene and animal health. Veterinary Record 113:554-559.

Webster, A.J.F. (1981). Weather and infectious disease in cattle. Veterinary Record 108:183-187.

Whittlestone, P. (1976). Effect of climatic conditions on enzootic pneumonia of pigs. International Journal of Biometeorology 20:42-48.

Willeberg, P., Gerbola, M.-A., Madsen *et al.* (1978). A retrospective study of respiratory disease in a cohort of bacon pigs. Nordic Veterinary Medicine 30:513-525.

Willeberg, P., Gardner, I.A., Mortensen, S., and Mousing, J. (1994). Models for herd size effects in swine diseases. Proceedings of the 7th ISVEE Conference, Nairobi, Kenya. The Kenya Veterinarian 18:189-191.

Willson, P.J., Falk, G., and Klashinsky, S. (1987). Detection of *Actinobacillus pleuropneumoniae* infection in pigs. Canadian Veterinary Journal 28:111-116.

Willson, P.J., Schipper, C., and Morgan, E.D. (1988). The use of enzyme-like immunosorbent assay for diagnosis of *Actinobacillus pleuropneumoniae* infection in pigs. Canadian Veterinary Journal 29:583-585.

Yagihashi, T., Kazama, S., and Tajima, M. (1993). Seroepidemiology of mycoplasmal pneumonia of swine in Japan as surveyed by an enzyme linked immunosorbent assay. Veterinary Microbiology 34:155-166.

Yagishi, T., Nunoya, T., Mitui, T., and Tajima, M. (1984). Effect of *Mycoplasma hyopneumoniae* infection on the development of *Haemophilus pleuropneumoniae* pneumonia in pigs. Japanese Journal of Veterinary Science 46:705-713.

Zimmermann, W., Tschudi, P., and Nicolet, J. (1986). ELISA-Serologie in Blut und Kolostralmilch: eine

Möglichkeit zur Überwachung der enzootischen Pneumonie (EP) in Schweine-Betänden. Schweizer Archiv für Tierheilkunde 128:299-306.

Zimmermann, W. (1990). Experience of controlling porcine enzootic pneumonia within the eradication scheme of the Swiss Pig Health Service. Tierärztliche Umschau 45:556, 559-562.

CHAPTER 1.2

RISK FACTORS FOR RESPIRATORY DISEASES IN NEW ZEALAND PIG HERDS

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This Chapter is accepted for publication in the *New Zealand Veterinary Journal*. Figures 5-8 and Tables 8 and 9 have been added for additional information.

1. Abstract

Aims. A survey of lung lesions and risk factors for respiratory diseases was conducted in order to estimate the prevalence of respiratory diseases in the New Zealand pig population and to identify influential management practices.

Methods. Eighty-nine New Zealand pig farms with a minimum herd size of 50 sows participated in the survey. Abattoir data were recorded once in winter 1995 and once in summer 1996, and risk factor data were collected using a mailed questionnaire. A total of 6887 lungs was inspected.

Results. The prevalence of enzootic pneumonia, pleuropneumonia and pleurisy in winter was 63.4%, 2.7% and 19.1% respectively. Enzootic pneumonia was significantly less frequent in summer. The univariate risk factor analysis was consistent with earlier published evidence on the importance of environmental factors related to housing and management of the farm. The multivariate models for enzootic pneumonia and pleuropneumonia or pleurisy had a reasonably good predictive power of 81-91% for farms with high disease prevalence.

Conclusion. The results are useful to model the disease process on high-risk farms, which account for a considerable proportion of the New Zealand pig population.

Key Words. Enzootic pneumonia, Pleuropneumonia, Pig, Prevalence, Risk factor analysis.

2. Introduction

Despite intensive research and rigid control programmes over the last several decades, respiratory diseases have remained among the most prevalent and costly health problems in pig production world-wide. A survey conducted in New Zealand showed that pneumonia was the most commonly observed gross lesion in slaughter pigs from 46 herds (Christensen and Cullinane, 1990). It was also estimated that each 1% of lung affected by enzootic pneumonia reduced average daily weight gain by 2.2 g and increased the number of days to slaughter by 0.61 days under New Zealand conditions (Christensen, 1995). These reductions in growth performance were calculated to result in a loss of N.Z. $ 1 for each 1% of lung affected.

As these figures suggest a considerable problem with respiratory diseases in pigs in New Zealand, an industry-wide survey was conducted to provide representative prevalence estimates for respiratory disease lesions in the New Zealand pig population, and to conduct a risk factor analysis in order to suggest possible improvements to farm management.

3. Material and methods

3.1 Farm recruitment

Farms were recruited from among about 800 registered pig producers in New Zealand. Within this population, eligibility for the study was limited to farms with at least 50 sows and producing 30 or more slaughter pigs per week, as a minimum of 30 lungs was required for a statistically reliable estimate of the prevalence of disease (Pointon *et al.*, 1990; Davies *et al.*,

1995). Herds in this size category produce about 90% of the pork raised in New Zealand. All farmers were contacted by mail in June 1995. They were sent a questionnaire together with a covering letter explaining the objectives of the study. Written consent was obtained to collect slaughterhouse information on pigs coming from these farms. As an incentive for collaboration, a report with the findings of the lung check as well as an evaluation of the farm with respect to environmental parameters and the risk of respiratory diseases was offered. All farmers who did not respond to the first mailing were sent a reminder.

3.2 Abattoir data recording

Slaughter pigs from all recruited farms were checked for respiratory lesions at the abattoir to which the farmer was routinely shipping the pigs. Data were collected twice, once in summer and once in winter, to assess seasonal differences.

Lungs were checked at the viscera table. Carcasses which could not be identified with the farm of origin were excluded. Then the surface of the lung was visually inspected for lesions. The tissue was palpated and the type of lesion assessed. The observed lesions were recorded in accordance with the description by Christensen and Mousing (1992; TABLE 4, FIGURE 5, FIGURE 6).

TABLE 4. Definition of macroscopic lung lesions in slaughter pigs

Lesion Type	Description	Recording
Enzootic pneumonia	Lobular catarrhal pneumonia with a cranioventral location and red, plum or grey colour, meaty or firm, fibrous structure	0-55
Pneumonia - not enzootic pneumonia	Lobular disseminated catarrhal pneumonia not cranioventrally located, red, purple or grey colour	Present/absent
Pleuropneumonia	Fibrinous/necrotising pneumonia, caudodorsally located with or without local fibrinous pleurisy with or without necrosis and abscesses	Present/absent
Pleurisy	Fibrinous or fibrous pleurisy, with or without other lesion	Present/absent
Abscess	Disseminated or isolated capsular lesions with or without other lesions	Present/absent

Lungs were scored with respect to the amount of tissue affected by enzootic pneumonia (EP). For this purpose, a scoring protocol was used as described by Goodwin and Whittlestone (1971). Each lobe was assigned a maximum number of possible points, the total of all lobes adding up to 55. Ten points were allocated to each apical and cardiac lobe, five points to the intermediate lobe and five points to each leading edge of the diaphragmatic lobes. For every lung lobe, the proportion of surface area of tissue affected with pneumonic lesions was estimated. The figures from all lobes were then added up to a total lung score ranging from 0 to 55.

If lungs could not be completely removed from the rib cage due to pleuritic adhesions, the lung was recorded pleurisy-positive and the other data categories recorded as missing if they were not observed in the available tissue. Because this event was rare, the resulting underestimation of lesions other than pleurisy appeared to be acceptable.

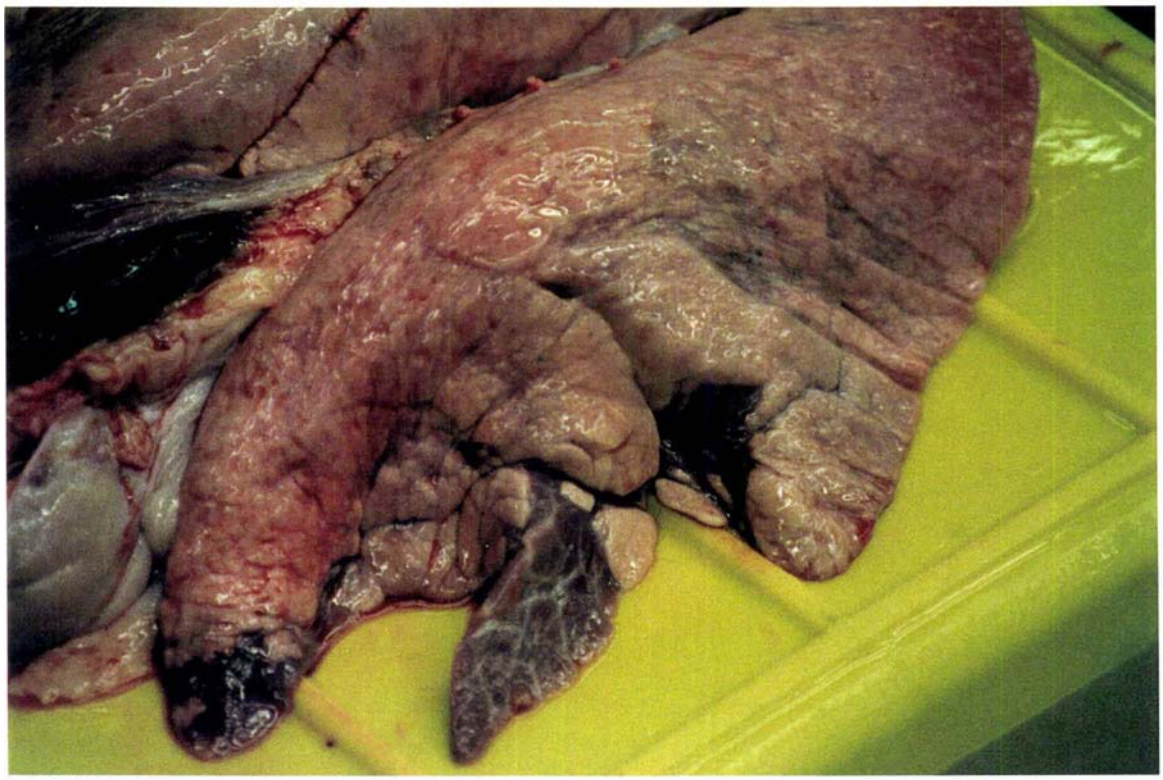

FIGURE 5. Typical lesion classified as 'enzootic-pneumonia-like lesion'

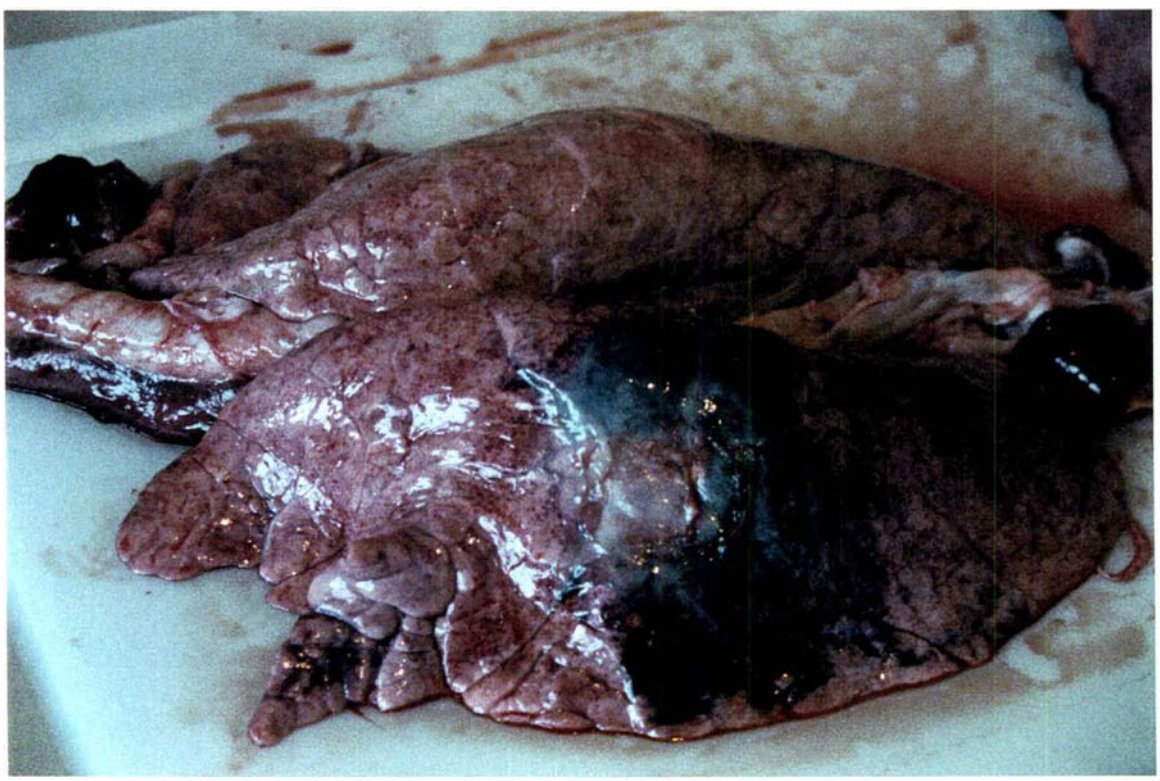

FIGURE 6. Typical lesion classified as '*Actinobacillus pleuropneumoniae*-like lesion'

If lungs could be completely removed from the carcass, all lesions (including pleurisy) were recorded as described above.

As many pigs as possible were examined from each farm. Small batches were examined completely, while in large ones at least 30 animals were examined. These were usually the first consecutive ones.

3.3 Farm data collection

A questionnaire (APPENDIX A) was sent to farmers together with the initial contact letter. The questionnaire was divided into three sections: general farm information (address, phone number), information on pig slaughter (name of abattoir, weekday of kill, number of pigs sold per week) and farm management information. The questionnaire consisted of closed questions, requiring either a number to be filled in or a yes/no answer. For some questions a choice of possible answers was offered out of which one had to be ticked. The answers to some questions were based on the farmer's own measurements (for example, temperature) or a subjective assessment (for example, hygiene status of the farm). All questions were carefully worded using simple non-technical expressions. The questionnaire was discussed with five farmers before it was mailed in order to avoid misunderstandings.

Farmers were provided with stamped return envelopes. If missing values occurred in a questionnaire, the farmers were either contacted by phone or letter to discuss the question. The farms were not visited.

3.4 Data management and analysis

Data was captured at the slaughter line using a hand-held computer (HUSKY FS2, Unilink Computers Inc., Clearwater, Florida) running data entry software (EpiInfo v. 6.0, Centers for Disease Control, Atlanta). The data was downloaded to a personal computer and imported into a data management system (Microsoft Access v. 7.0).

Descriptive statistical analysis was performed using statistical software packages (NCSS v. 6.0.12, Number Cruncher Statistical Systems, Kaysville, Utah; STATISTICA v. 5.0, StatSoft Inc., Tulsa). Logistic regression models with and without random effects were fitted using EGRET (Statistics and Epidemiology Research Corporation, Seattle).

Abattoir results were first pooled across all farms, and analysed separately for winter and summer data. They were then grouped by farm and re-analysed using 'farm' as a stratifying variable. The prevalence of the different lesion categories was calculated. Pleuropneumonia lesions and pleurisy were combined into one group 'pleuropneumonia/pleurisy' (PLPN), within which each lung was categorised as affected by pleuropneumonia, pleurisy or both.

Data from the questionnaire were screened for missing values and extreme values before starting the risk factor analysis. Variables with more than 10% missing values were excluded from multivariate analysis.

For risk factor analysis, the outcome at the farm level was the prevalence of affected lungs in winter (number of affected lungs / number of examined lungs). The categories EP and PLPN

were considered. First, univariate logistic models were fitted for each potential risk factor. If pairs of risk factors were highly correlated with each other ($r > 0.8$), only one was used. The assumption of linearity was checked for all continuous variables as described by Hosmer and Lemeshow (1989). Then, quartiles were used to group continuous variables into four categories.

Each variable with a statistical significance of $p < 0.05$ at the univariate level was considered for multivariate analysis. A forward stepwise selection procedure with an entry level of $p = 0.05$ was used. One-way interactions were tested for risk factor combinations that made biological sense or for which scientific evidence was available. A random effect was added to the final model, and the coefficients were recalculated. If previously significant variables became insignificant in the random effects model, they were removed, provided that the removal did not significantly change the deviance of the model at the 5% level.

To assess the predictive accuracy of the final models, the proportion of farms for which the prevalence was predicted within 10% and 5% of the observed value was calculated. Additionally, the model's capability to correctly classify a farm in one of three groups (farm with low, medium or high respiratory disease prevalence) was tested. The prevalence categories used for EP were: low ≤ 10%, medium 11-40%, high > 40%. For PLPN the following categories were used: low ≤ 3%, medium 4-20%, high > 20%.

4. Results

Of 312 farms, 116 (37.2%) returned a completed questionnaire. These farms routinely sent their pigs to 16 different abattoirs. Three small and remote abattoirs could not be included because of time and budget constraints, so five South Island farms were excluded from the analysis. Due to logistical problems at the abattoirs, slaughterhouse data could not be collected for all farms. For this reason, 22 farms were excluded (16 from the North Island, six from the South Island). Of the remaining 89 farms, nine produced only slaughter pigs and did not have any sows on the farm. Eight of these were in the South Island. All these farms were included in the study.

The 13 abattoirs visited were scattered over the North and South Islands of New Zealand (FIGURE 7). Small slaughterhouses with fully manual operation as well as large plants were included.

Data were collected in two rounds, one in New Zealand winter (31 July - 3 October 1995) and one in New Zealand summer (22 January - 20 March 1996). In the summer round, pigs from nine farms could not be re-checked at the abattoir for one of the following reasons: the farm was being repopulated (one farm), the farm had changed to selling weaners only (two farms), the farm had changed abattoir (two farms), or the farm was not shipping pigs in the week of data collection (four farms).

Enzootic pneumonia was the most frequently occurring lesion type (TABLE 5). However, 55% (winter) of the EP lesions were mild with a score < 6 and 35% were medium (score 6-20). Only 10% of all EP lesions were severe (score ≥ 20). In summer, the proportion of lungs with medium and severe EP lesions was even lower, 23% and 6%, respectively. This is reflected in the low average score calculated over all inspected pigs. Abscesses and not-EP-like pneumonia lesions were rare in both summer and winter. Pleuropneumonia lesions were more

often observed in summer than in winter. The prevalence of PLPN was similar in winter and in summer (TABLE 5).

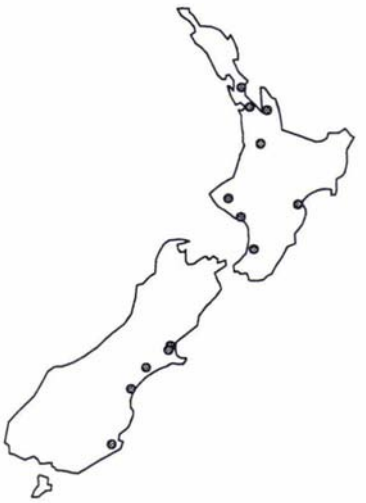

FIGURE 7. Location of abattoirs (n=13)

TABLE 5. The effect of season on the prevalence of respiratory lesions in slaughter-weight pigs in New Zealand

	Winter	Summer
Number of farms	89	80
Number of pigs inspected	3669	3218
Mean sample size	41.2	40.2
No. of lesions (%)	30.47	38.47[a]
Enzootic pneumonia (%)	63.37	52.22[a]
Mean score	5.03	3.91[a]
Abscess (%)	1.01	1.12
Pneumonia – not enzootic pneumonia (%)	2.15	2.30
Pleuropneumonia, no pleurisy	1.04	0.71
Pleuropneumonia and pleurisy	1.66	2.55
Pleuropneumonia, with or without pleurisy (%)	2.70	3.26
Pleurisy, no pleuropneumonia	16.22	14.48
Pleurisy, with or without pleuropneumonia (%)	19.13	19.33
PLPN[b] (%)	20.17	20.04
Multiple lesions of the above categories in one pig (%)	16.90	14.61[a]

[a]The difference between summer and winter was statistically significant ($p \leq 0.05$).
[b]PLPN = pleurisy and/or pleuropneumonia.

Prevalence figures for EP and PLPN calculated at the farm level are listed in TABLE 6 and distributions are shown as violin plots in FIGURE 8. Pigs from two farms in winter and from

four farms in summer were completely free of EP lesions. No herds with a certified EP-free status were included in the sample. In summer, farms had an EP prevalence that was on average 10.27% lower than in winter. The seasonal differences for both farm prevalence and average farm score were significant ($p \leq 0.05$). The following linear regression model describes the relationship between EP prevalence in summer and in winter:

$$EP\_Summer = 0.797*EP\_Winter \ (R^2 = 0.898).$$

TABLE 6. Occurrence of respiratory lesions in slaughter-weight pigs in New Zealand during winter 1995 (n=89) and summer 1996 (n=80)

	Mean	Median	S.D.	Min	Max
Enzootic pneumonia					
Farm prevalence	62.90/51.14	70.27/60.00	24.71/24.91	0/0	100/94.74
Farm mean of score	5.19/3.87	4.5/4.05	3.67/2.69	0/0	15.08/12.94
Pleurisy and/or pleuropneumonia					
Farm prevalence	19.41/19.70	12.50/11.11	20.52/22.89	0/0	78.95/83.33

On most farms PLPN lesions were less prevalent than EP. However, PLPN was more prevalent than EP on 5.6% of farms in winter and 15.0% in summer. In winter, 100.0% and in summer 83.3% of these farms were in the South Island, and some farms had an extremely high prevalence. The prevalence of PLPN was on average 1.39% lower in summer than in winter. This seasonal difference was not statistically significant.

The farm prevalence of EP and the average EP score per inspected pig were associated with each other (Spearman rank correlation coefficient = 0.917 for winter data, 0.825 for summer data). The average EP score increased exponentially with a higher EP prevalence in winter, but the relationship was linear in summer (FIGURE 9).

The relationship between the prevalence of EP and PLPN prevalence for the two sampling rounds is displayed in FIGURE 10, which shows a tendency for high PLPN prevalence to be associated with high EP prevalence. However, there are also farms for which this is not true. A PLPN prevalence of 40% and higher only occurred in South Island farms. This geographical difference was not observed with respect to the occurrence of EP.

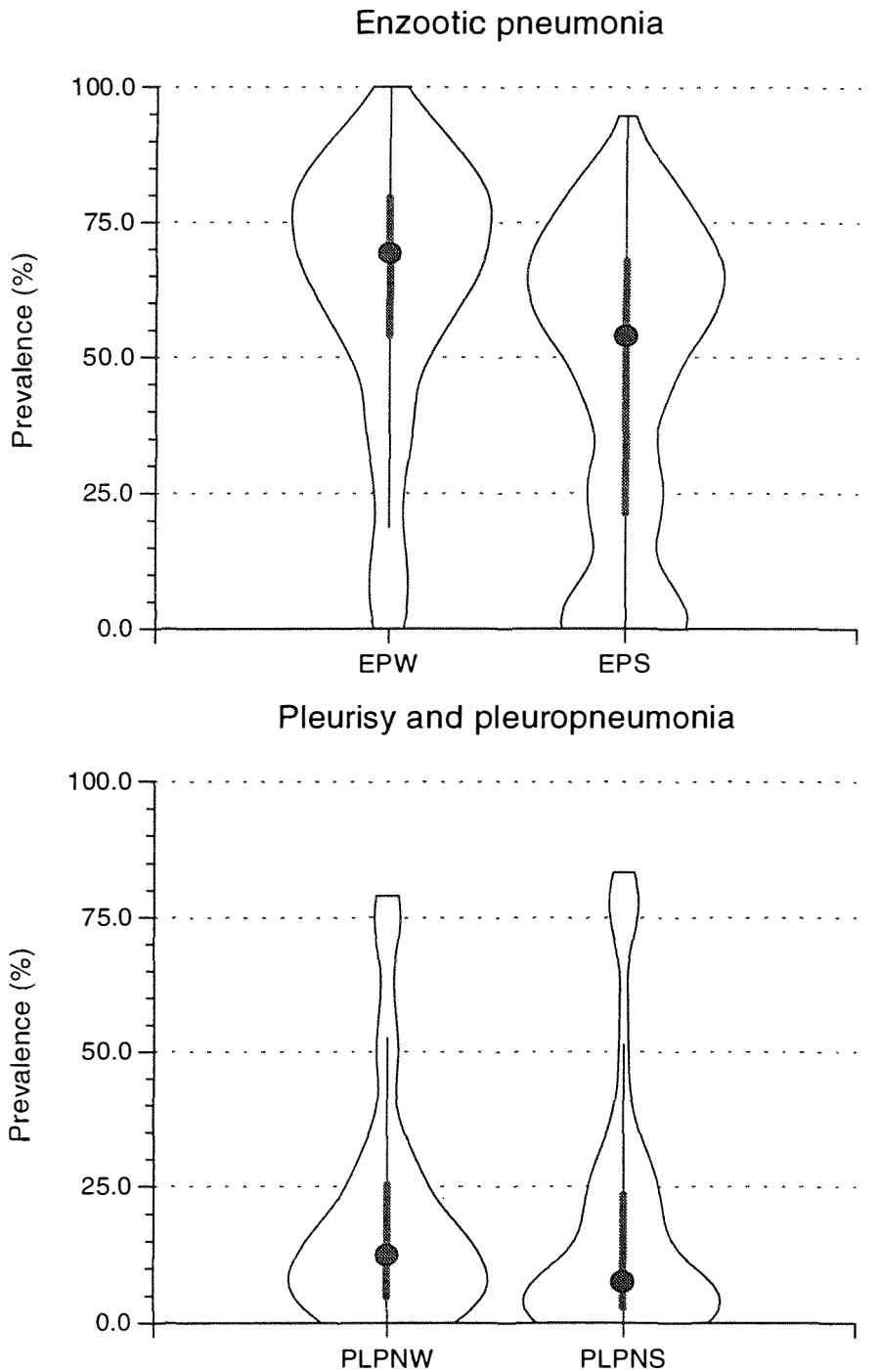

FIGURE 8. Violin plots for farm prevalence of enzootic pneumonia (EP) and pleurisy/pleuropneumonia (PLPN) in New Zealand pig herds (winter n=89, summer n=80)

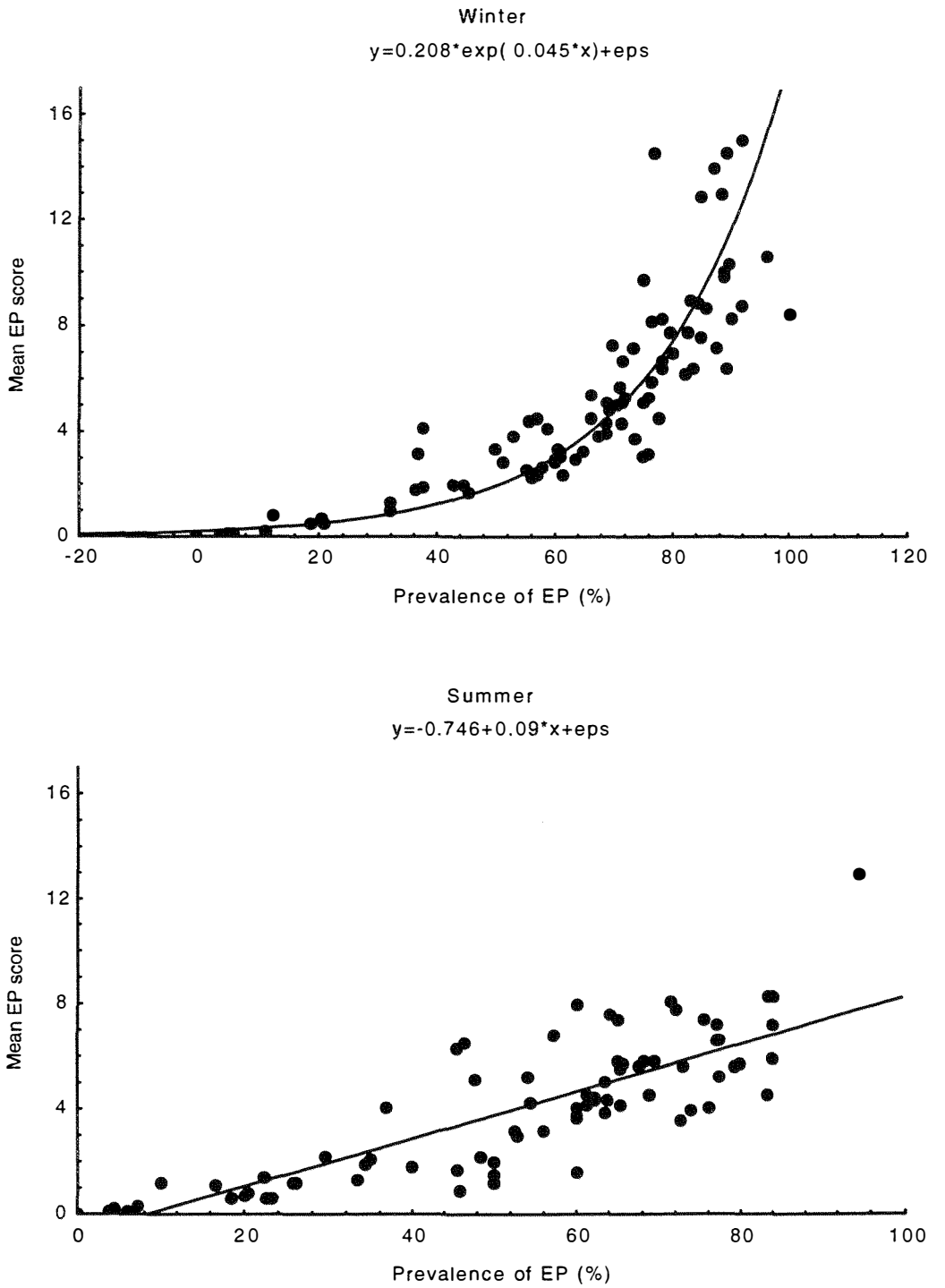

FIGURE 9. Association of enzootic pneumonia (EP) prevalence and mean EP score per inspected pig in New Zealand pig herds (winter n = 89, summer n =80).

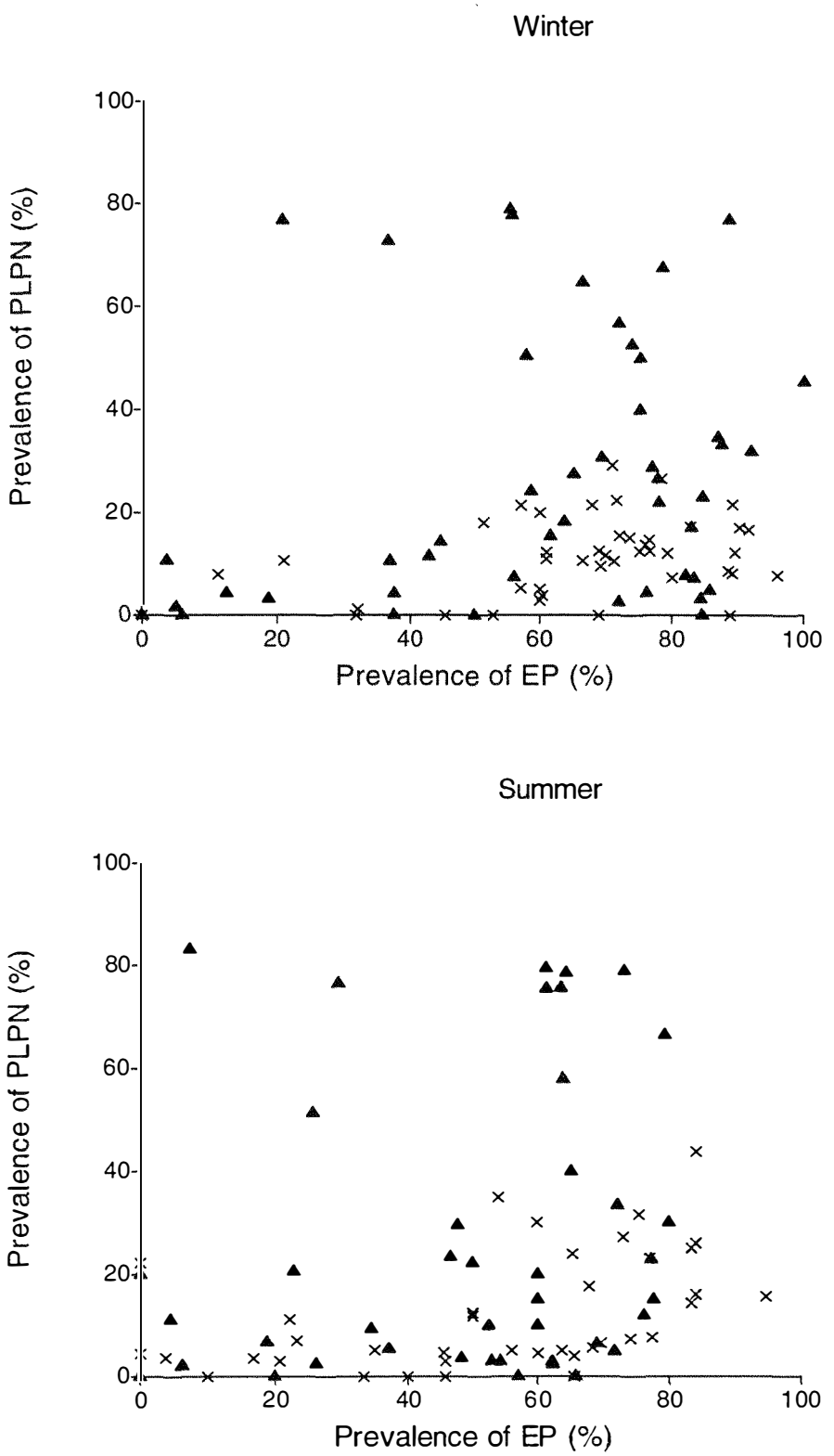

FIGURE 10. Relationship between prevalence of enzootic pneumonia (EP) and pleuropneumonia (PLPN) in New Zealand pig herds (X = North Island farm, ▲ = South Island farm; winter n = 89, summer n = 80).

TABLE 7. Descriptive statistics of continuous management variables of New Zealand pig farms

	n	25th percentile	Median	75th percentile
Number of sows	89	84.25	123.5	200.0
Number of fattening pigs	80	600.0	950.0	1575.0
Distance to next pig farm (km)	87	2.0	4.0	9.0
Average nursery temperature (°C)	49	22.0	25.0	27.0
Average number of nursery pigs per pen	84	15.0	17.5	20.75
Average area per nursery pig (m^2/pig)	78	0.22	0.3	0.40
Average number of nursery pigs per room	85	24.0	85.0	180.0
Average temperature (growers[a]) (°C)	50	18.0	19.0	20.0
Average number of growers per pen	89	12.0	15.0	18.0
Average area per grower (m^2/pig)	81	0.52	0.67	0.89
Average number of growers per room	87	120.0	270.0	425.0

[a]Growers = all growing pigs after they leave the nursery.

Descriptive results for continuous variables that were subsequently grouped into four categories are shown in TABLE 7. The quartiles described in this table are the classes used as input for the univariate analysis and the logistic regression analysis. Only one farmer practised vaccination against *Actinobacillus pleuropneumoniae*. This variable could therefore not be used in the univariate analysis. Also, the number of sows and the number of fattening pigs on a farm were highly correlated, and in the multivariate analysis only the number of fattening pigs was used.

At the univariate level, 27 of the analysed factors were significantly associated with the EP prevalence for at least one measurement level ($p \leq 0.05$, TABLE 8, TABLE 9). The strongest adverse effects (odds ratios > 2.0) were found for the following variables: farmer's attitude towards the importance of the environment, use of liquid manure in the nursery, and temperature in grower shed > 20.0°. The strongest protective effects (odds ratio <0.6) were associated with a good current environment (farmer's opinion), use of all-in/all-out system in the nursery, and with > 12 growers per pen.

With respect to PLPN, 31 tested factors were significantly related to the prevalence ($p \leq 0.05$, TABLE 8, TABLE 9). The associations were generally somewhat stronger with the strongest adverse effects (odds ratios > 2.0) associated with vaccination against EP, buying pigs from markets, free water access of growers, a farm size of > 600 finishing pigs and > 15 growers per pen. Strong protective effects (odds ratios < 0.6) were associated with a distance from the next piggery of > 1.575 km, an average temperature in the nursery > 27.0°, the use of medicated feed for growers, good hygiene (farmer's opinion), good current environment (farmer's opinion), automated ventilation in the nursery, frequent manure removal in both nursery and in grower houses. Three variables were excluded from subsequent analysis due to a large number of missing values (temperature in nursery, temperature for growers, average area per weaned pig).

TABLE 8. Farm management for North and South Island pig farms and odds ratios for risk factors for enzootic pneumonia (EP) and pleurisy/pleuropneumonia (PLPN) in New Zealand pig herds: Binary variables

Variable	n	North Island n=43 %	South Island n=46 %	EP O.R.	(95% C.I.)	PLPN O.R.	(95% C.I.)
Breeding-fattening farms	89	97.7	82.6*	n.s.[a]		0.82	(0.63-1.06)
Vaccination against EP	89	7.0	17.4	0.77*	(0.64-0.89)	2.98*	(2.45-3.61)
Coughing nursery	89	27.9	26.1	1.14	(0.98-1.34)	1.65*	(1.38-1.97)
Coughing at grower age	89	72.1	45.7*	1.79*	(1.56-2.06)	1.16	(0.98-1.37)
Diarrhea (any age group)	89	60.5	52.2	1.28*	(1.12-1.47)	1.13	(0.96-1.33)
Medicated feed (nursery)	89	76.7	69.6	1.28*	(1.09-1.51)	n.s.	
Medicated feed (growers)	89	25.6	26.1	1.26*	(1.08-1.46)	0.59*	(0.49-0.71)
Purchase policy	89						
no purchases		11.6	6.5	1[b]		1[b]	
breeding stock only		39.5	54.3	n.s.		n.s.	
buys from several farms		48.8	23.9*	1.32*	(1.15-1.54)	0.51*	(0.42-0.62)
buys from markets		0.0	15.2*	0.80	(0.62-1.02)	4.84*	(3.76-6.22)
Hygiene	89						
Could be better		18.6	15.2	1[b]		1[b]	
average		27.9	39.1	1.16	(0.94-1.43)	0.85	(0.69-1.07)
good		53.5	45.7	0.85	(0.70-1.04)	0.40*	(0.32-0.50)
Current environment	89						
Could be better		27.9	34.8	1[b]		1[b]	
adequate		44.2	43.5	1.00	(0.85-1.18)	0.99	(0.83-1.19)
good		27.9	21.7	0.54*	(0.45-0.65)	0.41*	(0.32-0.52)
Importance of Environment	89					n.s.	
not important		0	2.2	1[b]			
some importance		16.3	10.9	4.32*	(1.85-10.1)		
very important		83.7	87.0	2.79*	(1.20-6.30)		
Automated ventilation (nursery)	86	76.2	36.4*	n.s.		0.51*	(0.43-0.60)
Temperature threshold (nursery)	86	73.8	40.9*	n.s.		0.64*	(0.54-0.76)
Automated ventilation (growers)	89	39.5	21.7	n.s.		0.82*	(0.69-0.98)
Temperature threshold (growers)	89	60.5	54.3	n.s.		0.79*	(0.67-0.93)
Wet/dry feeding (nursery)	86	40.5	20.5*	0.68*	(0.59-0.78)	0.80*	(0.67-0.96)
Wet/dry feeding (growers)	89	72.1	43.5*	0.85*	(0.73-0.97)	n.s.	
Free water access (growers)	89	90.7	100*	n.s.		3.72*	(2.00-6.91)
Liquid manure (nursery)	86	97.6	88.6	2.40*	(1.65-3.48)	0.67	(0.46-1.06)
Liquid manure (growers)	89	69.8	82.6	0.87	(0.73-1.05)	2.00*	(1.54-2.57)
Frequency of manure removal (nursery)	86						

Variable	n	North Island n=43 %	South Island n=46 %	EP O.R.	(95% C.I.)	PLPN O.R.	(95% C.I.)
less than daily		47.6	61.4	1[b]		1[b]	
daily		40.5	27.3	1.14	(0.99-1.32)	0.78*	(0.66-0.93)
several times daily		11.9	11.4	0.70*	(0.54-0.91)	0.50*	(0.34-0.74)
Frequency of manure removal (growers)	89						
less than daily		30.2	47.8	1[b]		1[b]	
daily		60.5	39.1	1.44*	(1.30-1.60)	0.91	(0.77-1.07)
several times daily		9.3	13.0	1.46*	(1.27-1.68)	0.31*	(0.19-0.50)
Bedding used (nursery)	86	7.1	25.0*	1.25	(0.99-1.52)	1.58*	(1.22-2.04)
Solid pen separations (growers)	89	27.9	34.8	0.77*	(0.66-0.89)	1.25*	(1.05-1.48)
Sharing air space with other age group (nursery)	86	19.0	29.5	1.46*	(1.24-1.72)	1.42*	(1.18-1.70)
Sharing air space with other age group (growers)	89	39.5	34.8	n.s.		0.76*	(0.64-0.90)
All-in/all-out system (nursery)	86	64.3	54.5	0.62*	(0.54-0.72)	0.83*	(0.71-0.98)
All-in/all-out system (growers)	89	27.9	32.6	0.75*	(0.65-0.88)	n.s.	

*=p<0.05
[a]n.s.=not significant at p=0.2
[b]reference group

TABLE 9. Farm management for North and South Island pig farms and odds ratios for risk factors for enzootic pneumonia (EP) and pleurisy/pleuropneumonia (PLPN) in New Zealand pig herds: Continuous variables

Variable	n	North Island n=43 (SD)	South Island n=46 (SD)	EP O.R.	(95% C.I.)	PLPN O.R.	(95% C.I.)
Average number of sows	88	199 (150.5)	135* (142.5)				
2nd vs. 1st quartile				1.27*	(1.02-1.75)	1.28	(0.98-1.66)
3rd vs. 1st quartile				1.31*	(1.06-1.60)	0.85	(0.65-1.10)
4th vs. 1st quartile				1.26*	(1.04-1.53)	1.27*	(1.00-1.61)
Average number of fattening pigs	80	1,297 (1058.1)	1,144 (1,130.6)				
2nd vs. 1st quartile				0.80	(0.63-1.01)	2.30*	(1.65-3.20)
3rd vs. 1st quartile				1.23	(0.91-1.40)	2.17*	(1.60-2.95)
4th vs. 1st quartile				1.24*	(1.01-1.53)	2.33*	(1.73-3.14)
Average distance to next pig farm	86	7.65 (7.60)	5.49 (7.49)				
2nd vs. 1st quartile				1.14	(0.93-1.41)	0.63*	(0.49-0.82)
3rd vs. 1st quartile				0.66*	(0.55-0.79)	1.26*	(1.03-1.55)
4th vs. 1st quartile				0.91	(0.76-1.09)	0.59*	(0.46-0.74)
Average temperature (nursery) [°C]	49[b]	25.5 (2.20)	22.7* (1.95)	n.s.[a]			
2nd vs. 1st quartile						1.11	(0.82-1.50)
						1.65*	(1.21-2.26)

Variable	n	North Island n=43 (SD)	South Island n=46 (SD)	EP O.R.	(95% C.I.)	PLPN O.R.	(95% C.I.)
3rd vs. 1st quartile						0.55*	(0.39-0.76)
4th vs. 1st quartile							
Average temperature (growers) [°C]	50[b]	20.1 (1.75)	18.1 (1.62)				
2nd vs. 1st quartile				0.90	(0.72-1.14)	1.76*	(1.34-2.31)
3rd vs. 1st quartile				1.02	(0.82-1.26)	0.79	(0.59-1.10)
4th vs. 1st quartile				2.40*	(1.84-3.13)	0.81	(0.59-1.12)
Average area per weaned pig [m^2/pig]	78[b]	0.33 (0.15)	0.40 (0.25)				
2nd vs. 1st quartile				0.69*	(0.57-0.84)	1.15	(0.91-1.46)
3rd vs. 1st quartile				0.70*	(0.57-0.85)	0.69*	(0.53-0.90)
4th vs. 1st quartile				0.81	(0.65-1.02)	1.72*	(1.34-2.21)
Average area per grower [m^2/pig]	81	0.70 (0.28)	0.71 (0.32)				
2nd vs. 1st quartile				0.83	(0.69-1.00)	1.78*	(1.40-2.27)
3rd vs. 1st quartile				0.78*	(0.64-0.96)	1.47*	(1.13-1.91)
4th vs. 1st quartile				1.06	(0.85-1.32)	1.19	(0.90-1.59)
Average number of pigs per pen (nursery)	84	17 (4.9)	26* (19.6)				
2nd vs. 1st quartile				0.86	(0.70-1.07)	0.75*	(0.56-0.99)
3rd vs. 1st quartile				1.39*	(1.10-1.76)	1.62*	(1.22-2.15)
4th vs. 1st quartile				0.88	(0.71-1.11)	1.57*	(1.19-2.07)
Average number of pigs per pen (growers)	89	15 (4.3)	17 (10.0)				
2nd vs. 1st quartile				0.54*	(0.45-0.65)	1.57*	(1.26-1.97)
3rd vs. 1st quartile				0.77*	(0.64-0.94)	2.01*	(1.59-2.54)
4th vs. 1st quartile				0.86	(0.70-1.07)	1.32*	(1.01-1.72)
Average number of pigs per room (nursery)	85	118 (107.4)	132 (142.2)				
2nd vs. 1st quartile				0.94	(0.76-1.17)	0.76*	(0.59-0.99)
3rd vs. 1st quartile				1.17	(0.95-1.43)	0.59*	(0.46-0.75)
4th vs. 1st quartile				0.77*	(0.63-0.94)	1.03	(0.82-1.29)
Average number of pigs per room (growers)	86	272 (201.1)	297 (275.7)				
2nd vs. 1st quartile				0.73*	(0.59-0.91)	0.68*	(0.51-0.90)
3rd vs. 1st quartile				0.69*	(0.57-0.85)	1.90*	(1.50-2.40)
4th vs. 1st quartile				0.68*	(0.55-0.83)	0.99	(0.77-1.28)

*=$p<0.05$

[a]n.s. = not significant at p=0.2

[b]excluded from multivariate analysis due to missing values

Farms in the South Island had an increased risk of PLPN when compared with the North Island (OR = 2.8; 95% CI = 2.4-3.4). Some management differences between North and South Island were identified. Statistically significant ($p \leq 0.05$) differences were related to the type of farm (breeding-fattening more frequent in North Island v. fattening only more frequent in South Island), purchase policy (buying from several sources and from markets more prevalent in South Island), ventilation system (automated ventilation with temperature threshold in North Island v. natural ventilation in South Island), feeding (mainly dry feed in South Island v. mainly wet-dry feeding in North Island), use of bedding (more often used in South Island), temperature in the nursery room (lower temperatures in South Island) and the maximum number of pigs per pen (higher in South Island). These variables were also related to the prevalence of respiratory lesions, mostly PLPN. In other management areas, there was no geographical difference.

From the large number of input variables, only relatively few remained in the final logistic regression models (12 variables for EP, 11 for PLPN; TABLE 10 and TABLE 11). In the EP model, the strongest negative association was found between the prevalence of EP and the number of pigs per pen and per room in the nursery (higher numbers unfavourable), and a liquid manure system with slatted floors. All of these factors had odds ratios greater than 3.0. Among the protective factors the frequency of manure removal and the number of pigs per room (high numbers favourable) were most influential with odds ratios less than 0.3. In the PLPN model, the herd size (number of fattening pigs) and again the liquid manure system were the strongest negative risk factors (odds ratio > 3.0) and the number of pigs per pen in the nursery was a strong protective factor (higher number was favourable).

With the EP model, the prevalence of 39% of the farms was estimated within 10% of the true value and for 22% of the farms within 5%. The PLPN model was able to predict the prevalence of 76% of the farms within 10% of the observed figure and in 47% of the cases within 5%. When using prevalence categories, the EP model and the PLPN model correctly classified 86% and 53% of the farms, respectively. The sensitivity of the EP model was very poor (0%) for the low category, 43% for the medium and 100% for the high prevalence category, with specificity figures of 100%, 94% and 55%. For the PLPN model, sensitivity ranged from 14% to 81%, with the best result for the high category. The specificity was 93%, 60% and 90% for the low, medium and high category, respectively. The positive predictive value if a farm is classified in the high prevalence category was 91% for the EP model and 81% for the PLPN model.

TABLE 10. Random-effects logistic regression model for enzootic pneumonia in New Zealand pig herds (n = 76)

Variable	Coefficient (β)	OR	95%CI
Intercept	-1.467		
Number of pigs per pen (nursery)			
2nd v. 1st quartile	0.5650	1.76	1.06-2.93
3rd v. 1st quartile	1.082	2.95	1.77-4.93
4th v. 1st quartile	1.323	3.76	2.11-6.68
Frequency of manure removal (growers)			
daily v. less than daily	1.065	2.90	1.81-4.65
several times daily v. less than daily	0.7827	2.19	1.02-4.68
Frequency of manure removal (nursery)			
daily v. less than daily	-1.406	0.25	0.14-0.43
several times daily v. less than daily	–1.492	0.22	0.10-0.49
Number of pigs per pen (growers)			
2nd v. 1st quartile	-0.6638	0.51	0.35-0.76
3rd v. 1st quartile	–0.1205	0.89	0.58-1.36
4th v. 1st quartile	–0.0715	1.01	0.60-1.70
Number of pigs per room (growers)			
2nd v. 1st quartile	-1.297	0.27	0.14-0.55
3rd v. 1st quartile	–1.533	0.22	0.13-0.37
4th v. 1st quartile	–1.490	0.23	0.13-0.40
Hygiene			
Average v. could be better	0.8420	2.32	1.43-3.76
Good v. could be better	–0.6048	0.55	0.34-0.87
Current environment	n.s.[a]		
Adequate v. could be better			
good v. could be better			
Number of pigs per room (nursery)			
2nd v. 1st quartile	1.030	2.80	1.47-5.32
3rd v. 1st quartile	1.792	6.00	3.11-11.56
4th v. 1st quartile	0.4836	1.62	0.96-2.73
Average area per grower pig			
2nd v. 1st quartile	-0.3576	0.70	0.47-1.03
3rd v. 1st quartile	0.3951	1.49	0.94-1.34
4th v. 1st quartile	0.2451	1.28	0.77-2.12
Medicated feed (nursery)	0.8588	2.36	1.42-3.93
Liquid manure (nursery)	1.152	3.17	1.54-6.50
Wet/dry feeding (nursery)	-0.5523	0.58	0.44-0.76
All-in/all-out system (growers)	1.036	2.82	1.02-4.62
Random effect	1.153		

[a]n.s. = variable significant in ordinary logistic regression model but not in random-effects regression model.

TABLE 11. Random-effects logistic regression model for pleurisy / pleuro-pneumonia in New Zealand pig herds (n=75)

Variable	Coefficient (β)	OR	95%CI
Intercept	-3.522		
Hygiene			
average v. could be better	-0.3044	0.74	0.42-1.28
good v. could be better	–1.052	0.35	0.20-0.62
Island	1.090	2.97	1.82-4.88
Number of pigs per room (growers)			
2nd v. 1st quartile	-0.0821	0.92	0.47-1.81
3rd v. 1st quartile	0.4606	1.90	1.02-3.55
4th v. 1st quartile	--0.3115	0.73	0.39-1.36
Number of pigs per pen (nursery)			
2nd v. 1st quartile	-1.465	0.23	0.11-0.48
3rd v. 1st quartile	–0.5702	0.57	0.25-1.26
4th v. 1st quartile	–1.366	0.26	0.12-0.55
Average number of fattening pigs			
2nd v. 1st quartile	2.065	7.89	4.01-15.52
3rd v. 1st quartile	1.729	5.63	2.94-10.81
4th v. 1st quartile	1.730	5.64	2.82-11.30
Number of pigs per pen (growers)			
2nd v. 1st quartile	-0.2329	0.79	0.46-1.38
3rd v. 1st quartile	0.7307	2.08	1.07-4.02
4th v. 1st quartile	0.5731	1.77	0.87-3.62
Liquid manure (growers)	1.212	3.36	1.68-6.72
Bedding used (nursery)	0.8967	2.45	1.39-4.32
Solid pen separation (growers)	0.3815	1.47	0.91-2.36
Temperature threshold (nursery)	0.4495	1.57	0.94-2.61
Temperature threshold (growers)	-0.5364	0.58	0.35-0.98
Medicated feed (growers)	n.s.[a]		
Random effect	0.5754		

[a]n.s. = variable significant in ordinary logistic regression model but not in random-effects regression model.

5. Discussion

Respiratory lesions in slaughter-weight pigs are common in New Zealand. Gross pathological lesions observed at slaughter closely reflect the occurrence of specific pathogens (Grest, 1995; Pointon, 1995). The figures obtained for the occurrence of EP are similar to an earlier study which reported a 55% prevalence recorded over a period which included both winter and summer months (Christensen and Cullinane, 1990). The same authors reported a mean lesion score of 4.4, which again lies in-between the winter and summer values from this study. However, the highest score recorded by Christensen and Cullinane (1990) was only 11.7 while scores of 30 and higher were not rare in this survey. This difference is partially explained by the slightly different scoring protocol that we used (only the outer parts of the

lobes are considered) which over-all resulted in higher scores. Overseas studies on the frequency of EP also showed similar results. Enzootic pneumonia was found in Australia, Minnesota, Ontario and Switzerland in 30-50% (Pointon, 1995), 70% (Bahnson *et al.*, 1992), 75% (Wilson *et al.*, 1986) and 21-24% (Wunderli, 1993; Grest,1995) of slaughter weight pigs, respectively. The Swiss figure is smaller, which is probably due to a rigorous control programme. On a herd basis, the prevalence of EP was 63.5% in Switzerland (Grest, 1995).

The prevalence of pleuropneumonia lesions has not been assessed in New Zealand before. The disease was first diagnosed in 1989, although it had almost certainly been present for a number of years prior to its identification. Since then, it has been recognised throughout the country and typical lesions were observed in 2.7-3.4% of the pigs, a finding comparable with other countries. Pleuropneumonia was observed in 0.9-10.6% (Wunderli, 1993; Grest, 1995) of the inspected animals in Switzerland. In Minnesota and Queensland the prevalence was 0.6% and 0.2-0.5% of pigs, respectively (Bahnson *et al.*, 1992; Pointon, 1995). In Ontario, 11% of inspected slaughter pigs had pleuropneumonia or pleuritis (Wilson *et al.*, 1986). The herd level prevalence of pleurisy and pleuropneumonia in Switzerland was reported to be 20.1% and 8.9%, respectively. Pleurisy was recorded in about 19% of the pigs in our study. This type of lesion was observed in 14.2% of the pigs killed in Minnesota (Bahnson *et al.*, 1992) and in 12.9-20.5% of Swiss pigs (Wunderli, 1993; Grest, 1995). In Australia 19-50% of the pigs had pleurisy. Again, these figures are in a similar range.

A recent Swiss study showed that pleuropneumonia is a good indicator for *A. pleuropneumoniae* but localised pleurisy is more difficult to associate with an aetiological agent (Grest, 1995). Therefore, in this study the two lesion types 'pleurisy' and 'pleuropneumonia' were pooled for further analysis.

There was a significant geographical influence in the multivariate PLPN model but not in the EP model. Pleuropneumonia or pleurisy lesions occurred in 28.9% of South Island pigs but only in 12.5% of North Island pigs. The difference in PLPN between the islands may be related to the climate or different management practices as well as a clustered occurrence of the causal agent. Several housing variables were significantly related to the occurrence of PLPN. However, a number of these factors are also known to influence EP. Why only PLPN was found to be affected remains to be clarified.

The observed seasonal differences in respiratory lesions have been reported before (Done, 1991; Wongnarkpet, 1995). Enzootic pneumonia lesions are generally less prevalent in the warm, dry season, as the climate does not favour new infections and lesions acquired in winter mostly disappear before slaughter. As pleurisy lesions need some time to develop and do not tend to disappear quickly, winter infections are still detectable at slaughter. This effect can cause a similarly high prevalence in winter and summer or even a higher prevalence in summer. The conclusion from these comparisons is that respiratory problems in New Zealand pigs are occurring at a similar frequency and seasonal pattern as overseas.

An important step in tackling respiratory problems is the identification of risk factors. Many studies have been conducted with the objective to identify risk factors and their relative importance. A list of the most significant factors has been published by Christensen and Mousing (1992). These are:

> *Large herd size, high stocking density, non-SPF production system, introduction of pigs with unknown health status, continuous flow of*

pigs through facilities, badly insulated or poorly ventilated facilities with improper regulation of temperature and drafts, lack of partitions and pigs of different ages sharing same air space, pen dividers without solid partitions, insufficient regulation of climate.

In our study, information on these possible risk factors was collected using a mailed, self-completed questionnaire. The main advantage of this method is that it is cheap and fast but its disadvantage is that the clarity of the questions is crucial and that the validity of the responses is difficult to assess, particularly if the answer is based on a subjective opinion. A formal validation of the questionnaire results as suggested by Vaillancourt *et al.* (1991) was not performed in this survey. However, because most characteristics of the management of a pig farm are static and rather straightforward, answers given by the farmer should be reliable, particularly if only 'yes' or 'no' answers were required. Questions allowing continuous variables to be entered, such as 'number of pigs per pen', are more difficult to answer and more variable and therefore probably less reliable. It was thus decided that such variables would be re-coded in 4 classes according to their quartiles in order to eliminate some of the inherent uncertainty. Finally, questions requiring a subjective assessment of a process or situation, for example the hygiene status of a farm, have to be interpreted with care as they may be highly biased.

The univariate analysis conducted as part of this study confirmed the importance of environmental risk factors. Although it is well accepted that stocking density and the number of pigs per pen and per room have an influence on the occurrence of respiratory diseases (Done, 1991), the number of grower pigs per room (EP model) and the number of pigs per nursery pen (PLPN model) were significantly associated with contradictory effects in this study, indicating a favourable effect of high numbers. This is difficult to explain in terms of a causal hypothesis, because these variables may be intermediate variables related to certain housing systems and types of buildings. Also, particularly during the growing phase, pigs will typically be moved once or several times between different buildings and the value of these variables may change.

Both multivariate models mainly included previously expected risk factors. As an outcome variable, the prevalence of affected pigs in the winter was used, because this data set contained more farms and because it could be shown that the prevalence in summer and in winter are closely related. The inclusion of a random effect term significantly reduced the deviance of both models. Random effects should be considered in multivariate models whenever observations have a clustered structure and therefore cannot be considered independent (Curtis *et al.*, 1993). In this survey, pigs coming from the same farm have probably been exposed to similar risk factors and infective agents. A significant reduction of the deviance of a model through inclusion of a random effect term can be interpreted as a sign of relevant explanatory variables not having been recorded, or of inadequate measurement or control of certain variables (for example the herd effect) in the model (Collett, 1991). Due to the multifactorial nature of respiratory diseases, it is quite likely that additional variables could be relevant. Also, with a mailed questionnaire survey, continuous variables are more likely to be subject to some degree of measurement bias. Coefficients of random-effects logistic models can be interpreted as the logarithms of odds ratios and one then speaks of "the probability of a positive response given that the animal originates from a particular farm" (Curtis *et al.*, 1993).

In the current EP model, the risk of an animal showing gross lesions at slaughter was increased if the number of pigs per nursery pen was more than 15, if the number of pigs per nursery room was more than 24, or if there was a liquid manure disposal system (slatted floor) in the nursery. However, if manure was frequently removed in the nursery, a protective effect was achieved. The use of wet/dry feeding (dry feed is mixed with water as it is dispensed from the feeder) in the nursery also reduced the risk of showing EP lesions at slaughter. All these factors are likely to be related to the climatic conditions during the critical phase of disease spread or to the probability of contact between infected and susceptible animals (Done, 1991). The use of medicated feed in the nursery appears to be an indicator of respiratory problems because only problem farms are likely to adopt this treatment. Factors related to the growing phase of the pigs have a weaker effect because there is not so much disease spread during this time, and risk factors therefore have a more indirect effect on the disease. A high frequency of manure removal had an adverse effect during this phase. The unexpected results of stocking density-related factors from the univariate analysis were replicated and the earlier comments are also applicable to the multivariate model. Additionally, one variable (status of the current environment) was not significant in the final model, yet if it was removed the model's deviance was significantly increased. It was therefore left in the model, as it probably explains a significant amount of the variability. The apparently risk-increasing effect when comparing average hygiene status with sub-optimal hygiene may be attributed to biased farmer responses. No interaction terms between the current fixed effects seemed to be biologically plausible.

The risk for a pig to have PLPN lesions was increased if the farm was in the South Island, or if the farm had more than 600 fattening pigs. On the other hand, the risk was reduced if the farm had a good hygiene status. During the nursery phase, the use of bedding or an automatically controlled environment had a risk-increasing effect, while during the growing phase a liquid manure system or pens with more than 15 pigs were risk factors. An automatically controlled environment seemed to be advantageous for growing pigs. Again, some results were not in agreement with earlier published evidence and are interpreted as surrogate variables for other unmeasured factors.

The over-all fit of random-effects logistic regression models is difficult to estimate. No statistics are readily available, because the deviance will not necessarily have a χ^2-distribution. This means that after a random effect has been fitted, it is not possible to assess excess dispersion in the data (Collett, 1991). A general problem with both models is the limited number of farms in the study, and the lack of variation between these farms. Particularly with respect to EP, most farms had considerable disease problems and there were very few farms with a low prevalence. Consequently, the resulting model does not represent the low prevalence farms very well. However, as the sample reflects the national situation in New Zealand, most farms will have a high prevalence and the results are useful to model the disease process on those farms. This is supported by the high positive predictive values of both models if a farm is classified in the high disease prevalence category.

In summary, it can be concluded that similar risk factors are responsible for the high prevalence of respiratory diseases in pigs in New Zealand as in other intensive pig producing nations. In particular, factors related to stocking density and air quality such as manure management and general hygiene were found to be influential. The experience from overseas shows that, among the various possible control measures, the development of specific patho-

gen free (SPF) herds has been most effective (Christensen and Mousing, 1992; Kuiper *et al.*, 1994). However, SPF herds have difficulties in maintaining their disease-free status in regions with high pig densities, short farm-to-farm distances and complex trade structures. All these impeding factors are not as severe in the New Zealand pig industry. This approach seems therefore – in the long term - worth considering as a solution to the respiratory problem. Other control measures include vaccination programmes and medication.

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References

Bahnson, P.B., Pointon, A.M., Dial, D.G., and Marsh, W.E. (1992). Prevalence of lesions at slaughter at a Minnesota swine slaughter plant. Proceedings of the 12th IPVS Congress, The Hague, 585.

Christensen, N.H. (1995). Evaluation of the effects of enzootic pneumonia in pigs on weight gain and days to slaughter under New Zealand conditions. New Zealand Veterinary Journal 43:146-148.

Christensen, G., and Mousing, J. (1992).Respiratory system. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S., and Taylor, D.J. (eds,). Diseases of Swine. 7th Edition. Iowa State University Press, Ames, 128-162.

Christensen, N.H., and Cullinane, L.C. (1990). Monitoring the health of pigs in New Zealand abattoirs. New Zealand Veterinary Journal 38:136-141.

Collett, D. (1991). Overdispersion. In: Modelling Binary Data. Chapmann & Hall, London, 188-222.

Curtis, R.C., Mauritsen, R.H., Kass, P.H., Salman, M.D., and Erb, H.N. (1993). Ordinary versus random-effects logistic regression for analyzing herd-level calf morbidity and mortality data. Preventive Veterinary Medicine 16:207-222.

Davies, P.R., Bahnson, P.B., Grass, J.J., Marsh, W.E., and Dial, G.D. (1995). Comparison of methods for measurement of enzootic pneumonia lesions in pigs. American Journal of Veterinary Research 56:709-714.

Done, S.H. (1991). Environmental factors affecting the severity of pneumonia in pigs. Veterinary Record 128:582-586.

Goodwin, R.F.W., and Whittlestone, P. (1971). The comparative susceptibility of hysterectomy-produced, colostrum-deprived pigs and naturally born, enzootic-pneumonia-free pigs to enzootic pneumonia. Journal of Hygiene (Cambridge) 69:391-397.

Grest, P. (1995). Zur Prävalenz von Lungenläsionen bei Schlachtschweinen in der Schweiz [Prevalence of lung lesions in Swiss slaughter pigs]. Doctoral Thesis, University of Zurich, Zurich.

Hosmer, D.W., and Lemeshow, S. (1989). Model-building strategies and methods for logistic regression. In: Applied Logistic Regression. John Wiley & Sons, New York, 1989, 82-134.

Kuiper, C.J., Martens, M.R., and van Groenland, G.J. (1994). Specific pathogen-free health programs. Proceedings of the 13th IPVS Congress, Bangkok, 440.

Pointon, A.M. (1995). A review - Evaluation of the pig health monitoring scheme as an industry service. In: Hennessy, D.P., and Cranwell, P.D. (eds.). Manipulating pig production V. Proceedings of the 5th Biennial Conference of the Australasian Pig Science Association (APSA). Australasian Pig Science Association, Werribee, 232-248.

Pointon, A.M., Morrison, R.B., Hill, G., Dargatz, D., and Dial, G. (1990). Monitoring pathology in slaughtered stock: Guidelines for selecting sample size and interpreting results. Proceedings of the 11th IPVS Congress, Lausanne, 393.

Vaillancourt, J.-P., Martineau, G., Morrow, M., Marsh, W., and Robinson, A. (1991). Construction of questionnaires and their use in veterinary medicine. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Annual Meeting, London, 94-106.

Wilson, M.R., Takov, R., Friendship, R.M., Martin, S.W., McMillan, I., Hacker, R.R., and Swaminathan, S. (1986). Prevalence of respiratory diseases and their association with growth rate and space in randomly selected swine herds. Canadian Journal of Veterinary Research 50:209-216.

Wongnarkpet, S. (1995). The epidemiological investigation of pig diseases. PhD Thesis, Massey University, Palmerston North.

Wunderli, F. (1993). Makroskopische Lungenveränderungen bei Schlachtschweinen [Macroscopic lung lesions in slaughter pigs]. Swiss Vet 10:7-10.

CHAPTER 1.3

THE ROLE OF INFECTIOUS AEROSOLS IN DISEASE TRANSMISSION IN PIGS

(A LITERATURE REVIEW)

1. Introduction

Any process that results in the fragmentation of biological material will generate aerosols (Cox and Wathes, 1995). In diseased animals, sneezing and coughing can generate large amounts of airborne particles. Also, particles may originate from faeces and urine splashes. Diseased animals through their activities are a source of infectious aerosol and the inhalation of infectious aerosols by susceptible animals is a route of disease transmission in a number of viral and bacterial diseases. One of the first authors to realise the significance of this process and to investigate aerosol generation and behaviour was Flügge (1897). His opinion was that airborne transmission is possible with any infectious disease, but that it is more likely to occur in some diseases than in others.

In order to fully understand how diseases spread, it is therefore necessary to include research on the possible role of aerosols. Such investigations include aerosol sampling and analysis, both of which require a sound understanding of the behaviour of aerosols as well as the physical, chemical and biochemical factors which influence survival and infectivity of airborne bacteria and viruses (Cox, 1987). This chapter will review the techniques of aerosol investigation and the role of airborne infectious diseases in pigs. First some of the most frequently used terms will be defined.

2. Definitions

AEROSOL: An aerosol consists of solid or liquid particles suspended in air or other gaseous environment (Hirst, 1995). Dust, smoke and fog are examples of aerosols.

AEROBIOLOGY: Aerobiology is part of epidemiology and investigates the airborne transmission process for a variety of sources, particles and targets (Winkler, 1973).

BIOAEROSOL: A bioaerosol is an aerosol comprising particles of biological origin which may affect living organisms through infectivity, allergenicity, toxicity, pharmacological or other processes. Particle sizes may range from 0.5 to 100 μm (Hirst, 1995).

INFECTIOUS AEROSOL: Infectious aerosols form a subgroup of bioaerosols. Infectious aerosols carry pathogenic micro-organisms and therefore have the potential to transmit disease between individuals.

As the scope of this chapter is the review of the role of aerosols in infectious disease transmission, only infectious aerosols will be discussed. Therefore, the term 'aerosol' will in this context imply that the aerosol is infectious. The direct and indirect influence of non-infectious aerosols (gases, dust) on pig health have been discussed elsewhere (Groth, 1984; Gerber *et al.*, 1991; Hartung, 1994; Verstegen *et al.*, 1994) and will not be included. Infectious aerosols can also have allergic and toxic effects (Hartung, 1994; Wathes, 1994), which are undoubtedly important factors affecting particularly the health of housed animals such as pigs. A considerable amount of research has been conducted to cover these issues, the review of which is beyond the scope of this article.

3. The airborne pathway

The airborne disease transmission pathway includes three steps (Winkler, 1973): 1) aerosol generation ('take off' from the source), 2) aerosol transport to susceptible animals ('aerial transport'), and 3) inhalation of aerosols by susceptible animals ('landing' on the target). Disease transmission occurs if two requirements are met: a) infectious aerosols are inhaled by susceptible animals in sufficient concentration, and b) the infectivity of the inhaled aerosols is maintained. By its nature airborne disease transmission is a multifactorial process. The influence of various factors on the three steps in achieving airborne disease transmission in the context of farm animals have earlier been reviewed by Hyslop (1971), Hugh-Jones (1973), and Donaldson (1978). This chapter will provide an update on more recent research findings.

3.1 Factors influencing aerosol production

Airborne particles containing microorganisms can either originate from liquids as droplets or from dry matter. Droplets present a large surface to the air and evaporate quickly. They are thus reduced in size and weight and can remain airborne over long time periods. The residues of these evaporated droplets are called droplet nuclei (Wells, 1955). Droplet nuclei are small enough to be inhaled and therefore play a key role for airborne diseases with the pulmonary alveolus as a port of entry of infection.

Airborne microorganisms typically occur in clusters (Müller *et al.*, 1978). In one study at least 85% of airborne infectious particles contained 2 or more bacterial cells (Fišar *et al.*, 1990). The frequency distribution of the number of bacteria per cluster can be most closely fitted with a log normal distribution.

If airborne infectious microorganisms originate from dry matter they are likely to be associated with dust particles and the concentration of respirable dust may then be correlated with the concentration of respirable bioaerosol particles. However, if the airborne mircoorganisms have a different source than the dust particles, the two concentrations will not be related (Cormier *et al.*, 1990).

Aerosols are generated with particularly high efficiency by animals through sneezing and coughing. Knight (1973) observed that a sneeze in humans produces 1,940,000 particles with more than 75% being smaller than 2 µm. Coughing is less efficient with only 90,765 particles produced, out of which more than 95% are smaller than 2 µm. The size of the particles is relevant because it influences the time until they settle and also therefore the depth of penetration in the respiratory tract upon inhalation. The size of airborne particles is expressed as the aerodynamic diameter rather than their geometric diameter (Heber, 1995). The aerodynamic diameter is the diameter of a unit-density sphere with the same resistance to motion. Thus the effect of non-spherical shapes is accounted for.

Particles can also be suspended in normally exhaled breath though in lower concentrations. Depending on the activity of a subject, up to 4 particles per cm^3 can be excreted (human data, Fairchild and Stampfer, 1987). Furthermore, aerosols can originate from faeces or from urine splashes, including aerosols generated by spraying of slurry (Deans Rankin and Taylor, 1969; Boutin *et al.*, 1988). Other sources of aerosols are bedding and feed (Fišer and Kral, 1969). Aerosol generation is positively correlated with the level of animal activity (Pedersen, 1993;

Bönsch and Hoy, 1996). This induces diurnal rhythms in aerosol concentrations with highest levels during the day when animals are active, for example during feeding (Müller *et al.*, 1989; van Wicklen, 1989).

The concentration of infectious agents in aerosols is also directly proportional to the strength of the aerosol source. Indicators of the source strength are the number and concentration of infected animals on a farm (herd size, stocking density) or a region (pig density).

3.2 Factors influencing aerosol decay

After take-off, infectious aerosols are subject to both biological and physical decay. Biological decay includes factors that affect the survival (ability to replicate) of airborne microorganisms and/or their infectivity (ability to cause infection), survival being a prerequisite for infectivity (Cox, 1995). Factors influencing aerosol decay are typically characteristics of the micro-climate (indoors) or the atmospheric climate (outdoors).

The most important factor for biological decay is the change in water content. The ideal ambient relative humidity (RH) to maintain survival of airborne microorganisms varies with the nature of the agent (Cox, 1989). Viruses containing structural lipids (for example influenza virus) are hydrophobic and generally more stable than lipid-free viruses. Viruses with structural lipids survive best in dry air (RH <50-70%; de Jong *et al.*, 1973; Cox, 1995). Lipid-free viruses on the other hand (for example foot-and-mouth disease virus) are most stable in moist air.

Airborne bacteria have been shown to have narrow critical RH bands for survival, and some species are very sensitive to oxygen. Gram-negative bacteria are more stable at low RH as their phospho-lipid membranes most readily denature at mid to high RH (Cox, 1995). Unfortunately, the details of survival of airborne bacteria have not been studied in detail for many species.

How exactly RH affects airborne microorganisms is difficult to investigate but most authors agree that surface damage (for inactivation at high RH) and dehydration (for inactivation at low RH) are likely to be the most influential factors (de Jong *et al.*, 1973). This hypothesis is supported by evidence that survival can be greatly influenced by the composition of the suspending fluid prior to aerosol generation (Akers, 1973; Cox, 1995).

Further factors that may increase the biological decay of aerosols are radiation, ozone reaction products (also referred to as 'open air factor', OAF), air ions and pollutants. These factors are technically difficult to study and even less literature is available. However, OAF sensitivity has been related to the lipid composition of a virus, and foot-and-mouth disease virus as well as swine vesicular virus have been reported to be relatively OAF-resistant (Cox, 1987). This may be important to allow long-distance transmission.

Physical decay of aerosols depends on the time the particles remain suspended, which is influenced by particle size and particle deposition processes. Because air temperature and RH influence particle aggregation and net water flow, they also influence particle size and consequently particle concentration. The more hygroscopic a particle, the larger it gets in a humid environment and the faster is its sedimentation rate. Aerosols generally become unstable at a RH of 85% or higher (Beer *et al.*, 1975).

The influence of ambient temperature on airborne particle concentration has been studied in numerous articles and the authors generally agree that the concentration of airborne particles is increased at low temperatures (Fišer and Král, 1969; Heber *et al.*, 1988a; Butera *et al.*, 1991). Curtis and colleagues (1975a) quantified this relationship. They found that the common logarithm of the number of bacterial colony forming particles increases by 0.02 per Celsius degree decrease in median temperature for the day. Airborne bacterial concentrations were also found to be higher in winter than in summer (Fišer and Král, 1969; Hysek *et al.*, 1991). However, Jones and Webster (1981) found airborne particle concentration to be reduced in calf houses during cold, dry weather periods as opposed to mild, humid periods. The reduction particularly affected particles of respirable size. Because of the different management systems and ventilation regimes in these studies, the interpretation of the results is difficult as a consequence of many influential but uncontrolled factors. The direct comparison of results needs to be performed with caution.

The dilution effect of ventilation on aerosol concentrations has been a matter of dispute with some authors describing a reducing effect (Heber *et al.*, 1988a) and some authors describing no effect (Butera *et al.*, 1991). If a reducing effect was observed, higher ventilation rates seemed to reduce larger particles more rapidly than smaller ones (Pickrell *et al.*, 1993). Nillsson (1982, cited by Hartung, 1989) found an increase of dust levels when the ventilation rate was increased during periods of higher temperatures. It is clear that the effect of the ventilation strongly depends on the ventilation characteristics such as the incoming jet direction (Ikeguchi and Nara, 1992). Again, the impossibility to fully control these design effects may explain the contradictory results. Additionally, a modelling approach to explore the protective effects of building ventilation demonstrated that as the infection pressure rises (more infected animals) ventilation offers progressively less reduction of the aerosol concentration (Nardell *et al.*, 1991). This interaction needs also to be accounted for if the influence of ventilation is to be measured accurately.

The total number of airborne bacteria in pig houses is highly variable as it depends on a number of factors influencing aerosol generation and decay (see above). The results of a selection of the numerous published studies were summarised by Müller *et al.* (1989) and range from 200-300 colony forming units (CFU)/l air to several thousand CFU/l air. Spatial variability can introduce a bias to airborne particle counting (Conceicao, 1989; Barber *et al.*, 1991), and several measurements at different locations are necessary to determine the actual concentration.

Airflow models have been established to investigate the indoor distribution of aerosols (Smith *et al.*, 1993; Heber *et al.*, 1996; Hoff and Bundy, 1996). Airflow is not only influenced by the design of the building and by ventilation, but also by the animals. In fact, animal activity can be just as important in determining the spatial concentration of infectious particles as ventilation (Smith *et al.*, 1993).

Long distance transport of airborne microorganisms depends on atmospheric dispersion and associated dilution of the aerosol plume as well as deposition mechanisms. The 'footprint' of the infectious plumes may vary greatly in length over time and direction simulatenously. Plume dispersal is influenced by topography (Mason and Sykes, 1981) and by meteorological factors (Bartlett, 1973; Smith, 1983). Generally, longer transmission distances are achieved in a stable atmosphere. Turbulence is mainly generated by high wind speed and insolation (Pasquill, 1961). Different models have been used to predict plume dispersion. Traditionally, the

Gaussian dispersal model has been used (Müller *et al.*, 1978; Gloster *et al.*, 1981; Donaldson *et al.*, 1982a; Grant *et al.*, 1994). These models require as input the aerosol source strength, wind strength, diffusion parameters describing the stability of the atmosphere, the height of emission and an estimate for the biological survival as well as the sedimentation. The output is the aerosol concentration at different locations downwind. A computer model that was originally designed to predict the dispersion of toxic gases using a Gaussian distribution was able to predict the development of foot-and-mouth disease as well as Aujeszky's disease epidemics with reasonable accuracy (Casal *et al.*, 1997). More recently so-called puff models have been introduced, that can take into account topography (Mikkelsen *et al.*, 1984). Over short distances and in flat areas, both types of models produce similarly accurate results. When predicting long-range transmission however, the puff models are preferable due to their capability to model the three-dimensional space.

Airborne particles may be deposited by either wet deposition including precipitation in rain or fog or by dry deposition. The latter can either be due to gravitation or occurs when particles are being impacted onto surfaces by air currents. Particles with a size of 1 µm have a settling velocity of 0.003 cm/s (Cox, 1989). Particles of this size are therefore unlikely to settle at all and will only be removed from the air by other deposition processes, for example by filtration. When considering dry deposition, vegetation acts as a filter increasing deposition, provided wind speed is sufficiently high (Gregory, 1973). In the case of grass that is then ingested by animals, this can pose a considerable indirect infection hazard.

It has been argued that the efficiency of wet deposition by precipitation is significant only for sub-micron particles and over long transport distance (Chamberlain, 1970), while other authors consider wet deposition to be an important factor in general (Gregory, 1973).

In summary, long distance transport and survival of airborne agents are favoured by cool, damp, calm conditions in the absence of sunlight over flat, vegetation-free areas or water. If meteorological data is available, the form and concentration of the plume can be modelled. However, due to the complexity of the process predictions always need to be performed in close collaboration with a meteorologist (Smith, 1983).

3.3 Factors influencing aerosol inhalation and infection

When animals inhale aerosols, particles are deposited in the respiratory tract according to the particle size. Particles of a size of 6 µm or greater are trapped in the nose, while particles <2 µm may get as far as the lower respiratory tract and the alveoli (human data, Knight, 1973). Hygroscopic particles will increase in size as they pass through the saturated air in the respiratory tract. Hygroscopic particles of a size of 1.5 µm were found to be deposited in the nose, pharynx and secondary bronchi, tertiary bronchi to respiratory bronchi and alveolar ducts at ratios of 36, 1, 25, and 21%, respectively. A total of 83% of these particles were retained in the respiratory tract (human data, Knight, 1973).

The respirable size fraction (<5 µm) of aerosols and total bacteria counts in a pig house are highly variable. In three studies, the respirable fraction of total airborne bacteria was found to be 26%, 11-31% and 48%, respectively (Clark *et al.*, 1983; Curtis *et al.*, 1975b; Cormier *et al.*, 1990). Dust particle distributions inside a pig house seem to be log-normally distributed (Heber *et al.*, 1988b) with 50% of all particles <2.6 µm. Log-normal distributions were also

found when investigating airborne particle concentrations in other environments, both outdoors and indoors (Heber, 1995; Digiorgio *et al.*, 1996; Straja and Leonard, 1996).

The minimal infective dose for respiratory infection of animals exposed to infectious aerosols depends on the pathogenicity of the infectious agent and the susceptibility of the animal. The minimal infectious dose needs to be experimentally established using well-defined and controlled aerosols (Hensel *et al.*, 1993). Early data from tuberculosis experiments showed that for agents that are well adapted to airborne transmission the infective dose can be as low as 2 CFU (O'Grady and Riley, 1963). Donaldson *et al.* (1987) showed that there can be differences between virus strains and that low infective doses can induce subclinical infection. The time required until the minimal infective dose is accumulated depends on the respiratory volume, the concentration of organisms in the air and the clearance rate of the respiratory tract.

At the farm level, the probability of disease transmission also depends on the number and type of susceptible animals. The more animals inhaling aerosols the more likely is the infection of at least one of them. Therefore, herd size is a risk factor for airborne disease transmission (Willeberg *et al.*, 1994). Also, larger animals have a higher tidal air volume than smaller animals. The tidal air volume for a 25 kg pig has been reported to be 9.27 l/min (Brody, 1945). Higher tidal volumes again increase the possibility of inhaling the necessary number of airborne particles to transmit disease. For this reason pig farms are at lower risk of airborne FMD-infection than cattle farms (Sellers, 1971). Similarly, younger pigs are in terms of inhaled volumes at lower risk to contract airborne diseases than larger pigs. For this reason, the scale of heat producing units (HPU) was used as an approximation of the respiration volume (Laube, 1996; TABLE 12). HPUs relate to the physiological heat production of pigs and thus are proportional to their size and respiratory volume.

TABLE 12. Relationship between body weight of pigs and heat producing units

Body weight [kg]	HPU
>80	1
51-80	0.62
31-50	0.41
<30	0.24

The success of disease transmission may also depend on further indirect factors influencing the animals' immune response, such as disease status or environmental factors, some of which may be hard to measure. Wathes *et al.* (1989) for example demonstrated a relationship between cold-stress and susceptibility to aerosol infection with *Escherichia (E.) coli*.

With help of the factors influencing airborne disease transmission, the dynamics of the disease can then be modelled. Martin (1967) developed a mathematical model predicting the transmission pattern for respiratory disease in calves. This model included factors influencing the concentration of infectious particles (number of diseased animals, size of building, excretion rate), factors reducing the concentration of infectious particles (ventilation rate) and factors affecting the infection of susceptible animals (respiration volume, exposure duration, minimal infective dose). The infection was simulated in waves. The author acknowledges that

the model is not realistic because it assumes uniform distribution of infectivity, but it can be used to investigate the influence of the different parameters. Stochastic models should produce more realistic results, but to my knowledge such models have not yet been developed to simulate the spread of airborne diseases at the farm level.

4. Aerosol sampling

The most commonly used principles for aerosol sampling are 1) filtration, 2) impaction, 3) impingement, and 4) centrifugal sampling, each of which is briefly described in TABLE 13. Each technique has advantages but also a number of drawbacks (TABLE 13).

Different sampling techniques have been evaluated in pig houses and with pig pathogens (for example: Thorne and Burrows, 1960; Hurtienne, 1967; Donaldson *et al.*, 1982b; Crook *et al.*, 1989; Palmgren and Strom, 1989; Thorne *et al.*, 1992).

It is generally agreed that there is no single air sampling technique that is ideal under all circumstances and to meet all possible goals of a study (Hurtienne, 1967; Cox, 1987; Mouilleseaux, 1990). The European Commission organised a workshop on aerosol sampling in animal houses, which induced a series of recommendations (Wathes and Randall, 1989). The authors recommend cyclone samplers as the method of choice for most research objectives requiring air sampling in animal houses because of its high collection efficiency and the reduced collection stress. However, more research is needed to establish reliable aerosol sampling standards.

When sampling airborne microorganisms in animal houses, a number of factors can influence the results (Hartung, 1989). Due to the spatial variability of aerosol concentrations (Robertson, 1989; Conceicao, 1989; Barber *et al.*, 1991; Smith *et al.*, 1993; Mehta *et al.*, 1996), it is recommended to use sampling locations that are related to behaviour and height of the animals. Not only one but several sampling locations should be used. In order to account for the animals' activity pattern and temporal variation of airborne particle concentrations (Smith *et al.*, 1993), measurements should be performed over 24 hours. Factors known to influence aerosol concentrations have to be recorded: animals (species, type, number, age, stocking density, clinical disease history, behaviour and activity), building (orientation, dimensions, volume, lay out, floor type, pen wall design, ventilation system), feeding (method, equipment, feeding times and duration, type of feed, fat and water content of feed), manure system (type of bedding, removal system, quantity in building) and environment (temperature, relative humidity, ventilation rate, gas concentrations, air speed and direction).

TABLE 13. Air sampling methods and their characteristics (modified after Hecker and Meier, 1991)

Sampling method (Example)	Principle	Advantages	Disadvantages	References
Filter (Cellulose membrane filter)	While air is being pumped through the filter the particles are caught in the pores of defined size.	Simple and inexpensive equipment, very efficient.	Dehydration effects, potentially difficult removal of material from filter.	Fields *et al.* 1974; Lundholm, 1982; Hecker *et al.*, 1983; Palmgren *et al.*, 1986; Parks *et al.*, 1996
Impaction (Andersen viable sampler)	As a consequence of an abrupt change of the airflow direction, particles are impacted onto a solid surface, e.g. an agar plate.	Collection of several particle size categories is possible, if several stages are used in series where the air passes through perforated plates with different sized holes, high efficiency.	Risk of particle overload, limited sampling duration due to agar desiccation, expensive and time consuming.	Andersen, 1958; May, 1975; Lundholm, 1982; Mehta *et al.*, 1996
Impinger (AGI-30 all glass impinger)	Air is blown at high speed onto a liquid whereby airborne particles get impinged into the liquid.	No limited sampling duration, liquid sample can be used for a series of tests, separation of clustered bacteria.	Evaporation losses, need to analyse samples quickly, low flow rates, supplementation may be needed of impinger liquid, composition of collection liquid may be crucial for agent survival.	May and Harper, 1957; Lundholm, 1982; Hensel, 1994; Terzieva *et al.*, 1996;
Centrifugal sampler (Cyclone sampler)	Creation of a vortex by which particles are impacted upon a liquid or semi-solid collection surface on the sampler wall.	Liquid samples can be used for a series of tests, no limited sampling duration, method of choice for high volume sampling.	Evaporation losses, complicated technology, difficult calibration.	Errington and Powell, 1969; White *et al.*, 1975

5. Aerosol sample analysis

Once aerosols have been collected, the samples are analysed to measure the amount and type of microorganisms that have been caught. The choice of an appropriate approach depends on the study objective. Not all methods are possible with all sampling techniques. It also has to be decided, whether all microorganisms should be detected or only live organisms.

Plate counting: To investigate airborne bacteria, aerosols may be directly impacted onto culture plates, or collection liquids may be plated on agar immediately after sampling. Filters may also be placed directly on to agar plates for culture. After appropriate incubation microbial colonies from deposited particles are counted and, if the sampled air volume is known, the concentration of colony forming units (CFU) per m^3 air can be calculated. There is a limit in terms of contamination and of particle concentration for visual counting methods. If overgrowth occurs, the sampled air volume needs to be reduced. Plate count technique faces its limits for slow growing microorganisms and if specific identification of species is required. Also, up to 90% of microorganisms may to be viable but not culturable after aerosolisation (Heidelberg *et al.*, 1997), resulting in a severe underestimation of the bioaerosol burden. Therefore, alternative techniques should be used whenever possible.

Cell cultures: Aerosol collecting fluids that have been collected to detect airborne virus can be brought on to living cell culture monolayers susceptible to the virus under consideration. Infection of the cells leads to cell death and the formation of plaques. The number of plaque forming units (PFU) can then be counted and the concentrations titrated. Reliable results depend largely on the sampling conditions and the type of collecting liquid that is used (Bourgueil *et al.*, 1992a).

Microscopy (Lacey *et al.*, 1989; Morris, 1995): Light microscopy is a traditional and still important and relatively simple technique for direct visualisation of aerosol particles. In combination with fluorescence and specific antibody stains immunofluorescence microscopy allows precise identification of microorganisms. The limit lies in the resolution, which is about 0.2 mm. Scanning electron microscopes resolution lies around 10 nm, but this technique is expensive and labour-intensive. Recently new computer-based image analysis systems have been developed. These may offer new efficient ways for automatic sample analysis.

Antibody-based detection of specific microbial agents and assays for the detection of specific microbial nucleic acids (polymerase chain reaction [PCR]) or microbial products (enzymes, metabolites) have also been developed (Hensel and Petzold, 1995). Recently, new assays based on the investigation of molecular structures have been used for the analysis of air samples (Olsson *et al.*, 1996; also see CHAPTER 1.4). These techniques are very sensitive and specific. The fact that microorganisms do not have to survive the sampling process in order to be detectable by PCR is an advantage. These techniques help with previously difficult-to-detect pathogens (Eisenstein, 1990).

6. Airborne diseases in pigs

The documentation of the full airborne pathway of a specific disease ideally requires the investigation of the release of organisms in aerosol form by infected animals, specification of

survival requirements in the airborne state, and definition of minimal infective dose for susceptible animals by the aerosol route. Once these figures have been established, maximal transport distances can be calculated for given source strengths, meteorological conditions and target herds. Currently few pig diseases have been completely investigated with respect to these characteristics (TABLE 14) because the experiments required are technically complex and require expensive equipment.

More often, the hypothesis of airborne transmission is inferred indirectly based on epidemiological evidence. If disease transmission depends on risk factors such as herd size, distance to nearest infected herds, size of nearest herd and animal density in the area, it is likely that aerosols are involved as these factors are crucial for the determination of plume dispersal.

TABLE 14. Airborne infectious diseases in pigs: accumulated evidence

Disease	Field evidence	Isolation from air	% RH for best survival	Minimal infective dose
Foot-and-mouth disease	yes	yes	>60	2.6 $\log_{10}$ LD_{50}
Swine vesicular disease	no	yes	>55	?[a]
Aujeszky's disease (Pseudorabies)	yes	yes	55	4.5 $\log_{10}$ $TCID_{50}$
Influenza	yes	yes	?	?
Porcine respiratory and reproductive syndrome	yes	no	?	?
African swine fever	no	yes[b]	20-30	?
Classical swine fever	no	no	?	?
Porcine respiratory coronavirus	yes	yes	?	?
Enzootic pneumonia	yes	yes	<25 or >75[c]	?
Actinobacillosis	yes	yes	?	10^4-10^9 CFU/ml
Arthrophic rhinitis	yes	yes	75	?

[a]?=not known.
[b]Indirectly using mouse infection as a biological indicator.
[c]Established with other *Mycoplasma* spp.

6.1 Foot and mouth disease

Foot-and-mouth disease (FMD) is probably the most researched disease in terms of airborne virus transmission in veterinary medicine (Donaldson, 1979). Infected animals excrete FMD-virus during a period of time that may start before the first clinical signs can be detected. Up to 8.6 $\log_{10}$ $TCID_{50}$ virus particles are shed per pig per day (Sellers, 1971; Donaldson and Ferris, 1982). About 70% of the infectivity excreted into the air is associated with particles >6 μm, 19-24% with particles 3-6 μm, and 10-11% with particles <3 μm (Sellers and Parker, 1969; Donaldson *et al.*, 1987). Virus survival in the airborne state largely depends on air humidity. For FMD-virus survival is best at a high RH of >55-60% (Donaldson, 1972; Donaldson, 1973; Gloster *et al.*, 1981). Under such conditions and if suspended from milk, nasal fluid or cell culture fluid, FMD virus may remain viable with almost no decay over one hour (Donaldson, 1973; Barlow and Donaldson, 1973). In terms of long-distance transmission it has been calculated that, given a RH of 60% and a wind speed of 10m/s, virus could survive over 2.7 hours necessary to travel over 100 km (Donaldson, 1979). The minimal infective

dose for respiratory infection of pigs is 2.6 $\log_{10}$ mouse ID_{50} (Terpstra, 1972, cited by Donaldson, 1986). However, cattle become more readily infected via the airborne route because they have a larger tidal volume and will therefore inhale more infectious particles.

The transmission of FMD virus in aerosol plumes was modelled using mathematical dispersal formulae (Wright, 1969, cited by Donaldson, 1979; Gloster, 1982). Earlier work was based on the Gaussian dispersion function while later simulations used the puff model, which allows a three dimensional projection of the plume (Donaldson *et al.*, 1982a; Rumney, 1986; Sanson, 1993; Moutou and and Durrand, 1994). A critical step in the development of mathematical models is to validate them using field data. This has been successfully achieved for FMD transmission models (Donaldson *et al.*, 1982a; Donaldson *et al.*, 1988; Maragon *et al.*, 1994).

It was also shown that apart from infected animals, aerosols could origin from incineration of infected carcasses (Smith and Hugh-Jones, 1969), filling of milk tankers (Donaldson, 1973; Dawson, 1970) or splashes of milk or rain on infected ground (Gregory, 1971, cited by Donaldson, 1979). Aerosol risk from spreading infected faecal slurry is likely to be considerable, as faeces are highly contaminated (Donaldson, 1973; Donaldson, 1979).

6.2 Swine vesicular disease

Swine vesicular disease virus is excreted in aerosol form by infected animals for 2-3 days during the disease (Sellers and Herniman, 1974), but air concentrations are much lower than with FMD. The virus is also stable in aerosol form at RH >55% (Donaldson and Ferris, 1974). However, epidemiological outbreak data does not support the theory that airborne spread of the disease is very common (Donaldson and Ferris, 1974).

6.3 Aujeszky's disease

Aerosol transmission of Aujeszky's disease virus (ADV) was suspected by Gloster *et al.* (1984) and later demonstrated by epidemiological investigations in Denmark (Christensen *et al.*, 1990, 1993). The transmission of ADV for up to 9 km has also been reported from the UK (Taylor, 1988). It was shown that the status of neighbouring herds as well as the pig density in a region has an influence on the risk of a herd becoming infected with ADV (Leontides *et al.*, 1994a; Norman *et al.*, 1996; Auvigne and Hery, 1997). Herd size is also a risk factor for ADV infection (Leontides *et al.*, 1994b).

ADV was isolated from the air of rooms housing infected pigs (Donaldson *et al.*, 1983; Mack *et al.* 1986). The mean 24-hour excretion rate per pig was 5.2-5.3 $\log_{10}$ $TCID_{50}$. The survival of airborne ADV was best at 55% RH and at 4 °C (Schoenbaum *et al.*, 1990). Under such conditions a 50% decrease of the aerosol concentration occurs in <1 hour. A correlation was found between the concentration of airborne ADV in a room and the quantity of virus collected on nasal swabs from infected control pigs housed in the same room (Bourgueil *et al.*, 1992a).

It was found that the virus survives in slurry (Mack *et al.*, 1986; Bøtner, 1991), the spraying of which can therefore be a possible source of infectious aerosols. Furthermore, ADV was also isolated from dust (Vannier *et al.*, 1989), which may become airborne and generate in-

fectious aerosols. Gillespie *et al.*, (1996) demonstrated that pigs can be infected by aerosols with a total estimated dose of 4.5 $\log_{10}$ $TCID_{50}$. It was demonstrated that sentinel pigs exposed to air drawn from a room with infected pigs or housed in the same room as infected pigs, seroconverted (Donaldson *et al.*, 1983; Gillespie *et al.*, 1996).

The application of a Gaussian aerosol diffusion model showed that it fitted well to the spread of ADV in an area in the USA (Grant *et al.*, 1994). In this study, the model was applied in an area where 10 farms were infected with ADV during a cold weather period. These weather conditions were presumably associated with low ventilation rates. The distance between the farms was 1.3-13.8 km. Mortensen *et al.* (1994) showed that the use of meteorological data is useful for the prediction of airborne ADV infection. If such meteorological prediction models had sufficient sensitivity and specificity, they could be used to identify high-risk herds in an outbreak.

6.4 Influenza

Quantitative evidence of airborne transmission of influenza virus was provided by Schulman (1968). He developed an infection model where mice were exposed to either air drawn from cages housing infected mice or to artificially created infectious aerosols. The results of these experiments showed that the incidence of infection influenced by the airflow rate and by the relative humidity of the air. Infections were significantly more prevalent at low RH. Schulman was also able to isolate influenza virus from air samples collected in the environment of infected mice.

Very little information is available on airborne porcine influenza. Most research has concentrated on the zoonotic significance of the disease. Porcine influenza virus has the potential to infect humans as the following example demonstrates. Two people collecting nasal swabs from experimentally infected pigs developed symptoms despite wearing protective gear according to the standards of animal biosafety level 3 (disposable cloths, goggles, disposable gloves, hairnets and masks; Wentworth *et al.*, 1997). On one day only, a mask with lower protective capacity was inadvertently used. Thus, airborne transmission is likely to have occurred.

Comparative studies performed on different strains of influenza A virus have shown that human and porcine strains are similarly sensitive to decay in aerosol form and less resistant than avian and equine influenza viruses (Mitchell and Guerin, 1972). Human and porcine influenza virus survived for up to 15 hours at a RH of 15% and a temperature of 21°C.

6.5 Porcine respiratory and reproductive syndrome (PRRS)

Field evidence of airborne transmission of PRRS virus was reported by Robertson (1992). It was suspected that the virus could survive in the airborne state over distances up to 3 km. An influence of meteorological factors enhancing airborne transmission under conditions associated with low temperature, high humidity and low wind speed was also described (Komijn *et al.*, 1991, quoted by Albina, 1997). PRRS was first described in 1987 (Hill, 1990) and the hypothesis of airborne spread has not yet been fully investigated. Direct evidence of aerosol transmission is not available. In fact, it has been shown that under experimental conditions

airborne transmission is extremely difficult to achieve (Wills *et al.*, 1994), although aerosol infection is routinely used in infection studies (van Reeth, 1997). A field study investigating risk factors for PRRS infection in Denmark was also not able to demonstrate an influence of herd size (Mousing *et al.*, 1997), a risk factor commonly associated with airborne transmission. However, under experimental conditions piglets seroconverted after they had been exposed to air drawn from a cage housing infected animals (Torremorell *et al.*, 1996). However, only 3 of 16 pigs were infected and the development of antibodies was delayed when compared with the immune reaction in intranasally challenged pigs. This evidence supports at this stage the hypothesis that airborne transmission is probably not an important way of PRRS spread.

6.6 Classical and African swine fever

Donaldson and Ferris (1976) investigated the airborne survival of African swine fever virus (ASFV). The virus was not sensitive to a range of RH if exposed for a short time survival (1 s), but it was very sensitive to moist conditions when stored for 5 minutes. The optimal survival conditions seem to be at 20-30% RH. In an experiment, air was passed from a room housing infected pigs through ducting to a room with susceptible pigs, to assess the possibility of airborne transmission (Wilkinson *et al.*, 1977). In the same trial, susceptible pigs were also housed on a solid wood platform placed 2.3 m above infected animals. Both groups of recipient pigs were infected with ASFV and developed acute disease. However, attempts to isolate virus from the air in the room with infected pigs were not successful. It was concluded that airborne spread of ASFV is likely to be a problem in intensive housing systems.

Classical swine fever virus has been listed as possibly airborne by Falk and Hunt (1980), but this means of transmission is generally believed to be of minor epidemiological importance. The attempt to isolate classical swine fever virus from the air housing experimentally infected pigs has not been successful (see CHAPTER 2.2). Whether this was due to a lack of sensitivity of the air sampling system or to the absence of airborne virus could not be determined. Further studies under field conditions are required.

6.7 Porcine respiratory corona virus (PRCV)

The hypothesis of airborne transmission of PRCV was raised by an epidemiological study performed in Denmark (Flori *et al.*, 1995). In this survey it was found that the serological status of neighbouring herds and the distance to such herds are risk factors for a serologically PRCV-positive status of a herd. An increase of the distance from the nearest infected herd by 100 m was associated with a change in odds ratio of 0.85. Herd size was found to be a possible risk modifier. In Belgium, herd density was also described to be a relevant risk factor (Pensaert *et al.*, 1993). In the same report, an association between re-infection and distance to and herd size of the nearest pig farm was described. PRCV was also more readily introduced in farms in the colder seasons.

It has been shown that experimentally infected pigs produce airborne virus from day 1 to day 6 after infection (Bourgueil *et al.*, 1992b) and that aerosol sampling was particularly efficient

when protective agents were added to the collection fluid. Given this evidence, airborne transmission seems to be a likely way of infection for PRCV.

6.8 Enzootic pneumonia (EP)

Epidemiological studies of risk factors for EP transmission have suggested that airborne infection may be an important mechanism of disease spread between herds (Goodwin, 1985; Jorsal and Thomsen, 1988; Stärk *et al.* 1992a). The infection risk also seems to be climate-dependent which is another indicator of aerosol involvement (Stärk *et al.*, 1992b).

Early attempts to isolate *Mycoplasma hyopneumoniae* from air were indicative of its occurrence but failed to provide conclusive evidence (Tamási, 1973). Various avian mycoplasma strains have been recovered from aerosols up to 24 hours after generation (Beard and Anderson, 1967; Lloyd and Etheridge, 1974). This showed possible survival at 25°C and RH of 40-50%. Recently, *Mycoplasma hyopneumoniae* was isolated from the air with the help of a nested PCR assay (see CHAPTER 1.4).

Aerosol infection models for EP were successfully established by Jakab *et al.* (1991). An aerosol generated from culture medium containing 10^7 cells/ml induced minor lung lesions and a reduction in daily weight gain and *M. hyopneumoniae* could be isolated from the lungs of these pigs, although the animals remained clinically normal.

Furthermore, aerosol immunisation was described to be an effective way to protect animals (Murphy *et al.*, 1993). This is indicative of an agent well adapted to airborne transmission.

6.9 Pleuropneumonia

Although *Actinobacillus (A.) pleuropneumoniae* has not yet been isolated from the air in pig houses, there seems to be little doubt about the importance of aerosol transmission for this agent.

An aerosol infection model for *A. pleuropneumoniae* was recently developed (Hensel *et al.*, 1993; Hensel *et al.*, 1996; Jacobsen *et al.*, 1996). Aerosols of suspensions containing concentrations of 10^4 CFU/ml of biotype 1, serotypes 2, 5b and 6 induced lesions of haemorrhagic necrotising pneumonia. For the less virulent biotype 2 the infectious dose was 10^9 CFU/ml of suspension. This model is expected to be useful for virulence studies in the future.

Aerosol immunisation was investigated by numerous authors (Nielsen *et al.*, 1990; Bosse *et al.*, 1992; Loftager *et al.*, 1993; Hensel *et al.*; 1995). They found that aerosol-vaccinated pigs developed less severe pneumonia than non-vaccinated pigs. Hensel *et al.* (1995) found that inhalation of *A. pleuropneumoniae* may lead to an asymptomatic carrier stage in some pigs that could spread the disease under field conditions. This supports the epidemiological importance of the aerosol transmission.

6.10 Atrophic rhinitis

Atrophic rhinitis is another respiratory disease that has been investigated for airborne transmission as the degree of turbinate atrophy was found to be correlated with airborne bacteria concentrations (Robertson *et al.*, 1990). The two agents involved are *Pasteurella (P.) multocida* and *Bordetella (B.) bronchiseptica.*

In 29 out of 44 herds, Airborne *P. multocida* was found in small numbers, 32 CFU/m^3 (Bækbo and Nielsen, 1988). *B. bronchiseptica* was isolated from the air in commercial pig houses, too (Stehmann *et al.*, 1991a).

The biological decay of *P. multocida* and *B. bronchiseptica* were found to be 18-22 hours for 50% reduction when analysed on dry particle carriers. The influence of temperature and RH was found to be of little importance (Stehmann *et al.*, 1991b). In an aersosol chamber, the halftime of *P. multocida* and *B. bronchiseptica* strains at 23°C and 75% RH was 19 min and 56.7 min, respectively (Müller *et al.*, 1991).

Aerosol immunisation with *P. multocida* seemed to increase the alveolar clearance and possibly reduce the impact of a subsequent challenge (Müller and Heilmann, 1990).

6.11 Other diseases

The possibility of airborne transmission cannot be excluded for some enteric diseases, where aerosols could be generated from manure and water splashes during intensive cleaning or from manure and waste disposal practices. A study on risk factors for transmissible gastroenteritis showed an increased risk for seropositivity for herds with more than 2 farms in a 3 mile radius (Yanga *et al.*, 1995). A study on the transmission of *Salmonella* spp. within a calf unit revealed patterns more consistent with airborne spread than with transmission between contiguous pens (Hardman *et al.*, 1991). The fact that aerosol transmission of *Salmonella* is possible in chickens and calves (Clemmer *et al.*, 1960; Wathes *et al.*, 1988) and that the isolation of *Salmonella enteritidis* from the air of rooms housing chickens (Lever and Williams, 1996) indicates a possible role of the airborne infection route with this disease that might also be relevant for pig producers.

7. Prevention of airborne disease in pig production

Any measure reducing the number of airborne particles will directly reduce the risk of airborne disease transmission (Hartung, 1994). One has to distinguish between airborne disease transmission within a unit and between units. For within unit transmission, the following options are available.

A first step in aerosol reduction is dust prevention. As feed is the major source of airborne dust (Honey and McQuitty, 1979), possible measures to reduce the dust load could include adding tallow or soybean oil or water to the feed (Heber *et al.*, 1988a). A recent study showed that the application of a water-soybean oil emulsion aerosol reduced the concentration of airborne dust by 18% (Bönsch and Hoy, 1996). Also, excessive and unnecessary animal activity, such as movement of animals, should be avoided. Also the relative humidity of the air should

not drop below 60% (Hartung, 1994). Correctly designed ventilation systems and sufficient air space per animal (e.g. 3 m^3 per fattening pig) can help reduce particle concentration. The use of small sub-units with independent air spaces has also been advocated (Martin, 1967).

Air filtration combined with positive pressure ventilation has been studied as a second measure to reduce aerosols. Pigs housed in a room equipped with an air filter removing particles >5µm reached market weight significantly earlier (6-8 days) than the control group (Carpenter *et al.*, 1986). In the filter-equipped room, total particles, dust mass and bacterial CFU were significantly reduced. A similar study performed with veal calves reported a significant effect of air filtration on the number of treatments and total antibiotic usage (Pritschard *et al.*, 1981). However, such equipment is expensive and probably not practical under field conditions (Donaldson, 1978).

Other dust reducing measures such as dust control by air cleaning, electrostatic precipitation, dry filtration and wet scrubbing, were described by Carpenter (1987). The decontamination of the air by aerosol disinfectants (e.g. Narcosept 0.2%, chlorinated lime, Lugol's solition + 1% NaOH, 2% lactic acid, 0.1% Antigerm) is another option for short-term reduction of airborne bacteria (Sobih *et al.*, 1991).

A different approach to reducing within unit spread is the use of vaccines. It has been shown for enzootic pneumonia and for ADV that the shedding of airborne pathogens is reduced but not totally eliminated in vaccinated animals (Schatzmann *et al.*, 1996; Bourgueil *et al.*, 1992a).

The prevention of between unit spread of airborne diseases seems to be more difficult. Physical separation (housing) is not likely to be sufficient in order to avoid significant aerosol contact (Smith, 1983). Ideally, the geographical location of the unit should be selected in an area with low pig density and at a distance from neighbouring herds known to be infected with diseases subject to airborne spread. Müller *et al.* (1978) observed that a minimal distance between units of 150 m significantly reduces the aerosol challenge but cannot prevent airborne infections. As the choice of location will hardly ever be offered, other measures are needed. Again, air filtration or vaccination may be options. More promising seems the attempt to create areas free from specific diseases with the help of regional eradication programs (Laube *et al.*, 1996). Special attention should also be paid to slurry and dung spreading, as these operations may result in pollution at a distance of up to 600 m (Errington and Powell, 1969).

References

Albina, E. (1997). Epidemiology of porcine reproductive and respiratory syndrome (PRRS): An overview. Veterinary Microbiology 55:309-316.

Akers, T.G. (1973). Some aspects of the airborne inactivation of viruses. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection John Wiley & Sons, New York, 73-81.

Andersen, A.A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. Journal of Bacteriology 76:471-484.

Auvigne, V. and Hery, D. (1997). Analysis of the relationship between seroprevlence of Aujeszky's disease and pig density within the different areas of Brittany. Veterinary Microbiology 55:153-158.

Bækbo, P. and Nielsen, J. P. (1988). Airborne *Pasteurella multocida* in pig fattening units. Proceedings of the

10th IPVS Congress, Rio de Janeiro 51.

Barber, E.M., Dawson, J.R., Battams, V.A., and Nicol, R.A.C. (1991). Spatial variability of airborne and settled dust in a piggery. Journal of agricultural Engineering Research 50:107-127.

Barlow, D.F. and Donaldson, A.I. (1973). Comparison of the aerosol stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. Journal of general Virology 20:311-318.

Bartlett, J.T. (1973). Meteorological factors influencing the long range transmission of micro-organisms.Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection. IVth international symposium on aerobiology Oosthoek Publishing Company, Utrecht, The Netherlands, 385-391.

Beard, C.W. and Anderson, D.P. (1967). Aerosol studies with avian Mycoplasma. I. survival in the air. Avian Diseases 11:54-59.

Beer, K., Mehlhorn, G., and Arnold, H. (1975). Die bakterielle Kontamination der Stalluft und der Oberflächen in Milchviehställen. 2. Mitteilung: Die gesetzmässige Beeinflussung der Keimflora der Stalluft durch bioklimatische Faktoren. Monatshefte für Veterinär-Medizin 30:406-409.

Bönsch, S. and Hoy, St. (1996). Untersuchungen der Schwebstaubkonzentration bei Tiefstreuhaltung unter Berücksichtigung der Aktivität von Mastschweinen und bei Anwendung einer Aerosolapplikation. Berliner Münchner Tierärztliche Wochenschrift 109:388-391.

Bosse, J.T., Johnson, R.P., Nemec, M., and Rosendal, S. (1992). Protective local and systemic antibody responses of swine exposed to an aerosol of *Actinobacillus pleuropneumoniae* serotype 1. Infection and Immunology 60:479-484.

Bøtner, A. (1991). Survival of Aujesky's disease virus in slurry at various temperatures. Veterinary Microbiology 29:225-235.

Bourgueil, E., Hutet, E., Cariolet, R., and Vannier, P. (1992a). Air sampling procedure for evaluation of viral excretion level by vaccinated pigs infected with Aujeszky's disease (pseudorabies) virus. Research in Veterinary Science 52:182-186.

Bourgueil, E., Hutet, E., Cariolet, R., and Vannier, P. (1992b). Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion. Veterinary Microbiology 31:11-18.

Boutin, P., Torre, M., Serceau, R., and Rideau, P.-J. (1988). Atmospheric bacterial contamination from land-spreading of animal wastes: Evaluation of the respiratory risk for people nearby. Agricultural Engineering Research 39:149-160.

Brody, S. (1945). Bioenergetics and growth. Reinhold Publishing Corporation, New York.

Butera, M., Smith, J.H., Morrison, W.D., Hacker, R.R., Kains, F.A., and Ogilvie, J.R. (1991). Concentration of respirable dust and bioaerosols and identification of certain microbial types in a hog-growing facility. Canadian Journal of Animal Science 71:271-277.

Casal, J., Moreso, J. M., Planas-Cuchí, E., and Casal, J. (1997). Simulated airborne spread of Aujeszky's disease and foot-and-mouth disease. Veterinary Record 140:672-676.

Carpenter, G.A. (1987). Dust in livestock buildings - prevention and reduction. Environmental aspects of respiratory disease in intensive pig and poultry houses including the implications for human health Commission of the European Communities, Luxembourg, Publication No. EUR 10820 EN 100-110.

Carpenter, G.A., Cooper, A.W., and Wheeler, G.E. (1986). The effect of air filtration on air hygiene and pig performance in early-weaner accommodation. Animal Production 43:505-515.

Chamberlain, A.C. (1970). Deposition and uptake by cattle of airborne particles. Nature 225:99-100.

Christensen, L.S., Mousing, J., Mortensen, S., Soerensen, K.J., Strandbygaard, S.B., Henriksen, C.A., and Andersen, J.B. (1990). Evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus. Veterinary Record 127:471-474.

Christensen, L.S., Mortensen, S., Bøtner, A., Strandbygaard, S.B., Rønsholt, L., Henriksen, C.A., and Andersen, J.B. (1993). Further evidence of long distance airborne transmission of Aujeszky's disease (pseudorabies) virus. 132:317-321.

Clark, S., Rylander, R., and Larsson, L. (1983). Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. American Industrial Hygiene Association Journal 44 :537-541.

Clemmer, D.I., Hickey, J.L.S., Bridges, J.F., Schliessmann, D.J., and Shaffer, M.F. (1960). Bacteriologic studies of experimental air-borne salmonellosis in chicks. Journal of Infectious Diseases 106:197.

Conceicao, M.A.P. (1989). Spatial inhomogeneity of aerial pollutants in a poultry house. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, Report No. EUR 11877:38-43.

Cormier, Y., Tremblay, G., Meriaux, A., Brochu, G., and Lavoie, J. (1990). Airborne microbial contents in two types of swine confinement buildings in Quebec. American Industrial Hygiene Association Journal 51 :304-309.

Cox, C. S. (1987). The aerobiological pathway of microorganisms. John Wiley & Sons, Chichester.

Cox, C.S. (1989). Airborne bacteria and viruses. Science Progress 73:469-500.

Cox, C.S. (1995). Stability of airborne microbes and allergens. In: Cox, C. S. and Wathes, C. M. Bioaerosol handbook. CRC Lewis Publishers, Boca Raton, 77-99.

Cox, C. S. and Wathes, C. M. (1995). Bioaerosols in the environment. In: Cox, C. S. and Wathes, C. M. Bioaerosol handbook. CRC Lewis Publishers, Boca Raton, 11-14.

Crook, B., Travers, S.A., Lacey, J., and Topping, M.D. (1989). Airborne dust and micro-organisms in pig houses: sampling to determine human health effects. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, Report No. EUR 11877:44-48.

Curtis, S.E., Drummond, J.G., Kelley, K.W., Grunloh, D.J., Meares, V.J., Norton, H.W., and Jensen, A.H. (1975a). Diurnal and annual fluctuations of aerial bacterial and dust levels in enclosed swine houses. Journal of animal production 41:1502-1511.

Curtis, S. E., Drummond, J. G., Grunloh, D. J., Lynch, P. B., and Jensen, A. H. (1975b). Relative and quantitative aspects of aerial bacteria and dust in swine houses. Journal of Animal Science 41:1512-1520.

Dawson, P.S. (1970). The involvement of milk in the spread of foot-and-mouth disease: an epidemiological study. Veterinary Record 87:543-548.

de Jong, J.C., Trouwborst, T., and Winkler, K.C. (1973). The mechanism of virus decay in aerosols. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection. John Wiley & Sons New York, 124-130.

Deans Rankin, J. and Taylor, R.J. (1969). A study of some disease hazards which could be associated with the system of applying cattle slurry. Veterinary Record 85:578-581.

Digiorgio, C., Krempff, A., Guiraud, H., Binder, P., Tiret, C., and Dumenil, G. (1996). Atmospheric pollution by airborne microorganisms in the city of Marseilles. Atmospheric Environment 30:155-160.

Donaldson, A. I. (1972). The influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. Journal of general Virology 15:25-33.

Donaldson, A.I. (1973). The influence of relative humidity on the stability of foot-and-mouth disease virus in aerosol from milk and faecal slurry. Research in Veterinary Science 15:96-101.

Donaldson, A.I. (1978). Factors influencing the dispersal, survival and deposition of airborne pathogens of farm animals. Veterinary Bulletin 48:83-94.

Donaldson, A.I. (1979). Airborne foot-and-mouth disease. Veterinary Bulletin 49:653-659.

Donaldson, A.I. (1986). Aerobiology of foot-and-mouth disease (FMD): an outline and recent advances. Revue scientifique et technique de l'Office International des Epizooties 5:315-321.

Donaldson, A.I. and Ferris, N.P. (1974). Airborne stability of swine vesicular disease virus. Veterinary Record 95:19-22.

Donaldson, A.I. and Ferris, N.P. (1976). The survival of some air borne animal viruses in relation to relative

humidity. Veterinary Microbiology 1:413-420.

Donaldson, A. I., Gloster, J., Harvey, L. D. J., and Deans, D. H. (1982a). Use of prediction models to forecast and analyse airborne spread during the foot-and-mouth disease outbreak in Brittany, Jersey and the Isle of Wight in 1981. Veterinary Record 110:53-57.

Donaldson, A.I., Ferris, N.P., and Gloster, J. (1982b). Air sampling of pigs infected with foot-and-mouth disease virus: comparison of Litton an cyclone samplers. Research in Veterinary Science 22:384-385.

Donaldson, A.I., Wardley, R.C., Martin, S., and Ferris, N.P. (1983). Experimental Aujeszky's disease in pigs: Excretion, survival and transmission of the virus. Veterinary Record 113:490-494.

Donaldson, A.I., Gibson, C.F., Oliver, R., Hamblin, C., and Kitching, R.P. (1987). Infection of cattle by airborne foot-and-mouth disease virus: minimal doses with O_1 and SAT 2 strains. Research in Veterinary Science 43:339-346.

Donaldson, A.L., Lee, M., and Shimshony, A. (1988). A possible airborne transmission of foot and mouth disease virus from Jordan to Israel - a simulated computer analysis. Israel Journal of Veterinary Medicine 44:92-96.

Eisenstein, B.I. (1990). New molecular techniques for microbial epidemiology and the diagnosis of infectious diseases. The Journal of infectious Diseases 161:595-602.

Errington, F.P. and Powell, E.O. (1969). A cyclone sampler for aerosol sampling in the field. Journal of Hygiene Cambridge 67:387-399.

Fairchild, C.I. and Stampfer, J.F. (1987). Particle concentration in exhaled breath. American Industrial Hygiene Association Journal 48:948-949.

Falk, L.A. and Hunt, R.D. (1980). Overview of airborne contagion in animals. Annals of the New York Academy of Science: Airborne contagion (R. Kundsin ed.). 353:174-178.

Fields, N.D., Oxborrow, G.S., Puleo, J.R., and Herring, C.M. (1974). Evaluation of membrane filter field monitors for microbiological air sampling. Applied Microbiology 27:517-520.

Fišar, Z., Hýsek, J., and Binek, B. (1990). Quantification of airborne microorganisms and investigation of their interactions with non-living particles. International Journal of Biometeorology 34:189-193.

Fišer, A. and Král, F. (1969). Air temperature and air humidity effect on number of air bacteria in piggeries with a different feed technology. Acta Veterinaria (Brno) 38:579-587.

Flori, J., Mousing, J., Gardner, I., Willeberg, P., and Have, P. (1995). Risk factors associated with seropositivity to porcine respiratory coronavirus in Danish swine herds. Preventive Veterinary Medicine 25:51-62.

Flügge, C. (1897). Ueber Luftinfection. Zeitschrift für Hygiene und Infektionskrankheiten 25:179-193.

Gerber, D.B., Mancl, K.M., Veenhuizen, M.A., and Shurson, G.C. (1991). Ammonia, carbon monoxide, carbon dioxide, hydrogen sulfide, and methane in swine confinement facilities. Compendium of Continuing Education for the Practicing Veterinarian 13:1483-1488.

Gillespie, R.R., Hill, M.A., and Kanitz, C.L. (1996). Infection of pigs by aerosols of Aujeszky's disease virus and their shedding of the virus. Research in Veterinary Science 60:228-233.

Gloster, J. (1982). Risk of airborne spread of foot and mouth disease from the Continent to England. Veterinary Record 111:290-295.

Gloster, J., Blackall, R.M., Sellers, R.F., and Donaldson, A.I. (1981). Forecasting the airborne spread of foot-and-mouth disease. Veterinary Record 108:370-374.

Gloster, J., Donaldson, A.I., and Hough, M.N. (1984). Analysis of a series of outbreaks on Aujeszky's disease in Yorkshire in 1981-1982: The possibility of airborne disease spread. Veterinary Record 114:234-239.

Goodwin, R. F. W. (1985). Apparent reinfection of enzootic pneumonia-free pig herds: search for possible causes. Veterinary Record 116:690-694.

Grant, R.H., Scheidt, A.B., and Rueff, L.R. (1994). Aerosol transmission of a viable virus affecting swine: explanation of an epizootic of pseudorabies. International Journal of Biometeorology 38:33-39.

Gregory, P.H. (1973). The microbiology of the atmosphere. Leonhard Hill, Aylesbury.

Groth, W. (1984). Influence of animal housing microclimate on the health and performance of cattle and swine. Zentralblatt fur Veterinarmedizin, B 31:561-584.

Hardman, P. M., Wathes, C. M., and Wray, C. (1991). Transmission of salmonellae among calves penned individually. Veterinary Record 129:327-329.

Hartung, J. (1989). Practical aspects of aerosol sampling in animal houses. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, EUR 11877:14-23.

Hartung, J. (1994). The effect of airborne particulates on livestock health and production., In: Ap Dewi, I., Axford, R. F. E., Marai, I. F. M., and Omed, H. M. Pollution in livestock production systems CAB International, Oxon, 55-69.

Heber, A.J. (1995). Bioaerosol particle statistics. In: Cox, C. S. and Wathes, C. M. Bioaerosols handbook CRC Lewis Publishers, Boca Raton , 55-75.

Heber, A.J., Stroik, M., Nelssen, J.L., and Nichols, D.A. (1988a). Influence of environmental factors on concentrations and inorganic content of aerial dust in swine finishing buildings. Transactions of the American Association of Agricultural Engineers 31:875-881.

Heber, A.J., Stroik, M., Faubion, J.M., and Willard, L.H. (1988b). Size distribution and identification of aerial dust particles in swine finishing buildings. Transactions of the American Association of Agricultural Engineers 31:882-887.

Heber, A.J., Boon, C.R., and Peugh, M.W. (1996). Air patterns and turbulence in an experimental livestock building. Journal of Agricultural Engineering Research 64:209-226.

Hecker, W. and Meier, R. (1991). Surveillance of the count of organisms in the air within the scope of microbiological quality assurance of medicines. Pharmazeutische Industrie 53:938-947.

Hecker, W., Meier, R., Thevenin, J.-P., and Hartberger, K. (1983). Comparative examination between membrane filters of gelatin and cellulose esters as regards their suitability for the determination of the count of microorganisms in the air. Zentralblatt der Bakteriologie und Hygiene I Abt. Orig. B 177:375-393.

Heidelberg, J.F., Shahamat, M., Levin, M., Rahman, I., Stelma, G., Grim, C., and Colwell, R.R. (1997). Effect or aerolization on culturability and viability of gram-negative bacteria. Applied and Environmental Microbiology 63:3585-3588.

Hensel, A. (1994). Influence of serum and glucose additives on survival of *Actinobacillus pleuropneumoniae* aerosolised from the freeze-dried state. Applied and Environmental Microbiology 60:2155-2157.

Hensel, A. and Petzoldt, K. (1995). Biological and biochemical analysis of bacteria and viruses. In: Cox, C. S. and Wathes, C. M. Bioaerosol handbook Lewis Publishers, Boca Raton, 335-360.

Hensel, A., Windt, H., Stockhofe Zurwieden, N., Lodding, H., Koch, W., and Petzoldt, K. (1993). A porcine aerosol infection model for studying dose dependent effects caused by *Actinobacillus pleuropneumoniae* bacteria. Journal of Aerosol Medicine 6:73-88.

Hensel, A., Stockhofe Zurwieden, N., Ganter, M., and Petzoldt, K. (1995). Aerosol exposure of pigs to viable or inactivated *Actinobacillus pleuropneumoniae* serotype 9 induces antibodies in bronchoalveolar lining fluids and serum, and protects against homologous challenge. Veterinary Microbiology 47:27-41.

Hensel, A., Vanleengoed, L.A.M.G., Szostak, M., Windt, H., Weissenbock, H., Stockhofezurwieden, N., Katinger, A., Stadler, M., Ganter, M., Bunka, S., Pabst, R., and Lubitz, W. (1996). Induction of protective immunity by aerosol or oral application of candidate vaccines in a dose-controlled pig aerosol infection model. Journal of Biotechnology 44:171-181.

Hill, H. (1990) Overview and history of mystery swine disease (swine infertility respiratory sysndrome). Livestock Conservation Institute: Proceedings of Mystery Swine Disease Committee Meeting, Denver, Colorado, USA, 29-31.

Hirst, J. M. (1995). Bioaerosols: Introduction, restrospect and prospect. In: Cox, C. S. and Wathes, C. M. Bioaerosol handbook. Lewis Publishers, Boca Raton, 1-10.

Hoff, S.J. and Bundy, D.S. (1996). Comparison of contaminant dispersion modelling approaches for swine housing. Transactions of the American Association of Agricultural Engineers 39:1151-1157.

Honey, L.F. and McQuitty, J.B. (1979). Some physical factors affecting dust concentrations in a pig facility. Canadian Agricultural Engineering 21:9-14.

Hugh-Jones, M. E. (1973). The epidemiology of airborne animal diseases. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection. IVth international symposium on aerobiology Oosthoek Publishing Company, Utrecht, The Netherlands, 399-404.

Hurtienne, H. (1967). Vergleiche zwischen mehreren Verfahren zur Bestimmung des Keimgehaltes der Stalluft unter verschiedenen Bedingungen. Doctoral Thesis, Freie Universität Berlin, Berlin.

Hysek, J., Fisar, Z., Zizka, Z., Kofronova, O., and Binek, B. (1991). Airborne microorganism monitoring: a comparison of several methods, including a new direct counting technique. Zentralblatt für Mikrobiologie 146:435-443.

Hyslop, N. S. G. (1971). Factors influencing the epidemiology and epizootiology of airborne diseases. Journal of the American Veterinary Medical Association 159:1500-1507.

Ikeguchi, A. and Nara, M. (1992). Effect of ventilation on distribution of carbon dioxide concentration in windowless swine farrowing house. Journal of the Society of Agricultural Structures, Japan 22:9-17.

Jacobsen, M.J., Nielsen, J.P., and Nielsen, R. (1996). Comparison of virulence of different *Actinobacillus pleuropneumoniae* serotypes and biotypes using an aerosol infection model. Veterinary Microbiology 49:159-168.

Jakab, L., Rafai, P., Éliàs, B., Stipkovits, L., Albert, M., and Appel, J. (1991). Aerosol infection model with *Mycoplasma hyopneumoniae*. 7th International Congress on Animal Hygiene, Leipzig 687-692.

Jones, C.R. and Webster, A.J.F. (1981). Weather induced changes in airborne bacteria within a calf house. Veterinary Record 109:493-494.

Jorsal, S.E. and Thomsen, B.L. (1988). A Cox regression analysis of risk factors related to *Mycoplasma suipneumoniae* reinfection in Danish SPF herds. Acta veterinaria Scandinavica Suppl. 84:436-438.

Knight, V. (1973). Airborne transmission and pulmonary deposition of respiratory viruses. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection. IVth international symposium on aerobiology Oosthoek Publishing Company, Utrecht, The Netherlands, 175-182.

Lacey, J., Eduard, W., Karlsson, K., Palmgren, U., Strom, G., and Blomquist, G. (1989). Microscopic methods for enumerating microorganisms in filter samples. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, Report No. EUR 11877:63-68.

Laube, P. (1996). Simulation der flächendeckenden EP-Sanierung mit Hilfe eines geographischen Informationssystems. Doctoral Thesis, University of Zurich, Zurich.

Leontides, L., Ewald, C., Mortensen, S., and Willeberg, P. (1994a). Factors associated with circulation of Aujeszky's disease virus in fattening herds of an intensively vaccinated area of Northern Germany. Preventive Veterinary Medicine 20:63-78.

Leontides, L., Ewald, C., and Willeberg, P. (1994b). Herd risk factors for serological evidence of Aujeszky's disease virus infection in breeding sows in Northern Germany (1990-1991). Journal of Veterinary Medicine Series B 41:554-560.

Lever, M.S. and Williams, A. (1996). Cross-infection of chicks by airborne transmission of *Salmonella enteritidis* PT4. Letters in Applied Microbiology 23.

Lloyd, L.C. and Etheridge, J.R. (1974). Survival of mycoplasmas in aerosol particles. Victorian Veterinary Proceedings 32:39.

Loftager, M.K., Eriksen, L., Aasted, B., and Nielsen, R. (1993). Protective immunity following immunisation of pigs with aerosol of *Actinobacillus pleuropneumoniae* serotype 2. Research in Veterinary Science 55:281-286.

Lundholm, I.M. (1982). Comparison of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. Applied and Environmental Microbiology 44:179-183.

Mack, H., Wekerle, J., and Strauch, D. (1986). Isolation of Aujeszky's disease virus from pig faeces and air samples. Tierärztliche Umschau 41:32-38.

Maragon, S., Facchin, E., Moutou, F., Massirio, I., Vincenzi, G., and Davies, G. (1994). The 1993 Italian foot-and-mouth disease epidemic: Epidemiological features of the four outbreaks identified in Verona province (Veneto region). Veterinary Record 135:53-57.

Martin, H. (1967). Some considerations in dealing with respiratory disease in calves. Veterinary Record 79:255-261.

Mason, P.J. and Sykes, R.I. (1981). On the influence of topography on plume dispersal. Boundary-Layer Meteorology 21:137-157.

May, K.R. (1975). An "ultimate" cascade impactor for aerosol assessment. Journal of Aerosol Science 6:413-419.

May, K.R. and Harper, G.J. (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. British Journal of Industrial Medicine 14:287-297.

Mehta, S.K., Mishra, S.K., and Pierson, D.L. (1996). Evaluation of three portable samplers for monitoring airborne fungi. Applied and Environmental Microbiology 62:1835-1838.

Mikkelsen, T., Larsen, S.E., and Thykier-Nielsen, S. (1984). Description of the Risø Puff Diffusion model. Nuclear Technology 67:56-65.

Mitchell, C.S. and Guerin, L.F. (1972). Influenza A of human, swine, equine and avian origin: Comparison of survival in aerosol form. Canadian Journal of comparative Medicine 36:9-11.

Morris, K.J. (1995). Modern microscopic methods of bioaerosol analysis. In: Cox, C. S. and Wathes, C. M. Bioaerosol handbook Lewis Publishers, Boca Raton, 285-316.

Mortensen, S., Mikkelsen, T., Thyker-Nielsen, S., and Willeberg, P. (1994). Modelling the effect of exposure from neighbouring herds on the hazard of airborne infection with Aujeszky's disease virus with a meteorological dispersion model. The Kenya Veterinarian 18:183-185.

Mouilleseaux, A. (1990). Sampling methods for bioaerosols. Aerobiologia 6:32-35.

Mousing, J., Permin, A., Mortensen, A., Bøtner, A., and Willeberg, P. (1997). A case-control questionnaire survey of risk factors for porcine reproductive and respiratory syndrome (PRRS) seropositivity in Danish swine herds. Veterinary Microbiology 55:323-328.

Moutou, F. and Durrand, B. (1994). Modelling the spread of foot-and-mouth disease virus. Veterinary Research 25:279-285.

Müller, G. and Heilmann, P. (1990). Effect of local and systemic immunization of pigs on broncho-alveolar clearance of, and intrabronchial infection with *Pasteurella multocida*. Archiv für Experimentelle Veterinärmedizin 44:301-310.

Müller, W., Schneider, J., von Dossow, A., and Weisser, W. (1991). Tenazitätsprüfung von *Pasteurella multocida*- und *Bordetella bronchiseptica*-Stämmen in einer rotierenden Aerosolkammer - Ein Beitrag zur Epidemiologie der Schnüffelkrankheit. 7th International Congress on Animal Hygiene, Leipzig 668-674.

Müller, W., Wieser, P., and Kühme, H. (1978). Zur Frage der Ausbreitung von Luftkeimen in Tierställen. Zentralblatt der Veterinär-Medizin, Serie B 25:216-224.

Müller, W., Wieser, P., and v. Dossow, A. (1989). Sampling airborne bacteria in stables. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, EUR 11877:14-23.

Murphy, D.A., Van Alstine, W.G., Clark, L.K., Albregts, S., and Knox, K. (1993). Aerosol vaccination of pigs against Mycoplasma hyopneumoniae infection. American Journal of Veterinary Research 54:1874.

Nardell, E. A., Keegan, J., Cheney, S. A., and Etkind, S. C. (1991). Airborne infection. Theoretical limits of protection achievable by building ventilation. American Review of Respiratory Diseases 144:302-330.

Nielsen, R., Loftager, M., and Eriksen, L. (1990). Mucosal vaccination against Actinobacillus pleuropneumoniae infection. Proceedings of the 11th IPVS Congress, Lausanne, 13.

Norman, H. S., Sischo, W. M., Pitcher, P., Nesselrodt, A., and Day, R. L. (1996). Spatial and temporal epidemiology of pseudorabies virus infection. American Journal of Veterinary Research 57:1563-1568.

O'Grady, F. and Riley, R.L. (1963). Experimental airborne tuberculosis. Advances in Tuberculosis Research 12:150-190.

Olsson, M., Sukura, A., Lindberg, L.A., and Linder, E. (1996). Detection of *Pneumocystis carinii* DNA by filtration of air. Scandinavian Journal of Infectious Diseases 28:279-282.

Palmgren, U. and Strom, G. (1989). Sampling strategy and analysis of airborne microorganisms in the farmers' work environment and livestock buildings. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, Report No. EUR 11877:88-94.

Palmgren, U., Strom, G., Blomquist, G., and Malmberg, P. (1986). Collection of airborne micro-organisms on Nucleopore filters, estimation and analysis - CAMNEA method. Journal of Applied Bacteriology 61:401-406.

Parks, S.R., Bennett, A.M., Speight, S.E., and Benbough, J.E. (1996). An assessment of the Sartorius MD8 microbiological air sampler. Journal of Applied Bacteriology 80:529-534.

Pasquill, F. (1961). The estimation of the dispersion of wind-borne material. The Meteorological Magazine 90:33-53.

Pedersen, S. (1993). Time based variation in airborne dust in respect to animal activity. In: E. Collins, C. Boon Livestock Environment IV. 4th International Symposium 718-725.

Pensaert, M., Cox, E.v.D.K., and Callebaut, P. (1993). A sero-epizootiological study of porcine respiratory coronavirus in Belgian swine. Veterinary Quarterly 15:16-20.

Pickrell, J.A., Heber, A.J., Murphy, J.P., Henry, S.C., May, M.M., Nolan, D., Oehme, F.W., Gillespie, J.R., and Schoneweis, D. (1993). Characterisation of particles, ammonia and endotoxin in swine confinement operations. Veterinary and Human Toxicology 35:421-428.

Pritschard, D.G., Carpenter, C.A., Morzaria, S.P., Harkness, J.W., Richards, M.S., and Brewer, J.I. (1981). Affect of air filtration on respiratory disease in intensively housed veal calves. Veterinary Record 109:5-9.

Robertson, I.B. (1992). New pig disease update - Epidemiology of PRRS. Pig Veterinary Journal 29:186-187.

Robertson, J.F. (1989). Aerosol sampling in pig housing. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop: Luxembourg, Commission of the European Communities EUR 11877:95-101.

Robertson, J.F., Wilson, D., and Smith, W.J. (1990). Atrophic rhinitis: the influence of the aerial environment. Animal Production 50:173-182.

Rumney, R.P. (1986). Meteoroligal influences on the spread of foot and mouth disease. Journal of Applied Bacteriology 61:105S-114S.

Sanson, R.L. (1993). The development of a decision support system for animal disease emergency. PhD Thesis, Massey University, Palmerston North, New Zealand.

Schatzmann, E., Keller, H., Grest, P., Lorenz, D., and Burri, W. (1996). Field trials with a vaccine against porcine enzootic pneumonia (EP). Schweizer Archiv für Tierheilkunde 138:483-489.

Schoenbaum, M.A., Zimmerman, J.J., Beran, G.W., and Murphy, D.P. (1990). Survival of pseudorabies virus in aerosol. American Journal of Veterinary Research 51:331-333.

Schulman, J.L. (1968). The use of an animal model to study transmission of influenza virus infection. American Journal of Public Health 58:2092-2096.

Sellers, R.F. (1971). Quantitative aspects of the spread of foot-and-mouth disease. Veterinary Bulletin Weybridge 41:431-439.

Sellers, R.F. and Herniman, K.A.J. (1974). Airborne excretion by pigs of swine vesicular disease virus. Journal of Hygiene Cambridge 72:61-65.

Sellers, R.F. and Parker, J. (1969). Airborne excretion of foot-and-mouth disease virus. Journal of Hygiene Cambridge 67:671-677.

Smith, C.V. (1983). Airborne infection and livestock disease control: a meteorological appreciation. Philosophical Transactions of the Royal Society London B 302:543-557.

Smith, J.H., Boon, C.R., and Wathes, C.M. (1993). Dust distribution and airflow in a swine house. In: E. Collins, C. Boon (eds.) Livestock environment IV. 4th international symposium, 657-662.

Smith, L.P., and Hugh-Jones, M.E. (1969). The weather factor in foot and mouth disease epidemics. Nature 223:712-715.

Sobih, M.A., Dosoky, R.M., and Ismail, A. (1991). Decontamination of air in broiler houses by some aerosol disinfectants. Assiut Veterinary Medical Journal 23:142-150.

Stärk, K.D.C., Keller, H., and Eggenberger, E. (1992a). Risk factors for the reinfection of specific pathogen free pig breeding herds with enzootic pneumonia. Veterinary Record 131:532-535.

Stärk, K.D.C., Keller, H., and Eggenberger, E. (1992b). Climatic conditions and airborne transmission of *Mycoplasma hyopneumoniae*. Proceedings of the 12th IPVS Congress, The Hague, 559.

Stehmann, R., Mehlhorn, G., and Neuparth, V. (1991a). Characterization of strains of *Bordetella bronchiseptica* isolated from animal house air and the airborne infectious pressure generated by them. Deutsche Tierärztliche Wochenschrift 98:448-450.

Stehmann, R., Huster, A., and Neuparth, V. (1991b). Zur Tenazität ausgewählter pneumotroper Erreger im Staub und auf festen Keimträgern. 7th International Congress on Animal Hygiene, Leipzig 675-679.

Straja, S. and Leonard, R.T. (1996). Statistical analysis of indoor bacterial air concentration and comparison of four RCS biotest samplers. Environment International 22:389-404.

Tamási, G. (1973). Mycoplasma isolation from the air. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection John Wiley & Sons, New York, 68-71.

Taylor, K.C. (1988). Epidemiological aspects of Aujeszky's disease control in Great Britain., In: van Oirschot, J. T. Vaccination and Control of Aujeszky's disease. Kluwer Academic Publishers, Dordrecht, 185-196.

Terzieva, S., Donnelly, J., Ulevicius, V., Grinshpun, S.A., Willeke, K., Stelma, G.N., and Brenner, K.P. (1996). Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. Applied and Environmental Microbiology 62:2264-2272.

Thorne, H.V. and Burrows, T.M. (1960). Aerosol sampling methods for the virus of foot-and-mouth disease and the measurement of virus penetration though aerosol filters. Journal of Hygiene Cambridge 58:409-417.

Thorne, P.S., Kiekhaefer, M.S., Whitten, P., and Donham, K.J. (1992). Comparison of bioaerosol sampling methods in barns housing swine. Applied and Environmental Microbiology 58:2543-2551.

Torremorell, M., Pijoan, C., Janni, K., Walker, R., and Joo, H.S. (1996). Airborne transmission of PRRS virus and *Actinobacillus pleuropneumoniae* in swine nurseries. Proceedings of the 14th IPVS Congress, Bologna, 194.

Vannier, P., Madec, F., Tillon, J.P., and Monicat, F. (1989). Isolation of Aujeszky's disease virus from dust in a piggery with infected pigs. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Office for Official Publications of the European Communities, Luxembourg, EUR 11877:114-116.

van Reeth, K. (1997). Pathogenesis and clinical aspects of a respiratory porcine reproductive and respiratory syndrome virus infection. Veterinary Microbiology 55:223-230.

Verstegen, M., Tamminga, S., and Greers, R. (1994). The effect of gaseous pollutants on animals. In: Ap Dewi, I., Axford, R. F. E., Marai, I. F. M., and Omed, H. M. Pollution in livestock production systems CAB International, Oxon, 71-79.

Wathes, C.M. (1994). Air and surface hygiene. In: Wathes, C.M. and Charles, D.R. Livestock housing. CAB

International, Wallingford Oxon, 123-148.

Wathes, C.M., Miller, B.G., and Bourne, F.J. (1989). Cold stress and post weaning diarrhoea in piglets inoculated orally or by aerosol. Animal Production 49:483-496.

Wathes, C. M. and Randall, J. M., eds. (1989). Aerosol sampling in animal houses. Proceedings of a workshop held at the University of Bristol. Commission of the European Communities, Luxembourg, Report EU 11877.

Wathes, C. M., Zaidan, W. A. R., Pearson, G. R., Hinton, M., and Todd, N. (1988). Aerosol infection of calves and mice with *Salmonella typhimurium*. The Veterinary Journal 123:590-594.

Wells, W. F. (1955). Airborne contagion and air hygiene – and ecological study of droplet infections. Harvard University Press, Cambridge.

Wentworth, D.E., McGregor, M.W., Macklin, M.D., Neumann, V., and Hinshaw, V.S. (1997). Transmission of swine influenza virus to humans after exposure to experimentally infected pigs. Journal of Infectious Diseases 175 :7-15

White, L.A., Hadley, D.J., Davids, D.E., and Naylor, R. (1975). Improved large-volume sampler for the collection of bacterial cells from aerosol. Applied Microbiology 29:335-339.

Wicklen, G.L. van (1989). Respirable aerosol sampling in poultry and swine housing. In: Wathes, C. M. and Randall, J. M. Aerosol sampling in animal houses. Proceedings of a workshop Commission of the European Communities, Luxembourg, Report No. EUR 11877:117-122.

Wilkinson, P.J., Donaldson, A.I., Greig, A., and Bruce, W. (1977). Transmission studies with African swine fever virus. Infections of pigs by airborne virus. Journal of Comparative Pathology 87:487-495.

Willeberg, P., Gardner, I.A., Mortensen, S., and Mousing, J. (1994). Models for herd size effects in swine diseases. The Kenya Veterinarian 18:189-191.

Wills, R.W., Zimmermann, J.J., Swenson, S.L., Yoon, K.-J., Hill, H.T., Bundy, D.S., and McGinley, M.J. (1994). Transmission of PRRS virus by contact vs airborne exposure. Proceedings 75th Annual Conference of Research Workers in Animal Diseases, Abstract No. 242.

Winkler, K. C (1973). The scope of aerobiology. In: Hers, J. F. Ph. and Winkler, K. C. Airborne transmission and airborne infection. IVth international symposium on aerobiology. Oosthoek Publishing Company, Utrecht, The Netherlands, 1-11.

Yanga, S.T., Gardner, I.A., Hurd, H.S., Eernisse, K.A., and Willeberg, P. (1995). Management and demographic factors associated with seropositivity to transmissible gastroenteritis virus in US swine herds, 1989-1990. Preventive Veterinary Medicine 24:213-228

CHAPTER 1.4

DETECTION OF *MYCOPLASMA HYOPNEUMONIAE* BY AIR SAMPLING WITH A NESTED PCR ASSAY

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1. Abstract

This article describes the first successful detection of airborne *Mycoplasma hyopneumoniae* under experimental and field conditions, using a new nested PCR assay. Air was sampled using polyethersulfone membranes (pore size 0.2 µm) mounted in filter holders. Filters were processed by dissolution and direct extraction of DNA for PCR analysis. For the PCR, two nested pairs of oligonucleotide primers were designed using a *M. hyopneumoniae*-specific DNA sequence of a repeated gene segment. A nested PCR assay was developed and used to analyse samples collected in 8 pig houses with a history of respiratory problems. Air was also sampled in a mycoplasma-free herd. The nested PCR was highly specific and 10^4 times as sensitive as a one-step PCR. Under field conditions, the sampling system was able to detect airborne *M. hyopneumoniae* on 80% of farms with acute respiratory disease. No airborne *M. hyopneumoniae* were detected on infected farms without acute cases. Success of detection was increased if air was sampled at several locations within a room and at lower air humidity.

2. Introduction

Mycoplasma (M.) hyopneumoniae is the etiological agent of enzootic pneumonia (EP) in pigs and causing major economical losses in the pig industry worldwide (Ross, 1992). Several countries have established EP-free herds within national pig health schemes (Goodwin and Whittlestone, 1967; Keller, 1988). However, despite rigid biosecurity measures, many of these herds become re-infected with EP each year. As a consequence of these unexplained re-infections, the hypothesis of airborne agent transmission between farms has been tested in a number of field studies. . It was demonstrated that the risk of re-infection of EP-free herds was associated with the distance to conventional pig farms in the neighbourhood and the size of these farms as well as the concentration of pigs in the area (Goodwin, 1985; Stärk *et al.*, 1992; Thomsen *et al.*, 1992;). These risk factors indicate an airborne transmission process. However, the pathogen has never been isolated from the air. Early attempts by Tamàsi (1973) were only partially successful. An isolate that looked morphologically similar to *M. hyopneumoniae* colonies was described, but the identity of the organism remained inconclusive.

Accurate diagnosis of EP is essential to prevent infection from spreading. The most commonly used diagnostic methods are serological analyses such as direct or blocking ELISA (Nicolet *et al.*, 1980; Djordjevic *et al.*, 1994; Futo *et al.*, 1995; Mattsson *et al.*, 1995; Sørensen *et al.*, 1997), or direct detection of the organism in clinical samples by immunofluorescence (Giger *et al.*, 1977). Over recent years molecular genetic techniques have become available which have improved sensitivity and specificity (Mattsson *et al.*, 1995; Blanchard *et al.*, 1996). The molecular genetic methods benefit from the fact that the microorganisms do not have to be alive at the time of analysis. This makes the tools attractive for use in detection by air sampling techniques, for example air filtration, which may adversely affect survival.

Air filtration is one of the simplest and cheapest air sampling techniques available for the investigation of bioaerosols (Cox, 1987). It has successfully been used in combination with PCR assays (Olsson *et al.*, 1996). The objective of this project was to establish a highly sensitive and specific nested PCR method and to develop a filtration-based air sampling technique for the detection of *M. hyopneumoniae* in the air.

3. Material and Methods

3.1 Strain growth conditions and DNA extraction.

The porcine *Mycoplasma* and *Acholeplasma* strains used in this study are listed in TABLE 15. *M. hyopneumoniae* and *M. flocculare* were grown in Friis medium (Friis, 1971). The other strains were grown in B medium (Ernø and Stipkovits, 1973). The cells were cultivated until to the end of the exponential phase of growth, harvested by centrifugation at 20,000 *g* for 20 min, washed three times in TE buffer (Tris-HCl 10 mM, EDTA 1 mM, pH 7.5), and resuspended in 1/100 of the original volume of TE buffer. Titration of the viable cells/ml estimated as colony-forming units (cfu/ml) was performed by spreading samples of sequential 10 fold dilutions on solid Friis medium (Friis, 1971) and counting the colonies after 10 days incubation.

In order to obtain pure genomic DNA of mycoplasmal cultures, cells were harvested by centrifugation, washed in TE buffer and resuspended in 1/10 volume of TE buffer. A volume of 100 µl of resuspended cells (10^{10} cells/ml in TE buffer) was lysed by addition of 500 µl GES buffer (5 M guanidium thiocyanate, 100 mM EDTA, 0.5% N-Laurosylsarcosine (nastelle Sarcosyl)) for 10 min at room temperature, cooled on ice and then mixed with 250 µl 7.5 M ammonium acetate, pH 7.7. The lysate was then extracted 3 times with 500 µl [phenol:chloroform:isoamylalcohol, 49.5:49.5:1] (PCIA) (Fluka Chemicals, Buchs, Switzerland). The DNA was then precipitated by the addition of 0.7 volumes of isopropanol and collected by centrifugation at 10,000 *g* for 15 min at 4°C in an Eppendorf centrifuge. The DNA pellet was washed 3 times with 80% ethanol, dried, and resuspended in 100 µl TE buffer. DNA concentration was determined spectrophotometrically with a GeneQuantII™ (Model 2105, Pharmacia Biotech, Uppsala, Sweden).

3.2 Air sampling system

Air was sampled with polyethersulfone membranes (47 mm diameter) with a pore size of 0.2 µm (Supor200, Gelman Sciences, Ann Arbor, Michigan) mounted in filter holders (Schleicher & Schuell GmbH, Dassel, Germany). The air was pumped at a rate of 18.3-20.0 l/min using a vacuum pressure pump (Millipore, Bedford, Massachusetts). The airflow in the filter system was controlled with an in-line rotameter (Messerli Messtechnik, Riehen, Switzerland).

In order to determine the sensitivity of detection of mycoplasmas on the filters, we filtered 1 ml samples of a consecutively 10 fold diluted culture of *M. hyopneumoniae* strain NCTC10110 to obtain samples ranging from 10^6 to 0 viable cells/ml.

An experimental aerosol of *M. hyopneumoniae* was generated by nebulising a formaldehyde-inactivated culture in a closed chamber of 0,54 m^3 using a commercial nebuliser (DP10, DPMedical, Medela, Baar, Switzerland) with a vaporisation rate of approximately 2 ml/min.

TABLE 15. Porcine *Mycoplasma* and *Acholeplasma* strains used in this study and their reaction in the two steps of the nested PCR assay.

Species	Strain	Source	PCR result		
			Outer	Inner	Nested
M. hyopneumoniae	ATCC 25934T	type strain ATCC[a]	+	+	+
	NCTC 10110T	type strain NCTC[b]	+	+	+
	J (JF 184)	UK	+	+	+
	BQ 14	France	+	+[d]	+
	EP-S 924	Switzerland	+	+[d]	+
	EP-S 938	Switzerland	+	+	+
	EP-S 939	Switzerland	+	+	+
	EP-S 946	Switzerland	+	+	+
	EP-S 223	Switzerland	+	+	+
	232	Camden NSW, Australia	+	+	+
	YZ	unknown	+	+[d]	+
	Beaufort	Camden, NSW, Australia	+	+[d]	+
	Sue	Camden, NSW, Australia	+	+	+
	C173512	Camden, NSW, Australia	+	+	+
	OMZ407	Camden, NSW, Australia	+	+	+
	Hungary 1	Budapest, Hungary	+	+[d]	+
M. flocculare	Ms 42	type strain MRC[c]	-	-	-
	ZH1	Zürich, Switzerland	-	-	-
	ZH2	Zürich, Switzerland	-	-	-
	JF1628	Cambridge, UK	-	-	-
	JF7337	Cambridge, UK	-	-	-
M. hyorhinis	BTS-7	type strain MRC[c]	-	-	-
	SEP200	Stockholm, Sweden	-	-	-
	F44	Stockholm, Sweden	-	-	-
	31-53	Ottawa, Canada	-	-	-
M. hyosynoviae	S16	type strain MRC[c]	-	-	-
	M60	Stockholm, Sweden	-	-	-
A. axanthum	S-743	type strain MRC[c]	-	-	-
A. granularum	BTS-39	type strain MRC[c]	-	-	-
A. laidlawii	PG8	type strain MRC[c]	-	-	-

[a]American Type Culture Collection, Rockville, Maryland, USA
[b]National Collection of Type Cultures, London, GB
[c]Mycoplasma Reference Center, Aarhus, Denmark
[d]The DNA sequence of the 808 bp fragment was determined using the Taq Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems/Perkin Elmer) and oligonucleotides MHP950-2L and MHP950-2R.

The plume was sampled for 10 sec, 1 min and 6 min, using the sampling system described above. The experimental set-up captured approximately 1/10 of the volume of the evaporate per time unit.

3.3 Air-sample processing for PCR assay

The filters from the air samplings and the artificially contaminated filters were thoroughly dried, folded, and dissolved in 5 ml chloroform by vortexing in a 15 ml Falcon tube (catalogue no. 2059, Becton Dickinson, Lincoln Park, NJ, USA). Drying was necessary to ensure complete dissolution of the polyethersulfone membranes. The DNA was then extracted by the addition of 3.3 ml TE buffer and shaking for 10 min at room temperature. Phase separation was achieved by centrifugation for 10 min at 10,000 *g*. After recovery of the aqueous phase, the chloroform phase was extracted a second time with 2 ml TE buffer. Subsequently the aqueous phases were combined and extracted with 5 ml PCIA [Phenol-Chloroform-IsoAmylalcohol (49.5:49.5:1)]. After phase separation by centrifugation for 10 min at 10,000 *g*, the aqueous phase was recovered, mixed with 8 ml chilled ethanol and 400 µl Na-acetate 3 M pH 5.5 and cooled at -20°C for 10 min to precipitate the DNA. The precipitated DNA was recovered by centrifugation for 20 min at 10,000 *g*, dried and resuspended in 50 µl TE buffer.

3.4 Design of specific oligonucleotide primers and PCR reactions

The oligonucleotide primers used in the PCR reactions are based on the sequence data of the 1023 base pair (bp) repeated element MHYP1-03/950 (accession no. AF004388, GenBank/EMBL database) (J. Frey and S. Djordjevic, unpublished data). This sequence has been shown to be specific for the species *M. hyopneumoniae* and is present at 1 - 7 copies per chromosome. Seven copies of MHYP1-03-950 were shown to be present in *M. hyopneumoniae* type strain NCTC10110. The repeated element MHYP1-03-950 does not contain sequences typical for insertion sequences or known multycopy gene families. Southern blot analysis of genomic DNA of *M. flocculare*, *M. hyorhinis*, *M. hyosynoviae* and *A. laidlawii* with a labelled probe of MHYP1-03-950 did not show hybridization signals under low stringency conditions. (J. Frey and S. Djordjevic, unpublished data). Two nested pairs of oligonucleotides primers (TABLE 16) were designed using the primer analysis software OLIGO 4 (National Biosciences, Plymouth MN, USA). The outer primer pair (MHP950-1L / MHP950-1R) in the first reaction gave an amplification product of 913 bp, and the inner primer pair (MHP950-2L / MHP950-2R) in the second reaction gave a product of 808 bp. Both primer pairs were designed to be used at the same annealing temperature of 52°C for practical reasons.

TABLE 16. Oligonucleotide primers used in this study[a]

Primer	Sequence	Location (bp)[b]
MHP950-1L	aggaacaccatcgcgatttta	46 - 67
MHP950-1R	ATAAAAATGGCATTCCTTTTCA	958 - 937
MHP950-2L	ccctttgtcttaatttttgcaa	102 - 123
MHP950-2R	GCCGATTCTAGTACCCTAATCC	909 - 888

[a]All primers were annealed at 52°C.
[b]Based on nucleotide sequence AF004388.

The PCRs were carried out in a DNA thermal cycler (GeneAmp 9600, Perkin Elmer Cetus) in 50 µl reaction mix (10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.005% Tween 20, 0.005% NP-40 detergent, 170 µM concentration of each deoxynucleoside triphosphate, 0.25 µM of each forward and reverse primers, 1.25 units *Taq* polymerase) with 5 µl of extracted DNA from the filters or 2 ng of purified DNA (Miserez *et al.*, 1997) from cultured mycoplasmas as a template. The amplification consisted of 35 cycles at 94°C for 30 sec, 52°C for 30 sec and 72°C for 1 min. In the nested PCR assay, the first reaction was done with primers MHP950-1L / MHP950-1R. The second (nested) PCR reaction was performed with the primers MHP950-2L / MHP950-2R with 1 µl of amplification product from the first reaction as a template. The universal primers 16SUNI-L/16SUNI-R which amplify the 16SrRNA gene *rrs* (Kuhnert *et al.*, 1996) were used in control PCR reactions at the same amplification conditions. The PCR amplification products were analysed by gel electrophoresis on 0.7% agarose gels and visualised after staining with ethidium bromide on a UV transilluminator according to standard protocols (Ausubel *et al.*, 1990). Bacteriophage λ DNA digested with *Hin*dIII was used as molecular mass standard. The sensitivity of the method was determined by the analysis of artifically contaminated filters with 0.1 ml samples of a culture of *M. hyopneumoniae* NCTC10110 at 10^8 viable cells/ml which was sequentially diluted from 10x to 10^{10}x in culture medium. The same extraction protocols as for filters from air-sampling were used.

DNA sequence analysis of the 808 bp PCR products of the 'inner' reaction was done the PRISM™ Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems / Perkin Elmer Cetus, Norwalk, Conn.) using oligonucleotides MHP950-2L and MHP950-2R in an ABI Prism 310 Genetic Analyzer (Applied Biosystems / Perkin Elmer Cetus).

3.5 Field sampling

Air was sampled in pig rooms on 7 commercial farms, in 1 pig room in an animal clinic and in 1 room in a research facility (specific pathogen-free [SPF], negative control). Farms where EP was present were either SPF farms with a laboratory-confirmed re-infection with *M. hyopneumoniae* but whose pigs showed no acute clinical signs (3 farms) or conventional farms whose pigs had acute respiratory problems diagnosed by their local veterinarian (4 farms). A detailed description of farms and rooms is given in TABLE 17.

On each farm, 1-4 rooms housing growing pigs were chosen according to the occurrence of clinical signs (coughing). The following parameters were recorded for each room: air volume per animal, air temperature and humidity (aspiration psychrometer, Haenni & Co. Ltd., Jegenstorf, Switzerland), clinical signs of enzootic pneumonia (coughing), age of animals, subjective air quality, type of feed, housing system and ventilation system. Coughs were counted for 10 minutes and then counts were extrapolated to the number of coughs per 100 pigs per 10 minutes to make the figure comparable between rooms.

Each filter holder was positioned outside the pen, 30-50 cm above ground level and 10-20 cm from the animals (FIGURE 11). Air was pumped through the filter for 1, 10, 60 or 100 minutes, respectively. The sampled air volume was 18.3-20.0, 183-200, 1200, or 1830-2000 litres, respectively. After sampling, the filter membranes were transferred into petri dishes for transport.

TABLE 17. Description of Swiss pig farms and rooms sampled

	Room	Air volume (m^3/pig)	Air temperature (°C)	% relative humidity	Coughing[b]	Age (weeks)	Farm type
Farm 1	1[a]	16.25	21.0	60	25.0	mixed	Pig facility at animal hospital
Farm 2	1	2.31	18.6	84	0	11	Integrated farm, SPF[c] farm re-infected with EP[d]
Farm 3	1	6.00	20.0	65	0	mixed	SPF research unit (negative control)
Farm 4	1[a]	4.88	20.2	91	4.0	19-22	Growing farm, purchasing pigs from several sources, acute respiratory problems
	2	2.95	n.m.[e]	n.m.	3.0	15	
	3[a]	2.92	18.8	75	17.0	17	
	4[a]	2.83	n.m.	n.m.	10.0	14-16	
Farm 5	1	2.00	23.2	85	7.0	16	Integrated farm, SPF farm re-infected with EP
Farm 6	1	3.47	20.4	75	4.0	16-18	Integrated farm, SPF farm re-infected with EP
Farm 7	1[a]	2.44	21.2	72	13.0	12-15	Integrated farm, acute respiratory problems
Farm 8	1[a]	2.90	21.4	55	10.0	12-16	Growing farm, acute respiratory problems
Farm 9	1	1.79	20.0	66	16.0	14	Integrated farm, acute respiratory problems

[a]Rooms where positive air samples were collected.
[b]Number of coughs/100pigs/10min.
[c]SPF=specific pathogen free.
[d]EP = enzootic pneumonia.
[e]n.m. = not measured.

Three different sampling protocols were developed.

Protocol 1: Different sampling durations (1, 10, 100 minutes) at one location in the room were used (farms 1 and 2).

Protocol 2: 6 samples, each taken over 10 minutes, were collected at 6 different locations in one room (farms 4,5,6).

Protocol 3: a cumulative sample was collected over 60 minutes at different locations in the room with one filter only (farms 4,7,8,9).

Additionally, on farms 4,7,8 and 9 dust samples were collected in the same room where air samples were collected. Dust was scratched from surfaces and ventilation ducts at different locations in the room and transferred into a sterile plastic container. From each sample, 100 mg of dust was suspended in 1ml TE buffer and subsequently extracted by the same protocols used for air sampling with the filters.

FIGURE 11. Air sampling in the field

3.6 Statistical analysis

Data were analysed using the statistical package SPSS (v.7.5, SPSS Inc., Chicago, USA). To test for differences between groups, the χ^2 test and the Mann-Whitney-U-test were used. Logistic regression techniques were used to explore farm and room variables influencing the PCR outcome.

4. Results

4.1 Specificity and sensitivity of the nested PCR assay

In order to analyse the specificity of the individual PCRs and in particular of the nested PCR assay, 2 ng of purified DNA from 15 different strains of *M. hyopneumoniae* including the type strains NCTC10110^T and ATCC 25934^T, as well as DNA from several closely related porcine *Mycoplasma* and *Acholeplasma* species were used as templates in the reactions (TABLE 15). All strains of *M. hyopneumoniae* showed the expected DNA fragments in the individual PCR reactions and in the nested PCR, while none of the other *Mycoplasma* or

Acholeplamsa species showed any amplification product in either single or nested PCR assay (TABLE 15). DNA sequence analysis of the 808-bp fragment obtained from the inner PCR reaction obtained from a few selected strains with widely varying origins (TABLE 15) as well as from two field samplings revealed 99.7% identity of the nucleotides to those in the corresponding fragment MHYP1-03/950 from the type strain NCTC10110 (sequence AF004388). The fragments from strains S924, Hungary 1 and Beaufort, as well as those from the field samples differed from sequence AF004388 at two nucleotide positions that were different for each strain, while strains BQ 14 and YZ differed from sequence AF004388 at the same three nucleoside positions. In control PCRs with the universal prokaryotic primers 16SUNI-L and 16SUNI-R, a 1.4 kb PCR product from the 16S rRNA gene *rrs* was amplified from all *Mycoplasma* and *Acholeplasma* species tested.

In order to analyse the suitability of the method in air sampling, nebulised, formaldehyde-inactivated *M. hyopneumoniae* cells were sampled in order to obtain filters with approximately 700, 4000 and 24,000 cfu of *M. hyopneumoniae*. Nested PCR of the filter extracts (see Material and Methods) revealed the presence of *M. hyopneumoniae* on filters from all three samplings. For analysis of the sensitivity, different filters artificially contaminated with various amounts of *M. hyopneumoniae* NCTC10110 containing 7 copies of the target sequence were prepared as described and subjected to the nested PCR. FIGURE 12 shows the results of the electrophoretic analysis of the products of the first and second PCRs in the nested PCR assay. The detection limit of the first PCR reaction with the primers MHP950-1L / MHP950-1R is in the range of 10^4 viable cells/filter, yealding a clearly visible 913-bp band. However, when the PCR products of this first reaction were used as templates for the second PCR reaction in a nested PCR, the 808-bp fragment from the second step was clearly visible for samples containing as few as 1 viable cell/ml. No signal was detected on the filters with averages of 10^{-1} viable cells/ml or fewer.

4.2 Field sampling

The conditions under which air was sampled are described in TABLE 17 and PCR results are shown in TABLE 18. For farms where acute respiratory problems were present, the overall proportion of air filters with a positive PCR result was 53.6%, while none of the filters from re-infected SPF farms tested positive in the assay. Of the farms where acute respiratory problems were present, 80% had at least one positive filter result. All negative control samples tested negative (TABLE 18).

It was observed that larger sampling volumes were not necessarily associated with positive PCR results. The highest proportion of positive results was obtained with short sampling period (1 minute, 18.3-20.0 litres) and with the cumulative sampling protocol. In order to assess the potential inhibitory effect on the PCR of non-target organisms and other substances that could have been sampled when collecting large volumes, we have mixed equal volumes of extracts from filters of long samplings (200 litres and 1000 litres) with extracts from filters that gave a positive result and resubmitted the mixture to PCR. These samples also showed positive results without reduction of the signal intensity.

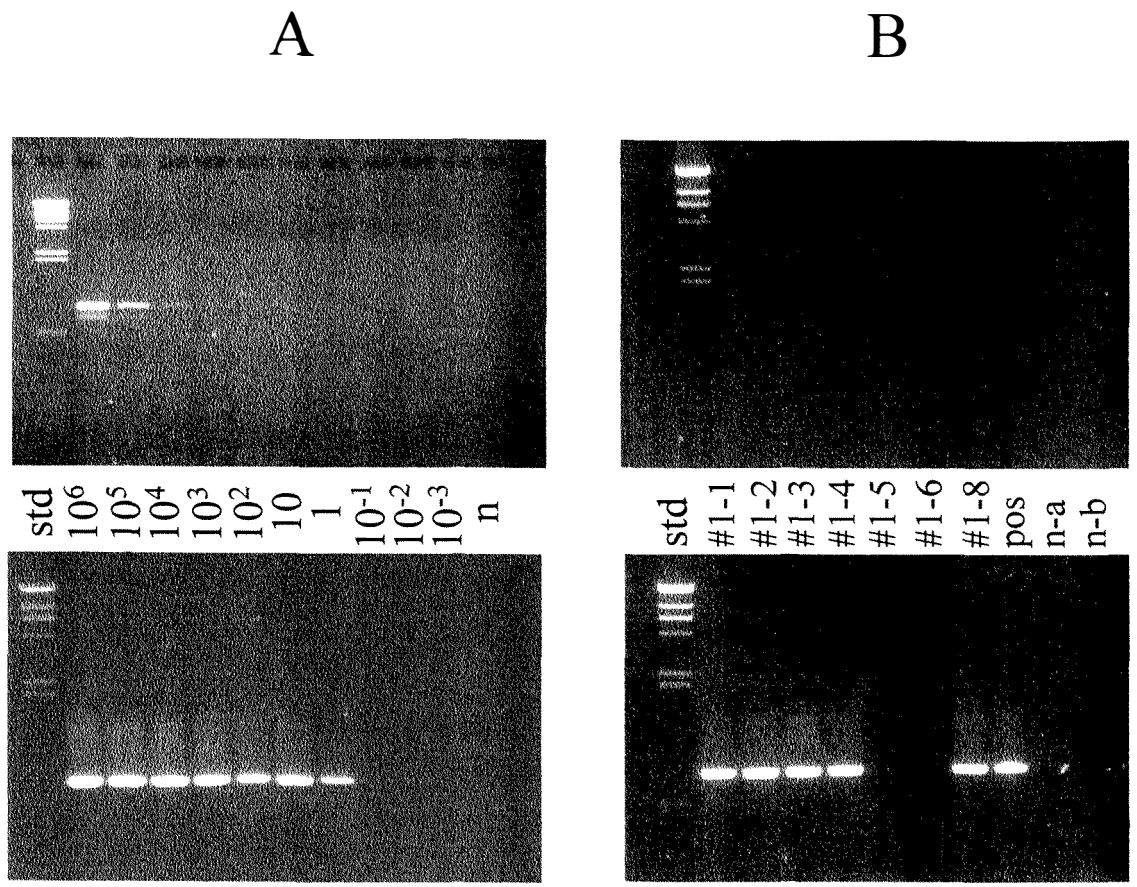


FIGURE 12. PCR analysis of air sampling - polyethersulfone membranes. The PCR amplification products from the first PCR reaction with primers MHP950-1L / MHP950-1R (upper photographs) and from the second PCR reaction with primers MHP950-2L / MHP950-2R (lower photographs) were analysed by agarose gel electrophoresis on 0.7% gels. A) Filters contaminated experimentally with known numbers of *M. hyopneumoniae* cells. The numbers between the two photographs are the average concentrations of viable cells/filter in the different samples. n, negative control, std, standard. Note that in some cases an additional PCR amplification product that was smaller than the 912-bp fragment was detected in low amounts in the outer PCR when high numbers of target organisms were used. This smaller PCR fragment, however, was not detected when purified *M. hyopneumoniae* genomic DNA was used. B) Filters from field sampling on a farm with pigs with respiratory problems. Lanes #1-1, #1-3, #1-4 and #1-6, 18-litre samples, lanes #1-2 and #1-5, 200-litre samples, lane #1-8, 2000-litre sample. Lanes n-a, negative control for buffers, lane n-b, negative control filter, std, standard, pos, positive control.

TABLE 18. Results for air samples analysed with a nested PCR to detect DNA of *Mycoplasma hyopneumoniae*

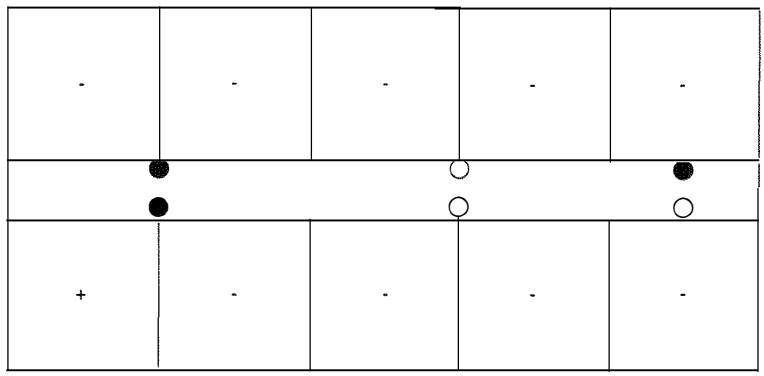
Air volume sampled (litres)	Re-infected SPF herd without acute problems		Herd with acute respiratory problems			
	Number of air samples #	Number of positive samples %	Number of air samples #	Number of positive samples %	Total	Negative controls # %
18.3-20.0	1	0	5	80.0	6	
183.0-200.0	13	0	16	43.8	29	5 0
>1000.0	2	0	3	33.3	5	1 0
Cumulativea (1098-1200)[a]	0	0	4	75.0	4	
Total by farm status	16		28	53.6	44	
% positive samples	0.0		53.6			
% positive farms	0.0		80.0			
Dust samples	0	0	4	25.0[b]	4	

[a]The same filter was used at 6 different locations in one room, sampling 183-200 l at each.
[b]The result from one dust sample was not interpretable.

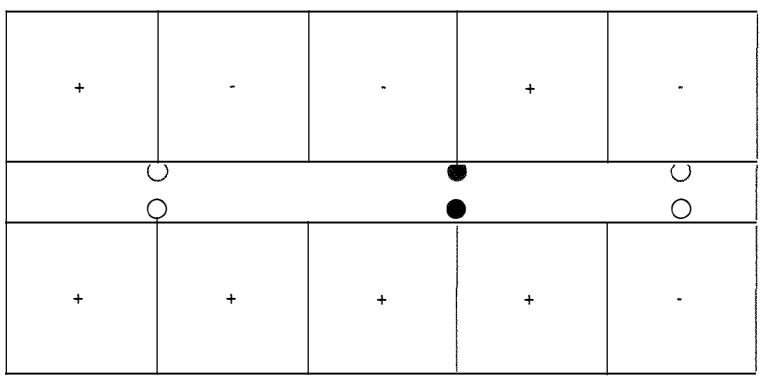
The results for the 6 samples collected at different locations in one room (protocol 2) showed that the distribution of mycoplasmas within a room did not seem to be uniform (FIGURE 13). Also, air samples with positive PCR results and pens where coughing pigs were observed during the sampling session were not clearly associated.

Because no samples from re-infected SPF herds were positive, further analyses with respect to factors influencing a positive PCR result was only performed for farms with pigs that displayed acute respiratory problems (28 filters). The likelihood of obtaining a positive air sample was influenced at the univariate level by the coughing intensity, age of the pigs and air humidity (TABLE 19). Samples with positive PCR results were more likely if a sample was taken in a room with higher coughing intensity, younger pigs and lower relative humidity. The likelihood of a positive test result was not significantly associated with the amount of air filtered, although filters that yielded positive results had a slightly higher mean volume. In a multivariate logistic regression model with stepwise variable selection, only air humidity was marginally significant with p=0.07 (OR=0.93, 95% C.I. 0.84-1.01).

Of 4 dust samples, 3 were negative and one yielded a smear on the agarose gel which was not investigated further and which was not interpretable (TABLE 18).



Farm 4, room 3



Farm 4, room 4

FIGURE 13. Charts of two pig rooms illustrating the distribution of positive PCR results from 6 air samples (200 l air sampled at each location. + = coughing observed in this pen, – = no coughing observed in this pen, ● = positive sample, ○ = negative sample)

TABLE 19. Factors associated with the outcome of a nested PCR assay to detect DNA from *Mycoplasma hyopneumoniae* in air samples (n=28)

Variable	Negative filters (n=13)			Positive filters (n=15)			p[a]
	Median	Mean	S.E.	Median	Mean	S.E.	
No. Coughs/100 pigs/10 min	10.0	9.62	2.22	13.0	14.87	2.43	0.14
% Relative humidity	75.0	75.69	2.88	72.0	68.33	2.54	0.06
Age of pigs (weeks)	14.0	15.38	0.59	13.0	14.40	0.57	0.12
Filter volume (litres)	200.0	416.92	124.57	200.0	465.20	156.16	0.62

[a]Man-Whitney U test, exact, two-tailed.

5. Discussion

This study documents the first successful attempt to definitely identify *M. hyopneumoniae* from the air in the vicinity of infected pigs. With an air sampling system using polyethersulfone membranes with a pore size of 0.2 µm, a simple method to recover DNA from the filters and a nested PCR assay, it was possible to detect DNA of *M. hyopneumoniae* under both experimental and field conditions.

PCR amplification-based tests for the detection of micro-organisms are generally highly sensitive, allowing in principle a single chromosome equivalent of a cell to be detected when pure DNA is used as template. In practice, however, this sensitivity is only very rarely achieved due to inhibitory substances in the samples to be tested, dilutions of the original samples by preparative procedures or loss of DNA during laborious extraction procedures. Typically for mycoplasmas, the sensitivity is on the order of 10,000 cells/reaction (Razin, 1994). This sensitivity of a one-step PCR is, however, not enough for the detection of micro-organisms in the air. In order to increase the sensitivity, nested (two-step) PCR have been developed for the detection of various micro-organisms, including several mycoplasmas (Razin, 1994). We have developed a highly sensitive nested PCR method based on the DNA sequence of a repeated gene segment of *M. hyopneumoniae* and used it to detect *M. hyopneumoniae* on filters used for air samplings. The method includes rapid dissolution of polyethersulfone filters and simultaneous extraction of DNA to be used for the nested PCR. Using filters that contained known numbers of *M. hyopneumoniae* cells (titrated as live cells), we observed a sensitivity of 1 viable cell/filter with the nested PCR compared with 10^4 viable cells/filter with one-step PCR. We had made a similar observation with a nested PCR used for the direct detection of *Mycoplasma mycoides* subsp. *mycoides* SC in clinical samples (Miserez *et al.*, 1997). Taking into account the dilution which is due to sample preparation and the small volume used for the first PCR step, the nested PCR was capable of detecting less than one viable cell per reaction. This is possible because prokaryotes contain more than one chromosome equivalent under exponential growth conditions. The gene target used for the PCR is the repetitive element MHYP1-03-950, which is present in 1-7 copies per chromosome of *M. hyopneumoniae*. This element is well conserved within the species of *M. hyopneumoniae* as shown by DNA sequence analysis, and it is absent from genetically and taxonomically related mycoplasmas. This explains the high detection limit and the high specificity of the nested PCR. Detection can be lower than one viable cell, which is of particular advantage in air sampling.

The field sampling showed that air sampling is most successful on farms with acute clinical problems (80% of these had at least one positive air sample). On farms with a higher rate of chronic disease associated with a lower prevalence of clinical signs, no airborne *M. hyopneumoniae* DNA was found in air samples. This indicates a low concentration of the agent under these circumstances. It is well known that *M. hyopneumoniae* cannot be isolated consistently easily during the entire course of the disease (Kobisch *et al.*; 1993; Sørensen *et al.*, 1997). Once the lung lesions start to regress at approximately 9 weeks after infection (Kobisch *et al.*, 1993), the isolation of mycoplasmas becomes difficult as their concentration in the tissue declines. It is therefore important to obtain samples from the right age group of pigs. In Switzerland, farmers wean pigs at the age of 4-6 weeks. At this stage, pigs will become infected if on a farm with EP problems and the best time to sample for airborne mycoplasmas would be

6-8 weeks later (Kobisch *et al.*, 1993), which is at an age of 10-14 weeks. In this study, the success in isolating *M. hyopneumoniae* DNA from an air sample was also associated with the age of the pigs. It appears that obtaining samples for somewhat younger pigs would have been advantageous. Also, in future studies, an assessment of the current infection status of the pigs at the time of air sampling (for example by analysing nasal swabs) would be desirable (Mattsson *et al.*, 1995; Sørensen *et al.*, 1997).

Coughing is considered the most reliable clinical indicator of EP infection (Sørensen *et al.*, 1993). Coughing starts about 2 weeks after infection and peaks after 5 weeks before gradually declineing (Kobisch *et al.*, 1993; Sørensen *et al.*, 1997). Because mycoplasmas become airborne through coughing, the concentration of infectious aerosol particles will also be higher when coughing is most prevalent. This is consistent with the results of this study, where more positive samples were obtained in rooms with a lot of coughing pigs.

The results also showed that the volume of air sampled is not the most important factor in the sampling protocol. Positive results were obtained with volumes as low as 20.0 litres, while some samples of >1000 litres were negative. Although we could exclude in our assay an inhibitory effect on PCR amplifications due to samplings of large volumes, it has to be noted that samplings of large volumes, in particular from highly contaminated air, could give false-negative results due to the inhibitory action on PCR by non-target DNA and other substances (Alvarez *et al.*, 1995). The result therefore is probably related to the fact that the distribution of airborne *M. hyopneumoniae* is not uniform within a pig room. The complex air circulation patterns within a pig room make it hard to predict where positive samples may be obtained. It has therefore earlier been suggested that air samples should be collected at a number of different sites within an animal room in order to obtain a representative result (Wathes and Randall, 1989). The cumulative sampling protocol used in this study seems to be the most suitable approach in this situation.

In this study air humidity was an influential factor as to whether infectious aerosols were detected in a pig room or not, with lower humidity being associated with positive sampling results. The relative humidity of the air influences particle aggregation and net water flow and therefore also particle size. The more hygroscopic a particle, the larger it gets in a humid environment and the faster is its sedimentation rate (Cox, 1995). In a drier environment particles will thus remain airborne over a longer time period, which will increase the airborne particle concentration and the chance of particle capture in air filters.

The alternative method of sampling dust instead of air was not successful. Only one of four dust samples was suspected of being positive in the PCR assay. Dust samples may contain organic inhibitors which have a negative effect on the PCR assay.

The results of this study using a nested PCR assay strongly support the hypothesis of airborne transmission of *M. hyopneumoniae*. However, questions with respect to the potential survival of airborne *M. hyopneumoniae* and maximum travel distances between farms remain to be investigated.

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References

Alvarez, A.J., Buttner, M.P., and Stetzenbach, L.D. (1995). PCR for Bioaerosol Monitoring: Sensitivity and Environmental Interference. Applied and Environmental Microbiology 61:3639-3644.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1990). Current protocols in molecular biology. Wiley Interscience, New York.

Blanchard, B., Kobisch, M., Bove, J.M., and Saillard, C. (1996). Polymerase chain reaction for *Mycoplasma hyopneumoniae* detection in tracheobronchiolar washings from pigs. Molecular and Cellular Probes 10:15-22.

Cox, C.S. (1987). The aerobiological pathway of microorganisms. John Wiley & Sons, Chichester.

Cox, C.S. (1995). Physical aspects of bioaerosol particles. In C.S. Cox, and C.M. Wathes (eds.), Bioaerosol handbook CRC Press Lewis Publishers, Boca Raton, 15-25.

Djordjevic, S.P., Eamens, G.J., Romalis, L.F., and Saunders, M.M. (1994). An improved enzyme linked immunosorbent assay (ELISA) for the detection of porcine serum antibodies against *Mycoplasma hyopneumoniae*. Veterinary Microbiology 39:261-273.

Ernø, H. and Stipkovits, L. (1973). Bovine mycoplasmas: Cultural and biochemical studies. Acta Veterinaria Scandinavica 14:436-449.

Friis, N.F. (1971). Sensitivity of *Mycoplasma suipneumoniae* to penicillin-G. Acta Veterinaria Scandinavica 12:120-121.

Futo, S., Seto, Y., Okada, M., Sato, S., Suzuki, T., Kawai, K., Imada, Y., and Mori, Y. (1995). Recombinant 46-kilodalton surface antigen (P46) of *Mycoplasma hyopneumoniae* expressed in *Escherichia coli* can be used for early specific diagnosis of mycoplasmal pneumonia of swine by enzyme-linked immunosorbent assay. Journal of Clinical Microbiology 33:680-683.

Giger, T., Bruggmann, S., and Nicolet, J. (1977). Immunologische Methoden zum Nachweis von *Mycoplasma suipneumoniae* in Gefrierschnitten und Bronchialabstrichen. Schweizer Archiv für Tierheilkunde 119:125-134.

Goodwin, R.F.W. (1985). Apparent re-infection of enzootic-pneumonia-free pig herds: search for possible causes. Veterinary Record 116:690-694.

Goodwin, R.F.W., and Whittlestone, P. (1967). The detection of enzootic pneumonia in pig herds. I. Eight years general experience with a pilot control scheme. Veterinary Record 81:643-647.

Keller, H. (1988). The Swiss Pig Health Service (PHS). Proceedings of the 10th IPVS Congress, Rio de Janeiro, Brazil, 334.

Kobisch, M., Blanchard, B., and Le Potier, M.F. (1993). *Mycoplasma hyopneumoniae* infection in pigs: duration of the disease and resistance to reinfection. Veterinary Research 24:67-77.

Kuhnert, P., Capaul, S., Nicolet, J., and Frey, J. (1996). Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. International Journal of Systematic Bacteriology 46:1174-1176.

Mattsson, J.G., Bergstrom, K., Wallgren, P., and Johansson, K.E. (1995). Detection of *Mycoplasma hyopneumo-*

niae in nose swabs from pigs by in vitro amplification of the 16S rRNA gene. Journal of Clinical Microbiology 33:893-897.

Miserez, R., Pilloud, T., Cheng, X., Nicolet, J., Griot, C., and Frey, J. (1997). Development of a sensitive nested PCR method for the specific detection of *Mycoplasma mycoides* subsp. *mycoides* SC. Molecular and Cellular Probes 11:103-111.

Nicolet, J., Paroz, P., and Bruggmann, S. (1980). Tween 20 soluble proteins of *Mycoplasma hyopneumoniae* as antigen for an enzyme linked immunosorbent assay. Research in Veterinary Science 29:305-309.

Olsson, M., Sukura, A., Lindberg, L.-A., and Linder, E. (1996). Detection of *Pneumocystis carinii* DNA by filtration of air. Scandinavian Journal of Infectious Diseases 28:279-282.

Razin, S. (1994). DNA probes and PCR in diagnosis of mycoplasma infections. Molecular and Cellular Probes 8:497-511.

Ross, R.F. (1992). Mycoplasmal diseases. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, and D.J. Taylor (eds.), Diseases of swine. 7th Edition. Iowa State University Press, Ames, Iowa, 537-551.

Sørensen, V., Barfod, K., Feld, N.C., and Vraa-Andersen, L. (1993). Application of enzyme-linked immunosorbent assay for the surveillance of *Mycoplasma hyopneumoniae* infection in pigs. Revue scientifiaue et technique, Office International des Epizoories 12:593-604.

Sørensen, V., Ahrens, P., Barfod, K., Feenstra, A.A., Feld, N.C., Friis, N.F., Billehansen, V., Jensen, N.E., and Pedersen, M.W. (1997). *Mycoplasma hyopneumoniae* infection in pigs: Duration of the disease and evaluation of four diagnostic assays. Veterinary Microbiology 54:23-34.

Stärk, K.D.C., Keller, H., and Eggenberger, E. (1992). Risk factors for the reinfection of specific pathogen free pig breeding herds with enzootic pneumonia. Veterinary Record 131:532-535.

Tamàsi, G. (1973). Mycoplasma isolation from the air. In: J.F.Ph. Hers, and K.C. Winkler (eds.) Airborne transmission and airborne infection. Oosthoek Publishing Company, Utrecht, The Netherlands, 68-71.

Thomsen, B.L., Jorsal, S.E., Andersen, S., and Willeberg, P. (1992). The Cox regression model applied to risk factor analysis of infections in the breeding and multiplying herds in the Danish SPF system. Preventive Veterinary Medicine 12:287-297.

Wathes, C. M., and Randall, J. M. (eds.) (1989). Aerosol sampling in animal houses. Proceedings of a workshop held at the University of Bristol, 26-29 July, 1988. EU Report 11877. Commission of the European Communities, Luxembourg.

CHAPTER 1.5

ALTERNATIVE METHODS TO SOLVE CLASSIFICATION PROBLEMS IN COMPLEX DATA SETS

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1. Introduction

Classification problems are analogous to the problem of diagnosis in medicine and therefore very frequent in veterinary epidemiology research. As part of an epidemiological study, measurements are commonly made at the animal or farm level. Based on these measurements we would like to predict the class this animal or farm is in with respect to, for example, actual disease status (diseased vs. non-diseased) or disease risk status (high risk vs. medium risk vs. low risk.).

Traditionally, parametric statistical techniques were used to achieve this objective. For classification problems, logistic regression and discriminant analysis are probably the most commonly used analytical techniques (Hand, 1992). However, they are based on assumptions with respect to the characteristics of the data set, which in reality, are often difficult to fulfil. The inherent structure and complexity of a data set can thus affect the suitability for conventional data analysis (Morgan and Sonquist, 1963; Burke, 1996). The complexity of a data set is particularly influenced by the following characteristics:

High dimensionality: A large number of explanatory variables is a problem that is well recognised in standard statistical analysis in general and specifically in veterinary epidemiology (Dohoo *et al.*, 1996; Martin, 1997). Some techniques require multivariate normality of the covariates and are therefore not suitable for the analysis of categorical variables. With other methods dimensionality is greatly increased by the need to introduce dummy variables for categorical variables. Therefore, most popular multivariate analytical procedures allow for the use of a dimensionality reduction procedure, for example stepwise variable selection.

Multicollinearity and interaction: Relationships among the independent variables can be a problem particularly in parametric analyses, which typically assume independence. However, effect modification by explanatory variables is a significant characteristic of a data set and needs to be measured. Unfortunately, the introduction of interaction terms in standard statistical analysis is cumbersome and often neglected (Morgan and Sonquist, 1963).

Nonhomogeneity and nonlinearity: Different relationships can hold between variables in different parts of the measurement space. Most parametric analysis techniques however assume linearity of the effects.

If a complex data set is not suitable for statistical analysis with conventional techniques for one or several of the above reasons, alternatives need to be considered. The objective of this article is to describe non-parametric methods, which are more flexible in terms of the underlying data structure and thus are appealing complementary tools for data analysts. The use of these techniques is illustrated using a field data set and the results are compared with a multinomial logistic regression model.

2. Recursive partitioning (classification trees) and machine learning

The principle of recursive partitioning is to group the observations in the data set based on the explanatory variables by applying a set of decision rules or successive splits. The terminal subsets of the data each form a particular partition of the data space. These segments are mu-

tually exclusive and exhaustive (segments do not overlap and each observation is in exactly one segment). The result of recursive partitioning can be graphically represented as a tree diagram with nodes and terminal leaves. Tree diagrams can be interpreted intuitively. This helps in communicating the results.

The objective of the classification tree analysis can be prediction (classification of future cases) or discovery of predictive structure in a data set (exploratory data analysis). In the latter case we are trying to understand which variables or interactions are driving the classification process. It can be used as a detector of interaction and provide important insight into otherwise hidden data structures (Magidson, 1994).

Tree-structured classification is a recursive and iterative procedure that requires the specification of the following elements (Breiman *et al.*, 1984):

1. A set of splitting rules using the predictive variables. Continuous, ordinal, nominal or binary variables can be used, but limitations exist for certain tree-growing methods. Most classification tree techniques will perform binary splits only based on the value of only one variable.
2. A rule for selecting the best split at any node. At each node an algorithm searches across all variables and produces a sequence of most efficient splits. It then compares the best single variable splits and selects the best from them using a goodness of split criterion. The different tree growing methods are distinct in terms of their goodness of split criterion.
3. A criterion for choosing the right-sized tree (stopping rule). Some techniques use a procedure called pruning, which is a process of removing nodes and branches from a tree in order to make it simpler while maintaining high accuracy of classification. Pruning algorithms use measurements of accuracy while penalising larger tree size. The result is the simplest and smallest tree that gives the maximum accuracy.
4. A rule for assigning every terminal node to a class.

The construction of classification rules is based on a training data set, which contains observations with their 'true' classification. The accuracy of a classifier is ideally assessed using new test data with observations that the classifier has not yet seen. Unfortunately, in most cases such additional data is not available and the original data set needs to be used for both classifier construction and testing of the classifier. If the data set is large enough (of order 1000 observations), a smaller subset can be set aside for testing (Henery, 1994a). The result from classifications in the sub-groups is used to calculate the classification error. Frequently a set consisting of 1/3 of all cases is being used although there does not seem to be a theoretical justification for this split (Breiman *et al.*, 1984). A second technique allowing for moderate-sized samples is *m*-fold cross-validation. The data set is divided into *m* subsets, each of which is tested by the classifiers derived from the remaining *m-1* subsets (Henery, 1994a). Finally, for small data sets bootstrapping can be used to calculate error estimates. The bootstrap is a non-parametric procedure that re-uses the original data set of size *n* to obtain new data sets also of size *n* by re-sampling with replacement (Henery, 1994a). Some observations can thus be used several times for training. The test data consists of all observations not used in the training set.

Examples of recursive partitioning algorithms are CART (**C**lassification **A**nd **R**egression **T**rees; Breiman *et al.*, 1984) and CHAID (**Chi**-squared **A**utomatic **I**nteraction **D**etector; Kass, 1980; Magidson, 1994; based on AID by Morgan and Sonquist, 1963). Among all tree-structured predictors applied in the medical domain, CART has been most frequently used, probably because it is the oldest and most intensively researched method.

Classsification tree algorithms are also frequently used in the field of machine learning. Machine learning (ML) is an emerging technology for the automated discovery of patterns in large data sets (Cunningham, 1995) and has been widely used for knowledge acquisition during the design of expert systems. The acquired knowledge is represented as a classification tree and can be interpreted as a set of 'if-then' decisions (Feng and Michie, 1994). ML algorithms have also been applied in the agricultural domain (McQueen *et al.*, 1995). A distinct characteristic of ML algorithms is that the performance of the algorithm is not only based on the percentage of correctly classified observations but on the over-all information gain (Quinlan, 1993). Examples of machine learning algorithms are ID3 (Quinlan, 1986) and C4.5 (Quinlan, 1993).

3. Neural networks

Neural networks (NN) are data-driven connectionist models from the field of artificial intelligence applying non-linear analytical processes to solve pattern recognition and classification problems. NN consist of a network of interconnected processing units, the structure of which is based on the structure of the human brain (Carling, 1992).

Each processing unit (node) in a NN has an output value X that is usually bounded to lie between 0 and 1 (Hart, 1992). The output value is determined by the input units feeding into it. Each link between nodes is associated with a weight L, which can be either negative or positive. Under these conditions X is given by:

$$X = f\left(\sum X_i L_i + \theta\right)$$

where θ is a threshold or bias associated with the node and f(.) is some activation function. Most commonly used non-linear activation functions are the sigmoidal or logistic function and the hyperbolic tangent function.

Processing units within a NN can be arranged in a number of ways. In order to perform its task correctly, a NN needs to have the appropriate architecture, which is determined by the person designing the network, but the weights are obtained by training. During training, a number of example cases are fed through the network in order to obtain the correct weights. It is assumed that the training data consists of a representative sample of the population. The training data also supplies the desired classification (supervised learning).

The necessary size of the training set depends on the number of input features and weights to be determined (connections between nodes). Sample size calculations as such are not possible, but in general NN are very 'data hungry'. This means that to be trained they require a large number of example cases, even more than conventional multivariate techniques. In order to reduce the number of observations required, some method for dimensionality reduction may have to be used.

The most commonly used NN architecture in supervised learning is the multi-layer perceptron (FIGURE 14). It consists of n input nodes and m output nodes with at least one layer of hidden nodes. The information is fed forward through the network from the input to the output nodes. There are no rules as to how many hidden layers and nodes in the hidden layers a NN should ideally have. The adequate network architecture has to be determined through experimentation and observation (Cross *et al.*, 1995).

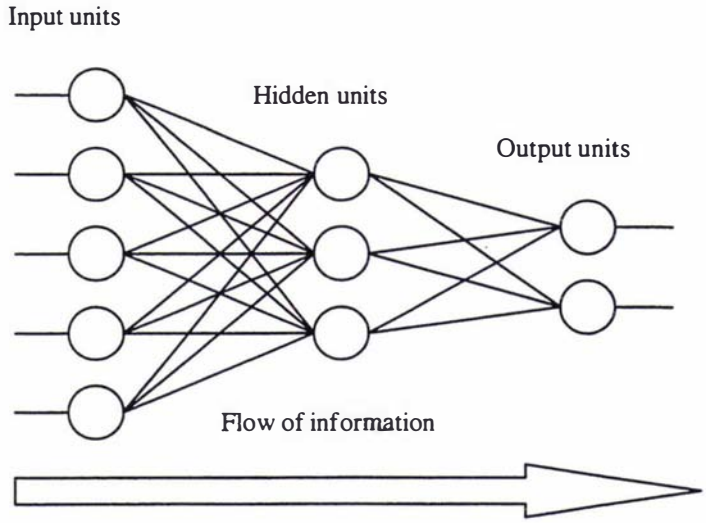

FIGURE 14. Diagram of a multi-layer perceptron (node O , link —)

In the multi-layer perceptron, training is achieved by minimising the square mean output error (measure of network performance) by backpropagation and using the generalised delta rule. The standard backpropagation algorithm uses two parameters to control the learning process. One is the *momentum term*, which causes the weight changes to be affected by the size of the previous weight changes. This is used to avoid local minima in the performance surface of the network. The *learning rate* tells the network how slowly to progress (proportion of error contributing to updating weights). Both values are between 0 and 1. For recommendations on how to set momentum and learning rate see Goodrace *et al.* (1996) and Swingler (1996).

The training stops when some stopping rule is met, for example the increase in precision falls below a certain target value.

To ensure integrity of testing, test data should not be used for training. Therefore a part of the initial data needs to be set apart for testing or additional data has to be collected. With small training data sets over-fitting may be a problem. The NN has then perfectly learnt the training data but cannot generalise to new data. As a general rule, the simpler the model the better it is at generalising to data it has not seen (Swingler, 1996).

4. Example: Classification of pig farms with respect to the prevalence of enzootic pneumonia

4.1 Data set

Data on enzootic pneumonia-type lesions in 89 pig farms were collected during 1995-1996 (Stärk *et al.*, 1996). The data set consists of the prevalence of gross lung lesions at slaughter as assessed during winter and of 40 farm variables (TABLE 20). The latter were collected in a postal survey. The outcome variable is the prevalence of pigs with lesions indicative of enzootic pneumonia. Farms were grouped into 3 classes according to the lesion prevalence: class 1 (prevalence $\leq$10.0%), class 2 (prevalence 11.0$\leq$x$\leq$40.0), and class 3 (prevalence >40.0).

4.2 Methods and software

4.2.1 Data pre-processing

In order to avoid problems associated with missing values, the original data set was completed using imputation. The missing data analysis feature of the statistical software package SPSS v.7.5 (SPSS Inc., Chicago, USA) was used. Three farms did not have weaned pigs and therefore all variables related to weaned pigs were missing. These observations were list-wise deleted (remaining 86 observations, 5 in class 1, 11 in class 2 and 70 in class 3). Two variables (WTEMP, GTEMP) had missing values in >30% of cases. These variables were excluded from the analysis. All the remaining variables with missing values were continuous variables. Stochastic regression imputation was used in SPSS v.7.5 (SPSS Inc., Chicago, USA) to replace missing values (Little and Rubin, 1987; Little and Schenker, 1995).

Method-specific data pre-processing is described for the different classification techniques below.

4.2.2 Multinomial logistic regression

All continuous variables were checked for linearity as described by Hosmer and Lemeshow (1989). They were then re-coded into four classes each using the quartiles as class borders. Categorical variables with more than 2 categories were labelled as factor variables.

TABLE 20. Variables used in the analysis of risk factors affecting the prevalence of enzootic pneumonia lesions in New Zealand pig herds

Name	Description	*Type*[a]	Name	Description	*Type*
AB1	In-feed antibiotics for weaned pigs	*Bin*	ISLAND	Farm in South Island (vs. North)	*Bin*
AB2	In-feed antibiotics for growing pigs	*Bin*	NEIGH	Distance to next pig farm (km)	*Cont*
COUGH1	Coughing in weaned pigs	*Bin*	PREV	Vaccination against *M.hyopneumoniae*	*Bin*
COUGH2	Coughing in growing pigs	*Bin*	PURCH	Purchase policy	*Cat(4)*
DIAR	Diarrhoea observed	*Bin*	SOWS	Number of sows	*Cont*
GAIAO	All-in/all-out system finishing pigs	*Bin*	SQUO	Air quality (subjective)	*Cat(3)*
GBED	Bedding used, finishing pigs	*Bin*	TYPE	Farm is finishing only facility (vs. mixed)	*Bin*
GFED	Wet-dry feed, finishing pigs	*Bin*	WAIAO	All-in/all-out system, weaned pigs	*Bin*
GMAN	Liquid manure, finishing pigs	*Bin*	WBED	Bedding used, weaned pigs	*Bin*
GPPP	Pigs per pen, finishing pigs	*Cont*	WFED	Wet-dry feed, weaned pigs	*Bin*
GPPR	Pigs per room, finishing pigs	*Cont*	WMAN	Liquid manure, weaned pigs	*Bin*
GREM	Frequency of manure removal	*Cat(3)*	WPPP	Pigs per pen, weaned pigs	*Cont*
GROW	Number of finishing pigs	*Cont*	WPPR	Pigs per room, weaned pigs	*Cont*
GSEP	Solid pen separation, finishing pigs	*Bin*	WREM	Frequency of manure removal	*Cat(3)*
GSHARE	Sharing room with other age group, finishing pigs	*Bin*	WSEP	Solid pen separation, weaned pigs	*Bin*
GSP	Space (m2) , finishing pigs	*Cont*	WSHA	Sharing room with other age group, weaned pigs	*Bin*
GTEMP	Temperature (°C) , finishing pigs	*Cont*	WSP	Space (m2) , weaned pigs	*Cont*
GVENT	Automated ventilation, finishing pigs	*Bin*	WTEMP	Temperature (°C) , weaned pigs	*Cont*
GWAT	Free water access, finishing pigs	*Bin*	WVENT	Automated ventilation, weaned pigs	*Bin*
HYG	Hygiene level (subjective)	*Cat(3)*	WWAT	Free water access, weaned pigs	*Bin*

[a]Bin=binary, Cont=continuous, Cat=categorical with number of classes in brackets

The multinomial logistic regression model was built using SAS v. 6.12 (Proc CATMOD). Standard methods of dimensionality reduction were applied as follows. Only variables with $p \leq 0.20$ at the univariate analysis level were candidates for the multivariate models (COUGH2, AB1, WVENT, WMAN, WREM, WBED, WSHA, WAIAO, GFED, GREM, GSHA, GROW4, NEIGH4). A stepwise forward selection procedure was performed using an entry level of $p \leq 0.05$. The parameters were estimated using the maximum-likelihood method. R^2 was calculated as described by Menard (1995). The final model was checked for potential interaction terms and used to construct the classification table. The latter was used to calculate classification accuracy, sensitivity, specificity and predictive values.

4.2.3 Tree classification algorithms

The CHAID, CART, ID3 and C4.5 algorithms as integrated in the SIPINA v. 2.02 knowledge discovery package (D.A. Zighed, L. Ponsard and R. Rakotomalala, E.R.I.C., Univeristé Lumière Lyon2, Bron, France) were used for the classification tree analysis. All explanatory variables were used as they were collected in the survey (no re-coding). The stopping rule for all schemes was a minimal leaf size of 5 observations and a χ^2 test of 0.05, where applicable. CART was used with two different splitting rules, the twoing and the gini goodness-of-split criterion (CART2 and CART1). The first tends to split into two nodes that are as pure as possible, and the latter tends to split off one small pure node and one large impure node (Breiman *et al.*, 1984).

A full tree was grown using all the observations in the data set. The performance of this tree was measured in terms of sensitivity, specificity and predictive values. The classification performance of each tree-growing scheme was also calculated using 15-fold cross-validation. The results were expressed as mean percentage correctly classified observations.

4.2.4 Artificial neural network

The software packages NeuroSolutions v.2.1 and NeuroSolutions for MS Excel v.1.0 (NeuroDimension Inc., Gainesville, USA) were the simulation environments used for training the neural network. A fully connected multi-layer perceptron with one hidden layer and 5 hidden units was trained. The learning rate and the momentum term were set to 0.25 and 0.7, respectively. All explanatory variables were used in the network as they were collected in the survey (no re-coding). Weighting of observations was applied in order to account for the unbalanced number of observations in the 3 classes. After the network was trained, sensitivity analysis was performed to identify influential variables. All variables with a relative influence on the outcome of <3.0% were excluded. The network was then retrained and tested using 15-fold cross-validation. Again, the performance was measured both in terms of the final network as well as in terms of average performance of repeated application of this technique.

4.2.5 Comparison of schemes

The schemes were compared using the technique metric multidimensional scaling available in the statistical software package NCSS97 (Number Cruncher Statistical Systems, Kaysville, Utah, USA). The following characteristics of the schemes were used: over-all correct classification proportion, correct classification averaged across outcome classes, sensitivity for each class and specificity for each class. For the purpose of comparison, two additional schemes were added, one representing an ideal classification scheme (IDEAL) with maximum values for all characteristics, and a baseline scheme (BASELINE), where all observations were classified in the group with the highest frequency (EP = high). The latter method is comparable to a single-rule ('All farms have a high EP prevalence') or baseline classifier.

5. Results

CHAID was the only classification scheme that could not conclusively classify all observations, because one of the final leaves contained an equal number of observations in two classes. By manually swapping the splitting variable which caused this problem with the one used on the next split, this could be avoided. The results of both the original tree (CHAID1) as well as the modified tree (CHAID2) are included in the results.

All classification methods applied in this study were able to correctly classify at least 84% of the farms (TABLE 21). However, there was considerable variability with respect to the class-specific performance of the schemes. Most schemes performed best in class 3 (high EP prevalence) with 91-100% correctly classified farms, and poorest in class 2 (medium EP prevalence) with 0-83% correctly classified observations. The MLR models achieved the best average correct classification proportion over all classes of 93 % followed by the modified CHAID2 tree with 85%. Sensitivity and specificity were explored as additional performance indicators. For class 1 (low EP prevalence) the specificity and the sensitivity both tended to be high, while in class 2 specificity was clearly higher than sensitivity for all schemes except for CHAID1, and with respect to class 3 sensitivity was higher than specificity.

TABLE 21. Comparative performance of classification schemes using data on enzootic pneumonia prevalence from 86 farms

Method	% over-all correct classified	Mean % correct per class	Sensitivity Class 1	Sensitivity Class 2	Sensitivity Class 3	Specificity Class 1	Specificity Class 2	Specificity Class 3
MLR[a]	96	93	100	83	97	100	97	88
ID3[b]	93	69	100	46	100	98	100	75
C4.5[b]	90	78	100	36	99	95	99	81
CHAID1[b,c]	84	58	0	82	97	100	97	81
CHAID2[b,d]	91	85	80	97	93	96	97	81
CART1[b,e]	86	60	80	0	100	96	100	44
CART2[b,f]	93	82	100	46	100	98	100	75
NN[g]	93	78	75	67	100	100	98	71
BASE-LINE	81	33	0	0	100	100	100	0
IDEAL	100	100	100	100	100	100	100	100

[a]MLR=multinomial logistic regression models.
[b]Stopping rules: size=5, χ^2=0.05.
[c]8 observations not classified.
[d]manually modified to avoid unclassified observations.
[e]using twoing rule for splitting.
[f]using gini rule for splitting.
[g]only 72 observations used for training, 14 for cross-validation. Increase of classification error in cross-classification data was used as stopping criterion.

The MLR model parameters are presented in TABLE 22. The variables COUGH2, WAIAO and WMAN were selected. The parameters for the comparison of outcome class 3 vs. 1 were reported to be 'infinite' by SAS, and standard error, χ^2 and p-value could not be calculated.

The R^2 of the model was 0.27. The –2 log likelihood was 68.18. With this model, 8 sub-populations of the data set were created, four of which had only 1-2 observations. The other four populations included 10, 13, 18, and 26 observations..

TABLE 22. Multinomial logistic regression models for the classification of 3 levels of enzootic pneumonia prevalence in 86 pig farms

Effect	Function[a]	Estimate	SE	χ^2	p
INTERCEPT	3 vs. 1	-13.3998	0.5410	613.44	0.0000
	3 vs. 2	-1.3691	0.6086	5.06	0.0245
COUGH2	3 vs. 1	5.2171[b]	n.c.[c]	n.c.	n.c.
	3 vs. 2	1.2149	0.4524	7.21	0.0072
WAIAO	3 vs. 1	-4.9231[b]	n.c.	n.c.	n.c.
	3 vs. 2	-0.8990	0.4741	3.59	0.0580
WMAN	3 vs. 1	-2.5248[b]	n.c.	n.c.	n.c.
	3 vs. 2	1.3698	0.6085	5.07	0.0244

[a]Compared outcome classes, 1=low prevalence, 2=medium prevalence, 3=high prevalence.
[b]Parameters are regarded as infinite.
[c]n.c.=not calculated.

The different classification schemes were next compared with respect to the number and type of variables that they used to achieve classification. All classification tree algorithms selected the variable COUGH2 for the first split (FIGURE 15). ID3 and CART2 (gini rule) produced an identical tree.

The ranking of variables in the NN was determined by sensitivity analysis. The 10 most influential variables are included in TABLE 23. They account for 79% of the variability in the outcome. The ID3, C4.5 and CART2 algorithms resulted in the inclusion ofsimilar variables, and there was a strong overlap with variables selected by the CHAID method. CART1 however (twoing rule) selected fewer and different variables. Stepwise MLR chose the same two variables first as ID3 and CART2, but it also used WMAN, a variable that none of the other schemes selected. Interestingly, all continuous variables that were used by multiple schemes were split at the same value (WSP 0.47 and 0.23, GPPP 12.5, GSP 0.68).

When looking at the performance during cross-validation, again, all schemes produced similar results. The average percentage of correctly classified farms was lower than with the tree that used all observations, and it was quite variable (TABLE 24). When comparing the number of rules or splits used by each scheme, CHAID and CART1 tended to require fewer rules, although this figure was highly variable particularly for CHAID. Cross-validation was not performed with MLR.

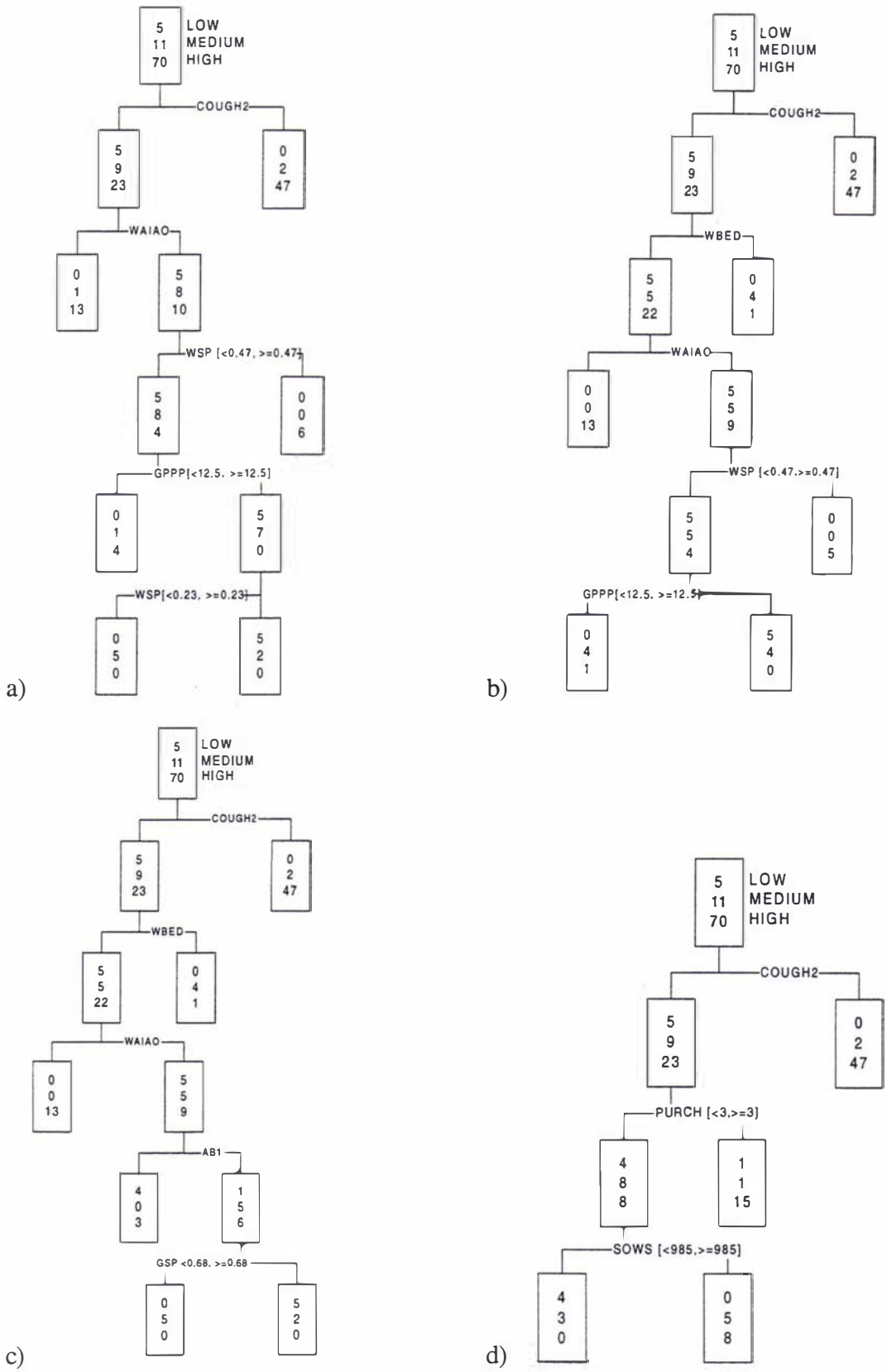

FIGURE 15. Classification trees grown using a) ID3 and CART (gini rule), b) C4.5, c) CHAID (manually modified to avoid unclassified observations) and d) CART (twoing rule).

TABLE 23. Ranking of variable selection in different classification schemes

	ID3	CART2[a]	C4.5	CHAID1	CHAID2[b]	CART1[c]	NN	MLR
COUGH2	1	1	1	1	1	1	6	1
WAIAO	2	2	3	4	3	-	9	2
WBED	-	-	2	2	2	-	-	-
WSP	3,5[d]	3,5[d]	4	-	-	-	-	-
GPPP	4	4	5	-	-	-	-	-
PURCH	-	-	-	-	-	2	2	-
AB1	-	-	-	3	4	-	-	-
GSP	-	-	-	5	5	-	-	-
SQUO	-	-	-	-	-	-	1	-
SOWS	-	-	-	-	-	3	-	-
HYG	-	-	-	-	-	-	3	-
WREM	-	-	-	-	-	-	4	-
GREM	-	-	-	-	-	-	5	-
WSHA	-	-	-	-	-	-	7	-
WVENT	-	-	-	-	-	-	8	-
WFED	-	-	-	-	-	-	10	-
WMAN	-	-	-	-	-	-	-	3

[a]CART2 was using the gini splitting criterion.
[b]CHAID2=CHAID1 manually modified to avoid unclassified observations.
[c]CART1 was using the twoing splitting criterion.
[d]Variable was used for two splits.

TABLE 24. Results of 15-fold cross-validation[a]

Method	% correct	SD	Rules	SD
ID3	0.79	0.20	5.60	1.02
C4.5	0.74	0.17	5.13	0.88
CHAID	0.73	0.19	4.60	1.02
CART1[b]	0.77	0.19	4.60	0.61
CART2[c]	0.79	0.19	5.47	0.72
NN	0.79	0.12	n.a.[d]	n.a.

[a]Stopping rules: size=5, χ^2=0.05; neural network: Increase of classification error in cross-classification data was used as stopping criterion.
[b]CART1 is using the twoing splitting criterion.
[c]CART2 was using the gini splitting criterion.
[d]n.a.=not applicable.

Multidimensional scaling was used to visually compare the performance of the different schemes . The first two dimensions accounted for 97.3 % of the variation and had a pseudo R^2 of 98.9 (stress factor 0.002). These two dimensions are plotted against each other in FIGURE 16. MLR is located closest to the IDEAL scheme, and CHAID2 and NN also are in the same quadrant, while all other schemes except for BASELINE are located in the diagonally opposite quadrant, at relatively close proximity to each other. With the data set used in this illustrated example, all information-based classifiers thus performed similarly compared with each

other, but not as well as MLR, NN and CHAID. The BASELINE scheme was shown not to be a competitive alternative.

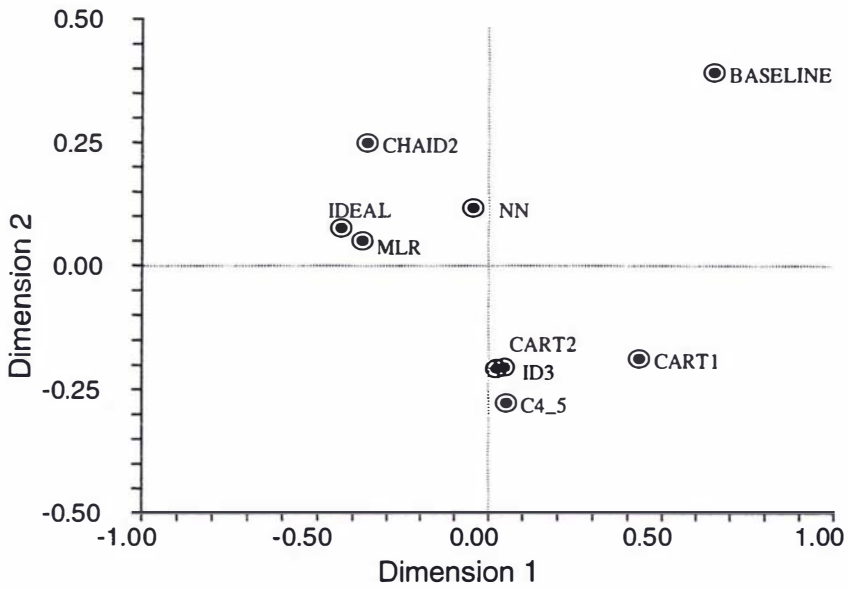

FIGURE 16. Graphical comparison of classification methods using multidimensional scaling

6. Discussion

In this study, two types of non-parametric classification methods, tree classifiers and neural networks, were compared with multinomial logistic regression when applied to data from an epidemiological field study. The selection of classification schemes included in this comparison was influenced by software availability. Particularly in the field of machine learning, algorithms become outdated rapidly due to the development of new and improved schemes. We acknowledge that ID3 and C4.5 are relatively old classifiers that are now being replaced by others. However, they have been used in comparative studies before and their behaviour is reasonably well researched, which justifies their inclusion. The same is true for CART and CHAID, which are established standards in the medical domain. Both of these techniques are now available in standard software packages such as STATISTICA (StatSoft Inc., Tulsa, USA) and SPSS (SPSS Inc., Chicago, USA), and appear to become the most accepted tree classifiers amongst the statistical community. Other non-parametric statistical classifiers listed by Molina *et al.* (1994), such as *K*-nearest neighbour, projection pursuit classification, non-linear discriminant analysis and multivariate adaptive regression splines (MARS) were not considered because these methods are not yet well established and not readily available to users who are not statisticians. On the parametric side, multinomial logistic regression is the method of choice for polytomous nominal dependent variables and predominantly categorical covariates (Menard, 1995).

The performance of a specific technique depends to a large extent on the type, size and quality of data set used. In our example we used field data from a survey that was characterised by an unbalanced number of observations in the different outcome classes, high dimensionality, a large proportion of nominal variables (non-linearity), non-linear continuous variables, as well as possibly noisy data. It therefore qualifies as a complex data set as described in the introduction. Jain and Chandrasekaran (1982) discussed the problem of an unbalanced number of observations across the different outcome classes. The probability of misclassification is minimised with equal sample sizes in all classes. NNs address this issue by using weights according to the frequency of a class, other classifiers however, particularly MLR do not offer a simple solution to this problem. Another problem is incomplete data. In order to make sure that all classification methods were using the identical data set, imputation was used to avoid the presence of missing values potentially influencing the comparison. Hence, this comparison did not allow assessing the effect of missing value data on the performance of the classification methods. In the case of logistic regression observations with missing values are commonly case-wise deleted. This can drastically reduce the number of available observations and consequently the power of the analysis. Some non-parametric classifiers such as CART include specific methods for dealing with missing values (Breiman *et al.*, 1984), and therefore have an advantage when analysing incomplete data. It should also be noted that if the training data is not representative of the patterns to be classified in the future, then the assessment of performance will be over-optimistic (Ripley, 1994)

From earlier empirical comparisons of classification algorithms, it is recognised that the often contradictory outcomes of published studies are almost impossible to reconcile because there are no agreed objective criteria by which the schemes should be judged (Henery, 1994b). For the statistical comparison of classification performance, suggestions on how to use principles of repeated measurements analysis have recently been described by Feelders and Verkooijen (1996). Concentration on misclassification however may not necessarily be the best approach in a clinical application. We therefore also used estimates of sensitivity and specificity for correctly identifying the different classes as a criterion for comparison and performed a multidimensional comparison. A similar approach was used by Michie *et al.* (1994).

With our data set, MLR was the best method with respect to both classification error as well as over-all performance. NN and CHAID produced similar over-all results. This was consistent with findings from the European multi-centre project StatLog, where 23 statistical, NN and machine learning classifiers were applied to 22 data sets (Michie *et al.*, 1994). The performance of the algorithms was compared using multidimensional scaling and hierarchical cluster analysis. It was shown that statistical and NN algorithms were performing equally well and preferred similar types of data sets while ML algorithms were better suitable to a different type of data set. Our results with respect to information-based schemes support this conclusion in that they show a distinct behaviour when compared with other classifiers. Decision tree algorithms in general seem to perform at the same level of accuracy, and it was speculated that they are out-performing classical statistical methods if the data set is multimodal (Michie *et al.*, 1994).

NN on the other hand can be expected to perform better than statistical methods only if non-linear relationships and interactions are present in the data structure. NN by nature provide little insight into the nature of the relationships between the variables they are based on ('black box'; Hart, 1992). Their application in medicine has therefore initiated discussions as

to the ethical aspects of this method. NN have also been criticised because of the lack of rules with respect to appropriate NN architecture and the need to experiment in order to identify the 'best' neural network (a quasi-alchemist method). Although research and improved understanding of the technique have reduced some of these concerns, NN technology is often being adopted only with some reluctance (Baxt, 1991; Cross *et al.*, 1995). Also, NN are extremely 'data hungry', because the algorithms perform better if applied to large data sets for training and testing. The EP data set used in this comparison only contained 86 observations. This is clearly not optimal for the development of a NN. As a rule of thumb, at least 3-5 times as many observations as there are weights included the network should be available (Jain and Chandrasekaran, 1982; Ripley, 1994). The small data set required us to use dimensionality reduction techniques for this comparison. However, if data requirements are fulfilled, NN are able to outperform classical classification methods in the case of many applications (Ripley, 1994; Baxt and Skora, 1996).

Although we concluded that MLR performed better than the other schemes, this result should be interpreted with caution. A potentially serious technical problem was that several coefficients of the MLR models could not be conclusively estimated. This could be caused by zero frequencies in the model or collinearity among the estimates (Anonymous, 1988). Indeed, some of the sub-populations created by the model represented only one or two observations, which creates a zero frequency in at least one of the outcome classes. As a result, the estimates in the model are likely to be unreliable. Also, MLR was not cross-validated, because cross-validation techniques are not commonly available in statistical packages and this technique is not part of the standard assessment of MLR model performance. Therefore, we do not know how well the model would perform when exposed to data it has not 'seen' previously. Results from the other schemes showed that there was considerable overlap in the cross-validation performance, and it is expected that MLR would have performed similarly as was suggested by results of Stewart and Stamm (1991). Other disadvantages of this technique include the extensive data pre-processing (testing for linearity with subsequent re-coding, univariate analysis) and post-processing (testing for interaction), as well as the difficult interpretation of the final models.

In other medical examples, ML methods have been found to produce simpler results that are at least as accurate as classification based on logistic regression (Stewart and Stamm, 1991; El-Solh *et al.*, 1997; Rodman Shankle *et al.*, 1997). Classification trees provide detailed insight into the data structure and inter-variable relationships, and thus provide a wealth of information superior to statistical methods. Some authors found tree classifiers preferable because of their practicality, the general robustness with respect to data structure and the ease of use and interpretation (Stewart and Stamm, 1991; Yamold *et al.*, 1997). In fact many authors described similar performance for classification trees and parametric classification methods and were unable to determine a superior scheme (Stewart and Stamm, 1991; Selker *et al.*, 1995).

Therefore, we believe that non-parametric techniques are alternative data analysis methods worth considering. Their flexibility and graphical output allow fast and easy data exploration and interpretation. Classification trees could be used to complement parametric techniques, because in many practical applications there will be no universally best method (Ripley, 1994, Selker *et al.*, 1995). For example, classification trees have been used in combination with logistic regression or survival analysis (Segal and Bloch, 1989; Schmoor *et al.*, 1993; Swan *et*

al., 1995). The choice of the appropriate method should also be based on considerations related to the application (Selker *et al.*, 1995). A range of computer applications using a combination of neural networks, decision trees and visualisation techniques recently became available to PC users (Hofland and Utsler, 1997). These techniques offer new approaches to the solution of classification problems that should be further explored by veterinary epidemiologists.

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References

Anonymous (1988). The CATMOD procedure. In: SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, USA, 191-282.

Baxt, W.G. (1991). Use of an artificial neural network for the diagnosis of myocardial infarction. Annals of Internal Medicine 115:843-848.

Baxt, W. G. and Skora, J. (1996). Prospective validation of artificial neural network trained to identify acute myocardial infarction. Lancet 347:12-15.

Breiman, L., Friedman, J. H., Olshen, R. A., and Stone, C. J. (1984). Classification and regression trees. Wadsworth International Group, Belmont, California, USA.

Burke, H.B. (1996): Statistical analysis of complex systems in biomedicine. In: Fisher, D., and Lenz, H.-J. (eds.) Learning from data. Artificial intelligence and statistics V, Springer, New York, 251-258.

Carling, A. (1992). Introduction to neural networks. SIGMA Press, Wimslow, U.K.

Cross, S. S., Harrison, R. F., and Kennedy, R. L. (1995). Introduction to neural networks. Lancet 346:1075-1079.

Cunningham, S. J. (1995). Machine learning and statistics: a matter of perspective Department of Computer Science, University of Waikato, Hamilton, New Zealand.

Dohoo, I. R., Ducrot, C., Fourichon, C., Donald, A., and Hurnik, D. (1996). An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Preventive Veterinary Medicine 29:221-239.

El-Solh, A., Mylotte, J., Sherif, S., Serghani, J., and Grant, B.J.B. (1997). Validity of a decision tree for predicting active pulmonary tuberculosis. American Journal of Respiratory and Critical Care Medicine 155:1711-1716.

Feelders, A. and Verkooijen, W. (1996). On the statistical comparison of inductive learning methods. In: Fisher, D. and Lenz, H.-J. eds. Learning from Data: Artificial Intelligence and Statistics V Springer-Verlag, New York, 271-279.

Feng, C., and Michie, D. (1994). Machine learning of rules and trees. In: Michie, D., Spiegelhalter, D.J., and Taylor, C.C. (eds.) Machine learning, neural and statistical classification, Ellis Horwood, New York, 50-83.

Goodrace, R., Neal, M. J., and Kell, D. B. (1996). Quantitative analysis of multivariate data using artificial neural networks: A tutorial review and applications to the deconvolution of pyrolysis mass spectra. Zentralblatt der Bakteriologie 284:516-539.

Hand, D. J. (1992). Statistical methods in diagnosis. Statistical Methods in Medical Research 1:49-67.

Hart, A. (1992). Using neural networks for classification tasks - some experiments on datasets and practical advice. Journal of the Operational Research Society 43:215-226.

Henery, R.J. (1994a). Methods for comparison. In: Michie, D., Spiegelhalter, D.J., and Taylor, C.C. (eds.) Machine learning, neural and statistical classification, Ellis Horwood, New York, 107-124.

Henery, R.J. (1994b). Review of previous empirical comparisons. In: Michie, D., Spiegelhalter, D.J., and Taylor, C.C. (eds.) Machine learning, neural and statistical classification, Ellis Horwood, New York, 125-130.

Hofland, P., and Utsler, J. (1997). Data mining at your desk. Byte No. 7, www.byte.com/art/9707/sec17/art1.htm.

Hosmer D.W., and Lemeshow, S. (1989). Model-building strategies and methods for logistic regression. In: Applied Logistic Regression. John Wiley & Sons, New York, 82-134.

Jain, A.K. and Chandrasekaran, B. (1982). Dimensionality and sample size considerations in pattern recognition practice. In: Krishnaiah, P. R. and Kanal, L. N. (eds.) Handbook of Statistics Vol. 2. North-Holland Publishing Company, Amsterdam, 835-855.

Kass, G.V. (1980). An exploratory technique for investigating large quantities of categorical data. Applied Statistics 29:119-127.

Little, R.J.A. and Rubin, D.B. (1987). Statistical andalysis with missing data John Wiley & Sons, New York.

Little, R.J.A. and Schenker, N. (1995). Missing data. In: Arminger, G., Clogg, C.C., and Sobel, M.E. (eds.) Handbook of statistical modelling for the social and behavioral sciences Plenum Press, New York, 39-75.

Magidson, J. (1994). The CHAID approach to segmentation modeling: Chi-squared automatic interaction detection. In: Bagozzi, R. P. (ed.) Advanced methods in marketing research. Blackwell Publishers Oxford, 120-159.

Martin, W. (1997). A structured approach for analysing survey data and making useful causal inferences. Epidemiologie et Santé Animale 31-32:13.01.1-13.01.3.

McQueen, R.J., Garner, S.R., Nevillmanning, C.G., and Witten, I.H. (1995). Applying Machine Learning to Agricultural Data. Computers and Electronics in Agriculture 12:275-293.

Menard, S. (1995). Applied logistic regression analysis. SAGE University Paper No. 106, Series: Quantitative Applications in the Social Sciences, SAGE Publications, Thousand Oaks.

Michie, D., Spiegelhalter, D.J., and Taylor, C.C. (eds., 1994). Machine learning, neural and statistical classification. Horwood, New York.

Molina, R., Pérez de la Blanca, N., and Taylor, C.C. (1994). Modern statistical techniques. In: Michie, D., Spiegelhalter, D.J., and Taylor, C.C. (eds.) Machine learning, neural and statistical classification, Ellis Horwood, New York, 29-49.

Morgan, J. N. and Sonquist, J. A. (1963). Problems in the analysis of survey data and a proposal. Journal of the American Statistical Association 58:415-434.

Quinlan, J. R. (1993). C4.5: Programs for machine learning. Morgan Kaufmann Publishers, San Mateo, California.

Quinlan, J. R. (1986). Induction of decision trees. Machine Learning 1:81-106.

Ripley, B.D. (1994). Neural networks and related methods for classification. Journal of the Royal Statistical Society B 56:409-456.

Rodman Shankle, W., Mani, S., Pazzani, M.J., and Smyth, P. (1997). Detecting very early stages of dementia from normal aging with machine learning methods. In: Keravnou, E., Garbay, C., Baud, R., and Wyatt, J. eds. Artificial Intelligence in Medicine. AIME'97 Springer, New York, 73-85.

Schmoor, C., Ulm, K., and Schumacher, M. (1993). Comparison of the Cox regression model and the regression tree procedure in analysing randomized clinical trials. Statistics in Medicine 12:2351-2366.

Segal, M. R. and Bloch, D. A. (1989). A comparison of estimated proportional hazard models and regression trees. Statistics in Medicine 8:539-550.

Selker, H.P., Griffith, J.L., Patil, S., Long, W.J., and Dagostino, R.B. (1995). A comparison of performance of mathematical predictive methods for medical diagnosis - identifying acute cardiac ischemia among emer-

gency department patients. Clinical Research 43:468-476.

Stärk, K.D.C., and Morris R.S. (1996): Risk factors for respiratory diseases in New Zealand pig farms. Proceedings of the Pig Programme, 2nd Pan-pacific Veterinary Conference, Christchurch, New Zealand, 37-40.

Stewart, P.W. and Stamm, J.W. (1991). Classification tree prediction models for dental-caries from clinical, microbiological, and interview data. Journal of Dental Research 70:1239-1251.

Swan, G.E., Carmelli, D., and LaRue, A. (1995). Performance on the digit symbol substitution test and 5-year mortality in the Western Collaborative Group Study. American Journal of Epidemiology 141:32-40.

Swingler, K. (1996). Applying neural networks - A practical guide Academic Press, Harcourt Brace & Company, London.

Yarnold, P.R., Soltysik, R.C., and Bennett, C.L. (1997). Predicting in-hospital mortality of patients with AIDS-related *Pneumocystis carinii* pneumonia: an example of hierarchically optimal classification tree analysis. Statistics in Medicine 16:1451-146

CHAPTER 1.6

RestiMATE[1] – THE DESIGN OF AN EXPERT SYSTEM FOR DIAGNOSING AND MANAGING RESPIRATORY DISEASES ON PIG FARMS

[1] RestiMATE is a module of PigWIN®Pro by FarmPRO Systems Ltd., Palmerston North

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1. Introduction

Pneumonia and pleurisy are widespread and economically important health problems in intensive pig production (Straw *et al.*, 1990; Christensen and Mousing, 1992). An assessment of the extent of the problem on a specific farm usually involves the inspection of pig lungs at the abattoir (Pointon *et al.*, 1992), which is time-consuming and sometimes difficult to arrange. If a respiratory problem has been diagnosed on a farm, potential interventions suitable to improve the respiratory health of the pigs then need to be identified. There are numerous reports in the literature investigating risk factors known to influence the occurrence of lung lesions in slaughter pigs on individual farms (CHAPTER 1.1). These factors are mainly related to management, husbandry and the environmental conditions the pigs are being kept under. However, the effect of individual factors and the consequence of interventions directed at specific factors are difficult to assess on an individual farm, due to complex interactions.

In order to overcome this problem, diagnostic aids for the veterinary practitioners have been developed in the form of a computer-supported guide RESPITE (Morrison and Morris, 1985) and a prediction model RESPIG (Turner *et al.*, 1993). RESPITE was a relatively crude diagnostic guide using 11 management and environment factors, each of which was measured in the farrowing shed, the nursery and the growing-finishing houses. By using some weighting factors this spreadsheet application calculated the prevalence of lung lesions at slaughter. The authors acknowledge that RESPITE was not a statistically valid prediction system but rather an educational tool. A somewhat different approach was used for RESPIG, which used probabilistic modelling for predicting both prevalence and severity of artrophic rhinitis in pigs. Environmental factors, animal factors and veterinary treatment were used as risk factors in the model to classify the farm in terms of environmental quality. This classification was subsequently used to adapt the probability of pigs changing states of infection. Both these computer-aided prediction tools do not appear to have been applied to independent field data or to have been validated in some other way.

More recently, Enting *et al.* (1995) started developing an expert system to diagnose health and welfare problems on pig farms. This system is based on expert interviews and uses knowledge engineering techniques for structuring the expert knowledge. The thinking routines of the experts are documented as interpretation models. The risk factors under consideration are compared with target values and classified as to whether they meet the target criteria or not. The reasoning process can thus be displayed as flow charts or decision trees. However, this system does not attempt to predict the prevalence of respiratory lesions. It appears to require very detailed data input including for example air quality measurements. At the time of writing, the plans to validate this system (Enting *et al.*, 1996) have temporarily been postponed.

In this chapter we describe a system called RestiMATE. RestiMATE uses risk factors to predict the respiratory health of pigs and gives advice on the management of respiratory diseases on an individual farm. RestiMATE develops the exploratory approach used in RESPITE into a true expert system. As an addition to RESPITE, the new system also gives advice on how the situation on a farm could be improved. In order to achieve these goals, the development of an expert system (ES) appeared to be most appropriate. Therefore, the application of expert systems in the domain of pig health and the principles of expert system development will now be described briefly.

Expert systems have been described as being useful in the field of pig health (Schreinemakers *et al.*, 1988). For example, ESs have been used for investigating litter size in pigs (Vos *et al.*, 1990) and reproductive problems (Wongnarkpet *et al.*, 1994). The advantage of the use of software-supported decision making is the quick access to and the consistent use of relevant expert knowledge concerning a specific health problem. This is particularly helpful with complex diseases that are influenced by a wide range of risk factors and their interactions, which may be difficult to assess for an individual farm.

Jackson (1992) defined an ES as

> *a computing system which embodies organised knowledge about some area of human expertise which enables it to perform as a skilful and cost-effective consultant.*

An ES is designed to perform procedures (tasks) in a specific expertise area (domain). ESs have proved to be useful for tasks such as data interpretation, categorisation problems (diagnosis), analysis of complex structures and planning. ES are most popular in domains where human experts are scarce, and they are increasingly being used as an alternative to written manuals (Jackson, 1992). Expert systems are a subgroup within the broader definition of knowledge-based systems.

ESs are part of the much broader field of Artificial Intelligence (AI). AI is a part of computer science investigating possibilities to emulate human intelligence as applied for example in problem solving by means of computer programs (Jackson, 1992).

The basic elements of an ES are a knowledge base, an inference engine and a user interface (FIGURE 17).

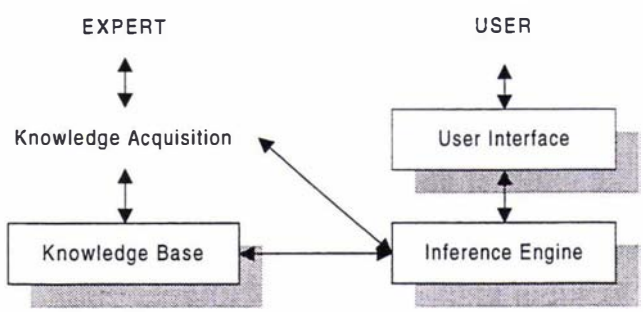

FIGURE 17. Structure of an expert system

Knowledge acquisition is the first step in designing an ES. The purpose of knowledge acquisition is to obtain the knowledge that is needed to build an ES (Scott *et al.*, 1991). Knowledge can be obtained by interviewing human domain experts or from published information.

Constructing an appropriate representation of the knowledge used by the expert system is a major undertaking in the development of an ES. Among the different approaches to knowledge representation the production rules with their IF...THEN structure appear to be particularly well suited to express the expert's rules of thumb (Jackson, 1992). The knowledge base contains specific information on facts (declarative knowledge, factual knowledge) and tasks (procedural knowledge, heuristic knowledge). Because the knowledge base is unlikely to be

perfect or complete, methods have been developed to make ESs capable to cope with uncertainty (conditional probability, certainty factors, fuzzy logic).

An ES uses heuristic knowledge to solve problems. Heuristics or 'rules of thumb' have been described as 'the art of good guessing' in the domain (Feigenbaum, 1993). This distinguishes these systems from other computer programs using algorithms or statistical models.

The inferencing is performed and controlled by the inference engine. Although this process involves manipulating rules and checking the results obtained, this mechanism is largely domain-independent (Jackson, 1992). 'Empty' inference engines (called 'shells') are commercially available for building ESs.

This chapter describes the design and features of the expert system RestiMATE. First, the problem is defined and then the ES design to solve this problem is described.

2. Problem definition

RestiMATE is used for classifying farms according to risk factors (farm variables) for respiratory diseases in pigs. It can identify problem areas on a farm and will make suggestions on what intervention(s) will most likely improve the situation. This system considers the following respiratory disease syndromes: Enzootic pneumonia (EP) and pleurisy / pleuropneumonia (PLPN).

3. Users

The envisaged users of RestiMATE are pig health professionals, who most frequently are veterinarians acting as consultants to pig producers. They want to run the program either on-farm using a laptop computer or in their offices. In the latter case, hard copies of the output are required for the farmer. All users have intermediate computer skills.

The veterinarians use the output of RestiMATE as a basis for discussing the health status of a farm and to consider possible interventions that could help reduce the prevalence of respiratory diseases. The use of RestiMATE is envisaged to be part of a general herd health visit.

4. System structure

The RestiMATE user is offered a choice of two classification methods. One is used for performing a herd evaluation based on field data collected from New Zealand pig herds (see CHAPTER 1.2). These data were used to develop an empirical classification tree. The second method is used to identify respiratory disease problems in general. The latter part is based on the reasoning process of a human domain expert. The user can run either one of these methods or both. The information flow within RestiMATE is schematised in FIGURE 18. Both methods use farm variables as input. They then compare the input with pre-defined target values and apply classification rules to produce output. The output and recommendations produced by the two methods contain different elements, as described below.

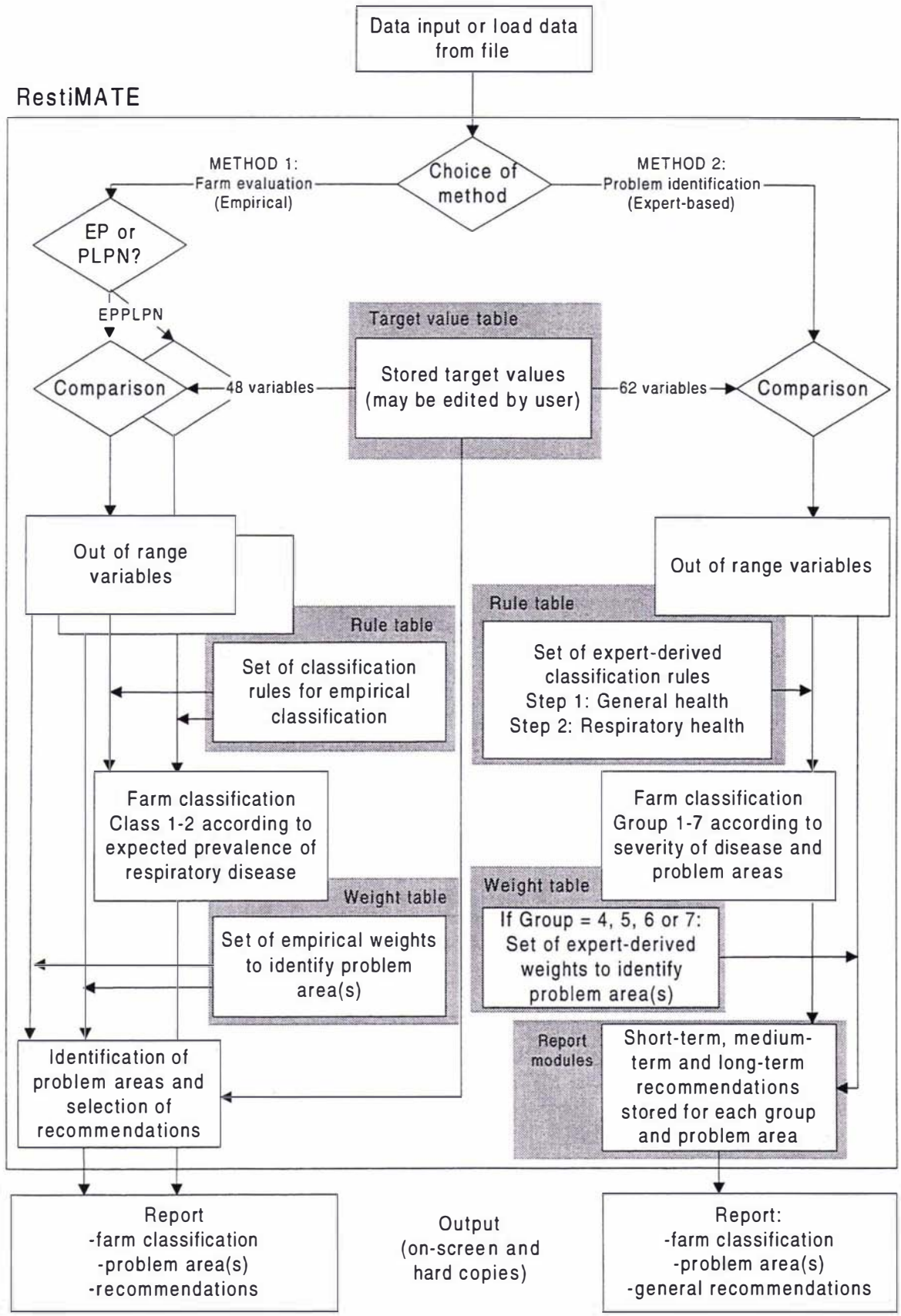

FIGURE 18. System architecture of RestiMATE

5. Input

In order to run RestiMATE a series of input responses is required by the system. The input consists of farm variables and target values. The farm variables are farm-dependent and entered by the user, while the target values are inherent to the system. However, the user can also modify the target values.

5.1 Farm variables

Farm variables are variables describing the characteristics of the management, husbandry system and health status of the farm to be evaluated by RestiMATE. One cluster of the farm variables (33 variables) was selected from published articles on risk factors for respiratory diseases in pigs. A second group of variables (47 variables) was identified by a domain expert during a series of 5 interviews. The expert had 32 years of practical experience in advising on disease control and pig health and 15 years of experience in respiratory disease research. The expert described the process of diagnosing respiratory disease on a pig farm and the evaluation of interventions in his own words. Diagnostic variables were extracted from the expert's descriptions. In a second step they were quantified and linked according to the expert's reasoning process. Some variables are used for diagnosing disease, some are used to identify problem areas in the management or husbandry system and a third group is used for weighting. For a list of all farm variables used by the system see APPENDIX B.

During a diagnostic session with RestiMATE, the values of the farm variables are entered interactively by the user using input screens. The screens are structured according to the following data types: general farm data, weaned pig data, growing pig data. Each screen displays a set of questions or statements allowing the user to key in or to select the appropriate answers (FIGURE 19)[1]. If the data has already been entered during a previous session, the file can be retrieved and edited.

[1] All prototype windows have been created in Microsoft Access97

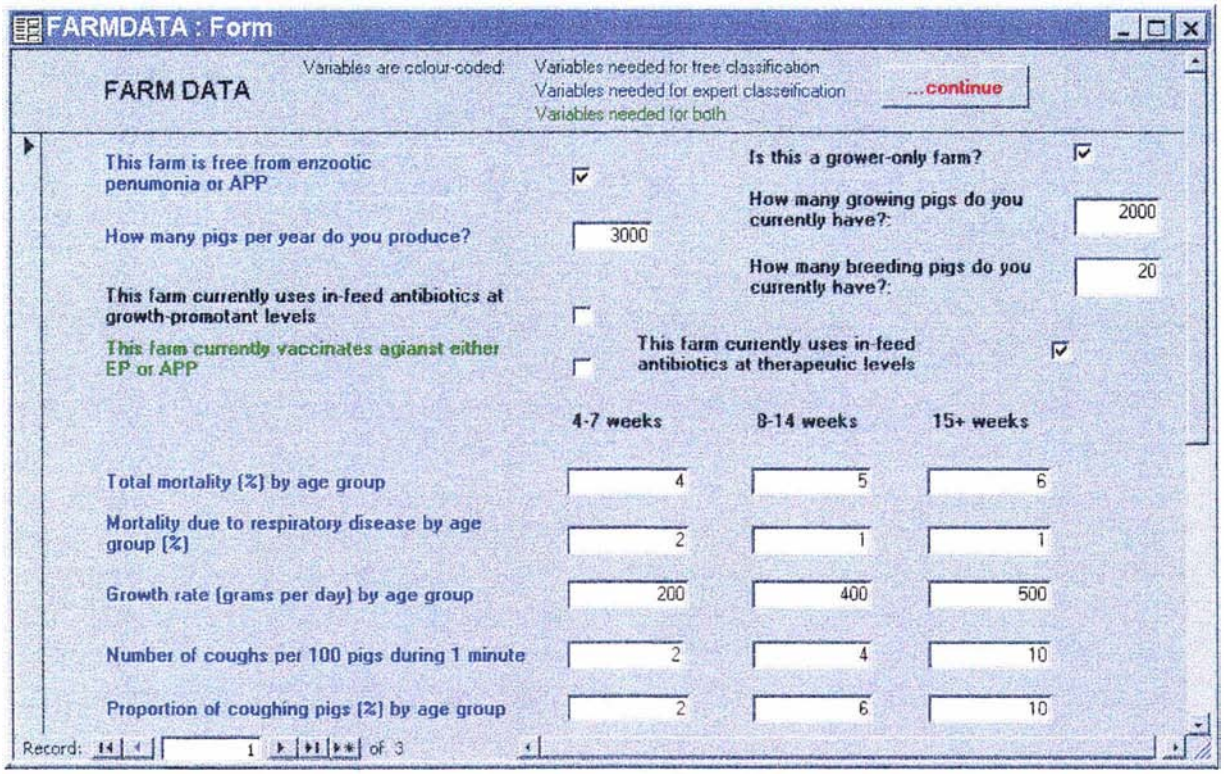

FIGURE 19. Example of data entry screen for general farm management area

5.2 Target values

For each farm variable a target value is stored in the target value table. The target value is the desired level for the variable. For example, the number of animals per room should be smaller than 200, therefore the target value or target range is any value below 200. An operator field defines whether the value needs to be <, = or > than the target value (TABLE 25). In a second field, an action value is stored. The action value defines the level where an intervention to correct the level of the variable is necessary. For binary variables, the target value and the action value are 0 or 1. For ordinal and continuous variables, target and action values can be any value defined for that variable. All target and action values are based on information taken from the literature or on expert opinion. The values obtained from the domain expert were reviewed by a second expert and modified in some cases to take account of both opinions.

These values are the default set of figures for RestiMATE to work with. However, the user is also be able to edit these values and to save his/her own set(s) of target values. Before the classification process is initiated, the user is asked to identify the target value set to be used.

For a complete list of all default target values see APPENDIX B.

TABLE 25. Structure of target value table and some examples of variables

ID	Full name	Level Tree	Level Expert	Op-erator	Tar-get value	Op-erator	Ac-tion value	Weight EP	Weight PLPN	Weight Expert
DIA	Diarrhoea in any age group	General	Step 1	=	0 (no)	=	1 (yes)	1.3	1.0	2.0
GRO	Number of growing pigs	General	-	<	500	>	1000	1.0	1.7	
WPPP	Number of weaned pigs per pen	Weaner	-	<	12	>	15	1.2	1.6	
GTEMP	Temperature in grower barn	Grower	-	>	18.0	<	15.0	1.0	1.0	
GSEP	Solid pen separations in grower barn	Grower	Environ-ment	=	1 (yes)	=	0 (no)	1.3	0.8	3.0

6. Processing

The objective of the processing step is to first classify the farm with respect to the risk of having a respiratory disease problem and second, to identify problem areas suitable for interventions.

6.1 Comparison of farm variables with target values

The first step in the classification is the comparison of the entered farm variables with the target values. Thus, out-of range values can be identified and flagged. Each variable can take one of the following states:

on target level

NOT on target level AND NOT on action level (= warning level)

on action level

Note that there is no warning level for binary variables.

This information is used for identifying problem areas and for selecting suitable interventions. They are also needed in the output. The information is stored until the output is produced.

6.2 Farm classifications

The scientifically most interesting part of the processing steps is the calculation of the farm classification because there are several approaches to how to solve this problem. Classifications can be performed using statistical techniques, classification trees, neural networks or

expert-derived classification rules (see CHAPTER 1.5). The two classification methods implemented in RestiMATE (empirical classification and expert classification) use different variables to classify a farm and different classification rules.

6.2.1 Empirical classification

The rules for the empirical classification were induced from field data using the CHAID algorithm (SIPINA v.2.02, University of Lyon 2, France) with subsequent translation of the tree into rules. The rules document variable patterns which identify farms with a specific disease level. As a result of the empirical classification procedure, the farm is classified in either of two groups for EP and PLPN (TABLE 26). However, no method will provide 100% correct classification. Therefore the probability of a classification being correct was determined based on the frequency of correct classification of farms in the field data set. The output window displays this probability value as additional information for the user to appreciate the degree of uncertainty related to the classification of a herd. The classification rules used by the empirical classification option are shown in TABLE 27. Note that the variable ISLAND is New Zealand-specific. Due to an increased occurrence of *Actinobacillus pleuropneumoniae* in the South Island, farms in the North Island have a geographical advantage. If RestiMATE was used in other countries, the classification rules would have to be adapted. The result of the classification process (group, probability to be in this group) is used in the output as explained in the next section.

TABLE 26. Farm classification groups for classification tree

	Enzootic pneumonia % prevalence	Pleuropneumonia and/or pleurisy % prevalence
Group1 (low prevalence)	0-40	0-10
Group2 (high prevalence)	>40	>10

6.2.2 Domain expert classification

These classification rules are designed to emulate the reasoning process of a human domain expert. They were developed in close collaboration with an expert. The reasoning is performed step-wise. The classification does not distinguish between EP and PLPN but is an overall assessment of respiratory health on a specific farm. First, the general health status of the farm is assessed, then the level of respiratory disease is diagnosed and the severity of the problem determined. The outcome of this process is the classification of the farm in one of seven groups (TABLE 28). Group 1 and Group 2 are for farms with no signs of respiratory diseases in pigs, Group 3 for farms with a problem in very young pigs, and Groups 4 to7 are for farms with increasing degrees of problems in older pigs.

There is also a set of weighting variables that are used. The assumption is that these variables tend to hide respiratory problems. Variables with this masking effect are the use of antibiotics and the use of EP vaccine. If these variables are present, the farm is moved into a higher severity group. As respiratory diseases tend to be less prevalent in summer, this is also taken

into account to classify the farm. The group numbers are temporarily stored and used for selecting the appropriate reports in the output. The rules are shown in TABLE 29.

TABLE 27. Rule base to classify farms with respect to the prevalence of EP and PLPN using a classification tree

```
EP
IF GCOUGH=1 and GROW<822
 THEN EP=HIGH {0.86}[1]
 ELSE
        IF GCOUGH=0 and WAIAO=1 and WSPACE>=0.47
        THEN EP=HIGH {1.00}
        ELSE
                IF GCOUGH=1 and FAT>=822
                THEN EP=HIGH with {1.00}
                ELSE
                        IF GCOUGH=0 and GPPP>=12.50 and WAIAO=1 and WSPACE<0.47
                        THEN EP=LOW {1.00}
                        ELSE
                                IF GCOUGH=0 and WAIAO=0
                                THEN EP=HIGH {0.93}
                                ELSE
                                        IF GCOUGH=0 and GPPP<12.50 and WAIAO=1 and
                                        WSAPCE<0.47
                                        THEN EP=HIGH {0.80}
```

```
PLPN
IF GROW<725.00 and NEIGH>=3.50 and WFED=1
THEN PLPN=LOW {1.00}
ELSE
        IF GROW<725.00 and NEIGH>=3.50 and WFED=0
        THEN PLPN=HIGH {0.90}
        ELSE
                IF GROW>=725.00 and GPPR<195.00 and WSPACE>=0.46
                THEN PLPN=LOW {1.00}
                ELSE
                        IF GROW<725.00 and NEIGH<3.50
                        THEN PLPN=LOW {0.93}
                        ELSE
                                IF GROW>=725.00 and GPPR<195.00 and WSPACE>=0.46 and
                                THEN PLPN=HIGH {0.67}
                                ELSE
                                        IF GROW>=725.00 and GPPR>=195.00 and NEIGH<9.00 and
                                        WSPACE>=0.26
                                        THEN PLPN=HIGH {0.84}
                                        ELSE
                                                IF GROW>=725.00 and WSPACE<0.26
                                                THEN PLPN=HIGH {0.95}
                                                ELSE
                                                        IF GROW>=725.00 and GPPR>=195.00 and
                                                        NEIGH>=9.00 and WSPACE>=0.26
                                                        THEN PLPN=LOW {0.60}
```

[1]For each rule the probability of correct classification is given.

TABLE 28. Farm classification groups for human expert

	Description
Group 1	Farm of high or average health status with no signs of respiratory disease
Group 2	Farm of low health status with no signs of respiratory disease
Group 3	Signs of respiratory disease observed in very young pigs (4-7 weeks old)
Group 4[1]	Signs of respiratory disease in pigs older than 14 weeks
Group 5[1]	Signs of respiratory disease in 8-14-week-old pigs
Group 6	As 4 or 5 but increased severity by weighting factors
Group 7	As 4 or 5 but increased severity by weighting factors

[1]If the assessment is done in summer, or if antibiotics or EP vaccine are used, the class number is increased by one, if any two or more of these conditions are true, the class is increased by two.

TABLE 29. Rule base for classifying farms with respect to EP

Step1: Assessment of health status

Consider: MORT1, MORT2, MORT3, GROWTH1, GROWTH2, GROWTH3, TREAT and GDIA

IF 0-2 of these variables are on action level
THEN health status = high
ELSE
IF 3-5 of these variables are on action level
THEN health status = average
ELSE
IF >5 of these variables are on action level
THEN health status = low

Step 2: Diagnosing respiratory disease problem

Consider: CPROP1, CPROP2, CPROP3, CC1, CC2, CC3, SIGN1

IF any of these variables on action level
THEN respiratory disease = yes
ELSE
IF SIGN2 and (MORT1 or MORT2 or MORT3) on action level
THEN respiratory disease = yes
ELSE respiratory disease = no

Step 3: Determining affected age class(es)

Consider: CPROP1, CC1, MRRESP1,

IF any of these variables on action level
THEN 4-7-week-old pigs affected for x (count) variables

Consider: CPROP2, CC2, MORRESP2,

IF any of these variables on action level
THEN 8-14-week-old pigs affected for x (count) variables

Consider: CPROP3, CC3, MORRESP3

IF any of these variables on action level
THEN >15-week-old pigs affected for x (count) variables

Step 4: Classification

IF respiratory disease = no AND health status = (high OR average)
THEN group = 1
ELSE
IF respiratory disease = no AND health status = low
THEN group = 2
ELSE
IF respiratory disease = yes AND affected age group = 4-7-week-old pigs only
THEN group = 3
ELSE
IF respiratory disease = yes AND most affected age group = 15+ weeks
THEN group = 4
ELSE
IF respiratory disease = yes AND most affected age group = 8-14-week-old pigs
THEN group = 5

Consider: SEASON, AB-USE, VACC

IF group 4 or 5 and any one of these variables on action level
THEN group +1
ELSE
IF any two or more of these variables on action level
THEN group +2
ELSE group = group

6.3 Identification of problem areas

Having first classified a farm with respect to respiratory disease risk, RestiMATE searches for problem areas, where interventions could be applied. The identification of problem areas is based on out-of-range values which were detected earlier in the analysis. Each farm variable is linked to a problem area on the farm (TABLE 25). The empirical method evaluates three problem areas (farm level, grower pig level, weaner pig level) and the expert-based method considers two areas (air flow, environment). When a farm variable is on action level it starts contributing to the over-all assessment of the area it belongs to. The more out-of-range variable in an area, the more weight is given to that area in terms of need for interventions.

6.3.1 Identification of problem areas when using empirical method

The problem can be related to the general farm management (11 variables), the weaned-pig management (11 variables) or the growing-pig management (11 variables). Not all variables in these areas are biologically equally important. This fact is accounted for by weighting the variables. As shown in TABLE 25, weights are stored for each variable for both EP and PLPN. These weights are equivalent to the odds ratios calculated for these variables in a univariate analysis (see CHAPTER 1.2). The value of the weight can be <0 (preventive factor, risk-reducing) or >1 (risk factor). If the weight for a value is $=1$ then the factor does not increase the over-all risk.

An over-all score for each area is calculated by multiplying the weights of all out-of-range variables. In the output the problem areas are colour-coded according to the level of the final value.

6.3.2 Identification of problem areas when using expert-based method

Problem areas are only evaluated if the most affected age group is 8-14-weeks-old or >14 weeks (groups 4,5,6 or 7). Not all variables are assigned to a problem area (APPENDIX B). The possible problem areas are 'air flow' (8 variables) and 'environment and hygiene' (11 variables). Weights are the same for both EP and PLPN. The weights are derived from the priorities that the domain expert assigned to these factors, ranging from 1 to 3, 3 being very important. The weights were then re-scaled to achieve the same possible maximal value in both problem areas. To identify the most important problem area, weights are added. The final report is specified according to the identified problem area. If both areas are equally important (same total figure of weights) then both are mentioned in the report.

6.4 Recommendation of interventions

When RestiMATE has identified problem area(s), the next step is to make some recommendations to the user on which risk factors could be considered for interventions and how they should be changed. The recommendation consists of a text string that is integrated in the final report.

6.4.1 Empirical classification method

For this step the variables tagged as being out-of-range by the earlier processing are used. From the variable table, RestiMATE retrieves a recommendation string. The recommendation is slightly different according to whether the variable is on warning or on action level (TABLE 30). All recommendations are based on research findings published in the literature (CHAPTER 1.2). For a complete list of all recommendations refer to APPENDIX B.

The recommendations are integrated in the output.

6.4.2 Expert-based method

If the user chose the expert-based methods, the recommendations are selected according to the previously determined group membership of a farm (group 1-7) and the identified problem areas rather than individual out-of-range variables. The full text of the modules can be found in APPENDIX C. A set of rules is used to combine the modules for the output (TABLE 31).

TABLE 30. Recommendation examples as they will be stored in the recommendation table of RestiMATE. Binary variables do not have a warning level.

ID	Full name	Recommendation Warning	Recommendation Action
DIA	Diarrhoea in any age group	n.a.[a]	
GRO	Number of growing pigs	Larger farms are at higher risk of suffering from airborne infectious diseases. No recommendation.	Large farms are at higher risk of suffering from airborne infectious diseases. No recommendation.
WPPP	Number of weaned pigs per pen	More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. You are getting close to this level. Try reducing the numbers.	More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. Try reducing the numbers.
GTEMP	Temperature in grower barn	The ideal temperature for growing pigs is 18 degrees Celsius. It is a bit too cold in your buildings.	The ideal temperature for growing pigs is 18 degrees Celsius. It is definitely too cold in your buildings.
GSEP	Solid pen separations in grower barn	n.a.	Solid pen separations help prevent disease transmission, Check whether this could be realised on your farm.

[a]n.a. = not applicable

TABLE 31. Rules for selecting advice components for reporting

IF	GROUP=1	THEN	advice sheet A	
IF	GROUP=2	THEN	advice sheet B	
IF	GROUP=3	THEN	advice sheet C	
IF	GROUP=4	THEN	advice sheet D	
IF	GROUP=5	THEN	advice sheet E	
IF	GROUP=6 or 7	THEN	advice sheet F	
IF	problem area=AIR FLOW	THEN		add report module 1
IF	problem area=ENVIRONMENT	THEN		add report module 2
IF	SIZE or PURCH on action level	THEN		add report module 3
IF	STATUS=free	THEN		add report module 4
IF	SIGN1 or SIGN2 on action level	THEN		add report module 5

7. Output

The output is provided in two forms: output screens and output reports. The output screens allows the user to interactively explore the results of the analysis. A hard copy of the output will be printed on demand. It consists of a report summarising the graphically displayed results.

7.1 Output of empirical classification method

The first window is tabulated and displays the farm classification and the probability of a correct identification for EP and PLPN (FIGURE 20). There will be three buttons for the possible problem areas. These buttons are colour coded according to the accumulated weights of the variables in the respective area. The buttons of the fields with the highest over-all weight are red. Other buttons are yellow. If there are no out-of-range-variables for an area, the button is blue.

The result for each problem area can be further investigated in terms of out-of-range values by clicking on the respective buttons. A new window will be displayed listing all out-of-range values. Again, there will be a colour-coding scheme using yellow for variables on warning level and red for variables on action level. Additionally a scale of different red values will be used according to the weight of a variable. This will help finding influential variables.

Recommendations are displayed for all out-of-range variables.

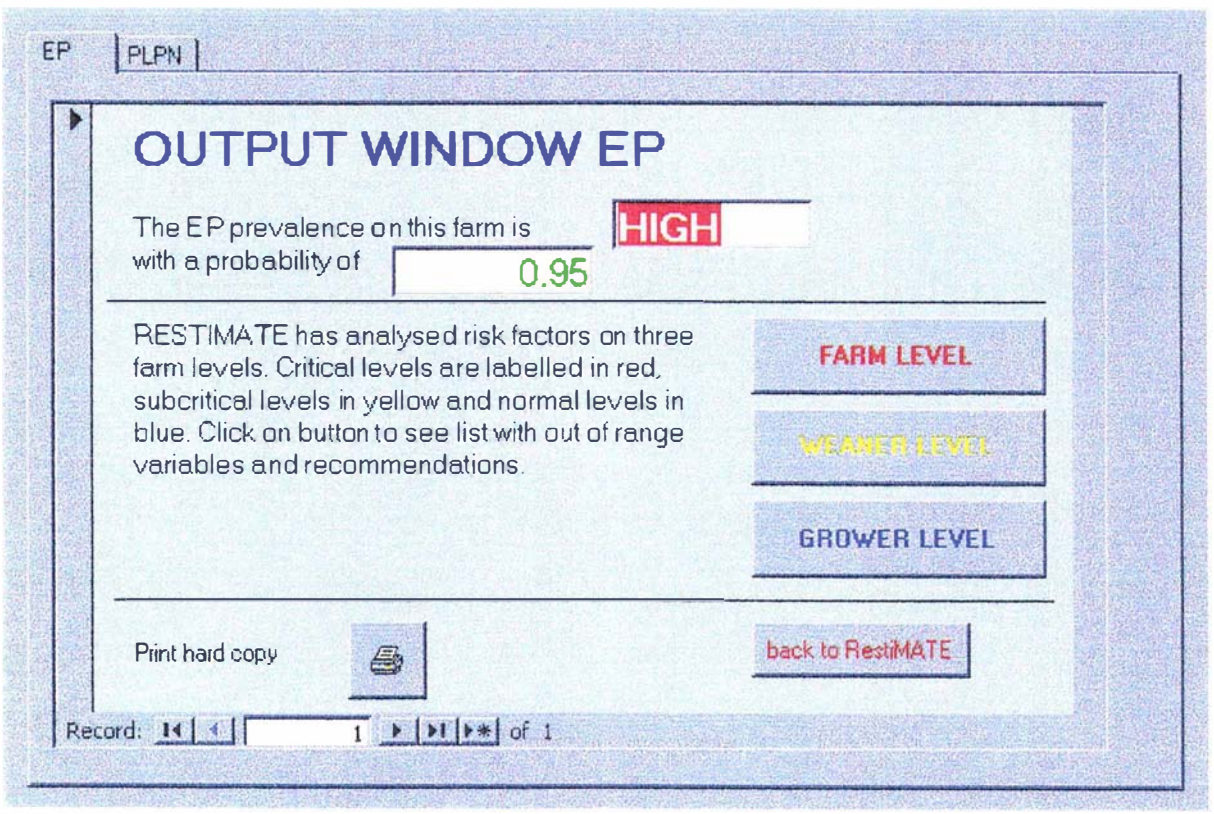

FIGURE 20. Example of an output window

7.2 Output of expert-based classification method

The output of the expert-based classification method consists of a report customised according to the classification of the farm using rules in TABLE 31. The last three rules are used to add notes to the report if there is an indication that the farm may have characteristics that

could have an influence on the validity of the results. For example, if a farm is known to be EP free, the observations should be interpreted differently.

The report modules are combined to a coherent text that can be read on screen or printed out (FIGURE 21). The advice is identical for all farms in one group and is therefore more general than with the empirical option.

Report on respiratory health status produced by RestiMATE

Date 17/11/97 **Date of last farm visit: 7/11/97**

Farm **Happy Pig Farm, Palmerston North**

General section

Note On this fann, signs of severe pneumonia and/or APP have been observed. It is recommended that the status of this farm is determined more specifically using slaughter checks or other diagnostic techniques. This issue hneeds to be discussed with the veterinary advisor.

Diagnosis section

This farm appears to have a severe problems with respiratory diseases.

Specific problem area section

The general environment and hygiene in the grower/finisher buildings appears to be a problem area. The stocking density needs checking. It is recommended that less than 200 pigs should share an airspace and less than 15 pigs should be housed in the same pen. Manure management is crucial to avoid an adverse environment. Possibilities to improve hygiene by all-in/all-out pig flow should be considered. Measures to avoid contact between pigs in different pens and of different age groups are likely to reduce the infection pressure.

Advice section

Short term The use of an antibiotic treatment at therapeutic level should be discussed with the veterinary advisor. If medication is already in use, product and dosage need to be reviewed.
Options for immediate improvement of airflow or reduction of stocking density by changes in the management need to be considered. All-in/all-out pig flow and cleaning and disinfecting between batches are essential.

Medium term Starting a vaccination programme is one option. Low-cost changes to buildings in order to improve air flow and general environment in the grower buildings may also be required.

Long term Permanent changes to buildings with respect to ventilation and sub-division of rooms need to be planned. Possibilities for eradicating respiratory diseases from this farm should be discussed with the veterinary advisor.

FIGURE 21. Example of a report produced by the expert-based classification method

8. Discussion

Respiratory diseases in pigs are a typical multifactorial problem, where a large number of risk factors and their interactions need to be taken into account (Christensen and Mousing, 1992;

CHAPTER 1.1). This is probably the reason why the use of computer-supported diagnosis tools in this specific situation has been explored by several research groups using different methods for knowledge elicitation, representation and reasoning. RestiMATE uses two approaches, an empirical one based on classification tree analysis and an expert-based one (TABLE 32).

TABLE 32. Comparison of tree-based and expert-based methods in RestiMATE

	Tree-based method	Expert-based method
Knowledge acquisition	Field data	Human expertise
Knowledge representation		
Number of input variables	48[a]	62[a]
Number of classes	3 EP, 3 PLPN	7
Number of rules		
Separate classification for EP and PLPN	Yes	No
Uncertainty	Yes	No
Level of detail in reporting	High	Low
Validation	No[b]	No

[a]15 variables also used by both methods.
[b]The tree-based method has been validated in principle by using cross-validation. For details see CHAPTER 1.5.

The usefulness of classification trees for diagnostic tasks has been documented in various domains, including the medical one (for example: Stewart and Stamm, 1991; Selker *et al.*, 1995; El-Solh *et al.*, 1997). The advantage of this approach is that rules can be derived from field data. Also, it is possible to express classification uncertainty. Provided that the data used for rule induction is representative of the data that the system will need to process in the future, classification trees provide valid prediction. However, if the data are not representative the potential to generalise the results will be limited. We used field data from farms that we believe are representative of the New Zealand pig industry (see CHAPTER 1.2). It is likely that the system will provide correct classification if used for New Zealand farms or farms that are similar to the New Zealand situation. The performance in other countries is unknown.

The second option in RestiMATE is expert-based, similar to ZOVEX (Enting *et al.*, 1995). However, we interviewed only one expert and a less structured approach to knowledge documentation was used. Problems related to knowledge elicitation from human experts are the selection of experts and how to deal with the lack of consensus. By using just one expert, the latter could be avoided. Yet the problem of how to define expertise remains unresolved. The classification rules are relatively simple, and the reports are pointing out general principles rather than giving tailored advice. Klein and Methlie (1995) stated that the function of an ES is not only to draw conclusions but also to explain its reasoning. With the currently broad classification classes and the general reports, the latter objective is probably only marginally fulfilled by the expert-based method within RestiMATE. We believe however, that within the framework of the current design the level of detail can be increased relatively easily. At this

stage this part of the system has not been applied to field data and therefore the accuracy of the classification is unknown.

The usefulness of any expert system clearly depends on the quality of the classifications made by the system. An important step in developing computer-aided diagnostic tools is therefore the validation of the output produced by the system. Classification sensitivity and specificity as well as the value of the advice have to be assessed using different data sets by different independent evaluators. In a next phase, RestiMATE therefore needs to be confronted with real data during field testing according to the principles for validating expert systems (O'Keefe *et al.*, 1987). An acceptable performance level needs to be defined so that the validation can be as quantitative as possible. In the case of RestiMATE the ES performance can be compared with slaughter check results and/or with the assessments of human experts. Not only the end-classification but the entire reasoning process and the recommendations should be taken into account. Changes in the target values and/or the variables used or in the reasoning process may be necessary in order to improve the prediction quality. Only after satisfactory results have been obtained can the system be distributed for general use.

A strong element of RestiMATE is its flexibility. It allows easy adjustments for conditions that may vary between areas or countries. Or individual users may choose to use their own set of target values for different risk factors to adjust the system in order to represent their personal judgement. Ultimately, the system will thus become a practice-oriented personal decision aid.

RestiMATE can also be used as an educational tool to train students and practitioners. It is anticipated to integrate RestiMATE into the pig farm management system PigWIN as an additional module.

References

Christensen, G. and Mousing, J. (1992). Respiratory System. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S., and Taylor, D.J. (eds.) Diseases of swine, 7th Edition. Iowa State University Press, Ames, Iowa, 138-162.

El-Solh, a., Mylotte, J., Sherif, S., Serghani, J., and Grant, B.J.B. (1997 May). Validity of a Decision Tree for Predicting Active Pulmonary Tuberculosis. American Journal of Respiratory and Critical Care Medicine 155:1711-1716.

Enting, J., Huirne, R.B.M., Tielen, M.J.M., and Dijkhuizen, A.A. (1995). ZOVEX: An expert system to help solving health and production problems on pig farms (project outlines). In: Udink ten Cate, A. J., Martin-Clouaire, R., Dijkhuizen, A. A., and Lokhorst, C. (eds) Artificial Intelligence in Agriculture Wageningen, 397-402.

Enting, J., Tielen, M.J.M., Huirne, R.B.M., and Dijkhuizen, A.A. (1996). Validation of ZOVEX, a knowledge-based system in swine health management. In: Graat, E. A. M. and Frankena, K. (eds.) Proceedings of the Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics Wageningen, 67-72.

Feigenbaum, E.A. (1993). Expert systems: Principles and practice. In: Ralston, A., and Reilly, E.D. The Encyclopedia of Computer Science 3rd Edition, Van Nostrand Reinhold Co., New York, 536-540.

Jackson, M. (1992). Understanding expert systems. John Wiley and Sons, Chichester.

Klein, M.R., and Methlie, L.B. (1995). Expert systems. In: Knowledge-based decision support systems with application in business. John Wiley and Sons, Chichester, 201-280.

Morrison, R.B. and Morris, R.S. (1985). RESPITE - A computer-aided guide to the prevalence of pneumonia in

pig herds. Veterinary Record 117:268-271.

O'Keefe, R.M., Balci, O., and Smith, E.P. (1987). Validating expert system performance. IEEE Expert 2:81-89

Pointon, A.M., Mercy, A.R., Backstrom, L., and Dial, G.D. (1992). Disease surveillance at slaughter. In: Leman, A.D., Straw, B.E., Mengeling, W.L., D'Allaire, S., and Taylor, D.J. (eds.) Diseases of swine, 7th Edition. Iowa State University Press, Ames, Iowa, 968-987.

Schreinemakers, J.F., Vos, M.L., Brand, A., Brée, D.S., and Verheijden, J.H.M. (1988). The introduction of expert systems in animal husbandry. The Veterinary Quarterly 10:281-288.

Scott, A. C., Clayton, J. E., and Gibson, E. L. (1991). A practical guide to knowledge acquisition. Addison-Wesley Publishing Company, Reading.

Selker, H.P., Griffith, J.L., Patil, S., Long, W.J., and Dagostino, R.B. (1995). A comparison of performance of mathematical predictive methods for medical diagnosis - Identifying acute cardiac ischemia among emergency department patients. Journal of Investigative Medicine 43:468-476.

Stewart, P.W. and Stamm, J.W. (1991 Sep). Classification tree prediction models for dental-caries from clinical, microbiological, and interview data. Journal of Dental Research 70:1239-1251.

Straw, B.E., Shin, S.J., and Yeager, A.E. (1990). Effect of pneumonia on growth rate and feed efficiency of minimal disease pigs exposed to *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae*. Preventive Veterinary Medicine 9:287-294.

Turner, L.W., Wathes, C.M., and Audsley, E. (1993). Dynamic probabilistic modelling of respiratory disease in swine, including production and economic effects. Livestock Environment IV American Society of Agricultural Engineers, 89-97.

Vos, M.L., Schreinemakers, J.F., Brée, D.S., and Verheijden, J.H.M. (1990). TEP – The electronic pig: a prototype of a knowledge-based computer system for swine herd health. Preventive Veterinary Medicine 9:95-106.

Wongnarkpet, S., Morris, R.S., Stern, M.W., and Pfeiffer, D.U. (1994). PIGFIX – a pig fertility investigation expert system. The Kenya Veterinarian 18:454-456.

PART II

Exotic infectious diseases

Example: Classical and African swine fever

CHAPTER 2.1

REVIEW OF THE EPIDEMIOLOGY OF CLASSICAL AND AFRICAN SWINE FEVER

1. Epidemiology of Classical swine fever

1.1 Introduction

Classical swine fever (CSF) or hog cholera is a highly contagious viral disease of *suidae*. The disease was first described in the 19th century in the United States and later in Europe, South America and South Africa (van Oirschot, 1992). In the United States, 13 % of the swine population died in 1886 due to CSF (Stalheim, 1988).

The disease is at present occurring world-wide. In 1995, 47 countries reported CSF outbreaks (OIE, 1995). Although most countries now have compulsory eradication or control programs in place, vast outbreaks have recently occurred in various countries and are currently occurring within the European Union (EU): Belgium 1993-1994 (Koenen *et al.*, 1996), Italy 1993-1995, Germany 1993-1996 and 1997, the Netherlands 1997, and Spain 1998. In New Zealand, the last CSF outbreak occurred in 1953. A serological survey conducted in 1995 confirmed that the New Zealand pig population is free from CSF infection (Horner, 1997).

The direct and indirect losses caused by the disease are severe, particularly in countries with a dense pig population. It has been estimated that the German outbreak from 1993-1995 caused economic losses of at least DM 1,500 million (Teuffert *et al.*, 1995).

1.2 Aetiology

CSF is caused by an enveloped, single-stranded RNA virus belonging to the *Pestivirus* genus being part of the family *Flaviridae* (Paton *et al.*, 1995). The virus has a diameter of 40-50 nm. Its genome sequence is 66% homologous to that of the bovine virus diarrhoea (BVD) virus, another member of the genus *Pestivirus* (Terpstra, 1991). This antigenic relationship leads to cross-reactions in various diagnostic test systems. Antigenetically distinct strains can be distinguished (Wensvoort *et al.*, 1989) and the strains can be grouped into different virulence categories (Carbrey *et al.*, 1980). Yet these two characteristics do not seem to be associated with each other.

CSF virus is very stable in protein-rich environment. It can be denatured by lipolytic agents and it is rapidly deactivated at pH values of <4 and >11 (Dulac and Mebus, 1992).

1.3 Affected species

The pig is the only natural host of CSF virus although wild boars (*Sus scrofa ferus*) are also susceptible to the disease (Dahle and Liess, 1992). In Sardinia a prevalence of 11 % seropositive wild boars was observed (Laddomada *et al.*, 1994). Simulations of the disease dynamics indicate that CSF will probably maintain itself in any small population of wild boars (Hone *et al.*, 1992).

1.4 Disease course

The course of CSF infection is dependent on the following factors: virus virulence, virus dose, transmission route and various host factors such as age (Koenen *et al.*, 1996) and possibly breed (Depner *et al.*, 1997a). The influence of host-related factors have probably been underestimated. They may be responsible for the range of clinical pictures that may be observed during a CSF outbreak (Depner *et al.*, 1997b)

The clinical signs range from peracute disease with high mortality to subacute, chronic or subclinical infection. The acute disease is characterised by a rapid course and atypical clinical signs such as anorexia, pyrexia, conjunctivitis, vomiting followed by constipation or diarrhoea. In the terminal stage, pigs shiver and develop a staggering gate, followed by posterior paresis and death after 10-20 days (Terpstra, 1991). Hyperaemia and haemorrhages in the skin as well as cyanosis of the ears are typical signs of CSF. In chronic cases, death does not occur before 30 days. Persistent infection is mainly characterised by retarded growth, continuous virus excretion and a lack of immune response (Carbrey *et al.*, 1980; Depner *et al.*, 1996). Symptoms linked to prenatal infection are prenatal death, mummification, malformations, congenital tremor and eventually death (Trautwein, 1986 cited by Liess, 1987).

When pregnant sows are infected with a low virulent strain, they may develop the 'carrier sow' syndrome (van Oirschot and Terpstra, 1977; Dahle and Liess, 1992). Diaplacental infection during early pregnancy (up to 70 days) leads to abortion. Later in gestation (70 - 90 days) the infection is transmitted diaplacentally to the foetuses which may develop persistent infection (van Oirschot and Terpstra, 1977). In one farm in a Dutch outbreak, 43% of the sows showed intra-uterine infection (Wensvoort and Terpstra, 1985). Persistently infected piglets die after a growth-retarded lifespan of up to several months.

The course of postnatal infection caused by low virulence virus is influenced by age, immune competence, and nutritional status (van Oirschot, 1992). Infection of young pigs with a low virulent virus leads to high mortality while older animals may recover (Carbrey *et al.*, 1966). The variability of response of individual animals to the same virus strain is considerable and not fully understood (Plateau *et al.*, 1980; Depner *et al.*, 1996).

The course of the disease in wild boars is similar to the one observed in domestic pigs (Depner *et al.*, 1995a). Altered behaviour patterns such as loss of shyness may also be observed (Loepelmann and Dedek, 1987).

1.5 Diagnosis

Due to the variable and atypical clinical signs which may easily be confounded with African swine fever and BVD (Terpstra and Wensvoort, 1988), conclusive diagnosis is impossible without laboratory confirmation (Young, 1970). Direct immunofluorescence is the laboratory method of choice for antigen detection, complemented by PCR techniques (van Oirschot, 1992). If virus is isolated from new-borne piglets, their dams must have been infected 4-6 weeks earlier. Virus may be isolated from clinically healthy and serologically negative pigs (Koenen *et al.*, 1992).

Antibody surveillance should not be started earlier than 35 days after the possible date of infection in order to ensure sufficiently high titers (Roberts, 1995). When investigating antibody titres, special techniques that can differentiate CSF and BVD virus infections have to be used. These techniques are based on the use of monoclonal antibodies (Wensvoort *et al.*, 1989). Few and low antibody titres can be interpreted as a consequence of recent infection while a high prevalence of high titres is indicative of an older infection process (Depner *et al.*, 1995b). During an outbreak, morbidity seems to be correlated with the proportion of serologically positive pigs and may therefore be an indicator of the spread and duration of the infection (Koenen *et al.*, 1996). Antibody screening will not help in early detection of infection because antibodies are often not present until 4 weeks after infection (Terpstra, 1987).

Molecular biological assays and antibody panels allow the grouping of isolates in geographical or temporal clusters (Wensvoort *et al.*, 1989; Edwards and Sands, 1990; Hofmann *et al.*, 1994; Vilcek *et al.*, 1997). This may be helpful in the epidemiological investigation of an epidemic. In the 1991-1994 outbreaks in Germany, three virus types were isolated (Kramer *et al.*, 1995). These could be related to different regions and all of them had also been isolated from wild boars confirming the association of the disease in domestic pigs with wildlife infection. The use of a nucleotid sequence database of CSF isolates could hence support the epidemiological investigation of CSF outbreaks (Haas *et al.*, 1997).

1.6 Virus transmission

Natural transmission within an infected herd commonly occurs through direct contact via the oral/nasal route. The minimal infectious dose for fatal disease with the Alfort strain was reported to be less than 10 $TCID_{50}$ per pig (Liess, 1987). The tonsils are the primary sites of virus replication from where it spreads to the regional lymphnodes and secondary target organs (Ressang, 1973). The incubation period ranges from 2-6 days after natural infection (Terpstra, 1991) and up to 7 days in experiments (Depner *et al.*, 1994). Viraemia and virus shedding occurs already during the incubation period (Depner *et al.*, 1994). High virus titers are first excreted in saliva and later and to a lesser extent in faeces, urine, nasal and lachrymal fluids (Ressang, 1973; Depner *et al.*, 1994). Virus can also be shed by insufficiently immunised pigs after challenge (Ressang *et al.*, 1972) and when using modified live-virus vaccines (Carbrey *et al.*, 1966). Piglets born to 'carrier sows' shed large amounts of virus for several months while not showing any clinical signs nor developing antibody response (van Oirschot and Terpstra, 1977). As a general rule virulent strains spread faster within a herd and induce higher morbidity than less virulent strains (Terpstra, 1991). Morbidity and mortality in natural outbreaks have been reported to range from 8-13 % and from 12-17 %, respectively (Carnero *et al.*, 1983).

Between farms, moving infected animals such as pigs in the incubation period, carrier sows or persistently infected pigs most frequently spreads the virus (TABLE 33). The herd incidence rate for a 24-month time interval in Germany was reported to be on average 1.12 % but varied significantly for the different herd types, ranging from 0.81 % for breeding herds to 1.38 % for fattening herds (Lorenz and Boorberg, 1986).

Fattening farms have been reported to be at higher risk than breeding and breeding-fattening farms (Carnero *et al.*, 1983; Küttler, 1984; Kramer *et al.*, 1995). The risk of becoming in-

fected seems furthermore to be particularly high in large herds and in high-density pig areas (Wachendörfer *et al.*, 1978; Küttler, 1984; Vannier *et al.* 1986; Terpstra, 1987; Caporale *et al.*, 1988). Lorenz and Boorberg (1986) reported that the risk is on average 30 times as high in herds with 700 or more pigs as in herds with up to 49 pigs. This is probably due to a high frequency of stock introduction from a number of sources. However, it has been suggested that small, non-professionally run farms might be at an equally high risk of infection due to a lack of information on preventive measures and little risk awareness (Kramer *et al.*, 1995).

TABLE 33. Sources of classical swine fever outbreaks in European epidemics

	Animal movement	Vehicles	Swill feed	Neighbourhood infection	Persons	Wild boar	unknown	Source
Germany 1971-1974[a]	4.1%	2.9%	19.7 %	n.r.[b]	45.1%	10.6 %	17.6%	Wachendörfer *et al.*, 1978
Germany 1994-1995	21.1 %	14.1%	5.8%	21.8%	-[c]	10.9 %	26.3%	Teuffert *et al.*, 1995
The Netherlands 1982-1985	14.8 %	1.8 %	n.r.	29.1 %	-	n.r.	54.3 %	Terpstra, 1987
- breeding/mixed farms	45.9 %	1.8 %	2.3 %	21.6 %	-	n.r.	29.4 %	
– fattening farms								

[a]subjective opinion of veterinary officers.
[b]n.r. = not recorded.
[c]recorded under neighbourhood infection.

Indirect spread of the virus through contaminated vehicles, people, instruments or feed is also possible (TABLE 33). It has been reported that veterinarians and slaughter personnel were involved in the spread of the disease (Küttler, 1984). The role of liquid manure and particularly slurry tanks has been discussed (Vannier *et al.*, 1986; Terpstra, 1987). Due to the fact that virus excretion in faeces is low and the agent is quickly inactivated in that type of environment this is considered an unlikely means of transmission.

When subclinically infected pigs are slaughtered CSF virus may enter the pork production chain. Untreated pork products act then as a reservoir for the infection (Terpstra, 1987). The survival of the virus is enhanced in this protein-rich environment and is particularly good in cooled and frozen products (TABLE 34).

TABLE 34. Persistence of classical swine fever virus in various pork products

Pork product	Treatment	Survival	Source
Parma ham	processed 112 d	yes	McKercher *et al.*, 1987
	processed 188 d	no	
Ham	cooked, chilled	no	Helwig and Keast, 1966
	uncooked, chilled	85 d	
Sausage casings	salted	86 d	Helwig and Keast, 1966
	unsalted	3 d	
	salted	147 d	McKercher *et al.*, 1980
Iberian ham Iberian shoulder	processed 252 d	no	Mebus *et al.*, 1997
	processed 140 d	no	
Iberian loin Serrano ham	processed 126 d	no	
	processed 140 d	no	

The feeding of garbage containing such products without prior heat treatment to susceptible animals is an important way of transmission. For a number of European outbreaks insufficiently treated swill containing contaminated pork or wild boar products have been assumed to be the source of infection (TABLE 33; Küttler, 1984; Williams and Matthews, 1988; Kihm, 1994).

Airborne transmission is considered to be of minor importance. However, some aerosol transmission trials were successful (Hughes and Gustafson, 1960). This type of transmission could occur between mechanically ventilated units over short distances (Terpstra, 1987) and the analysis of recent outbreaks has described distinct spatial movements of the disease, which suggests airborne spread (Roberts, 1995). Further research to specify its importance is required. Currently potential airborne spread is being covered by the transmission category 'local spread' or 'neighbourhood infection (TABLE 33). This category is a conglomerate of possible risk factors acting at a short distance of the infected property. It includes possible spread by rodents, birds, air or unspecified personal contact. It is used when a secondary outbreak without specific cause of infection occurs at short distance around an infected property, most often on a contiguous property. At present, the process of short distance transmission is not fully understood (Koenen *et al.*, 1996).

Seasonal peaks of disease occurrence in spring have been reported (Lorenz and Boorberg, 1986). The same was observed in Italy. This has been related to pig movement and production patterns rather than environmental factors (Caporale *et al.*, 1988).

The transmission of CSF from infected wild boar to domestic pigs is possible, although the importance of this pathway has not been fully quantified (Dahle and Liess, 1992; Laddomada *et al.*, 1994). This means of spread depends on the occurrence of infected wild boars in a specific region and its importance can therefore be highly variable in different outbreaks (TABLE 33). Imported meat from infected wild boars can also act as a source of virus introduction into a region (Krassnig *et al.*, 1995). Further investigations of a German epidemic revealed that garbage of American bases and other porcine slaughter offal had been used as baits during the hunting season (Wachendörfer *et al.*, 1978). Feeding on wild boar carcasses left behind by hunters might also infect free-ranging domestic pigs. On the other hand, wild boars may become infected if CSF-infected carcasses of domestic pigs are buried in places

accessible to predators (Aubert *et al.*, 1994). Infection in wild boars has also been related to vaccination of domestic pigs (Dahle *et al.*, 1993).

1.7 Prevention and control

Traditionally, CSF has been controlled by vaccination. For example, in the United States, an official vaccination program was in place for 50 years, before it was ceased and the disease eradicated (Stalheim, 1988). Cost-benefit analyses comparing vaccination with alternative control strategies were carried out in the European Union (EU) in the 70s, and an economic advantage of disease eradication was demonstrated (Ellis, 1972). The EU subsequently adopted the eradication strategy in 1980 (directive 80/217 EEC) and vaccination was to be stopped within 2 years (Edwards, 1989). Under current legislation in the EU, the use of vaccination to control CSF will result in an extended trade ban, as antibodies due to natural infection and vaccination cannot be distinguished. New vaccines that allow a distinction are likely to overcome this problem. However, such a vaccine may not become available in the near future, and if it does, it is questionable if it will be readily accepted as an alternative control policy (Rümenapf *et al.*, 1995). It is recommended that, if mass vaccination is applied as a means of outbreak control, monthly vaccination of 8-10 week-old stock and newly introduced stock should be continued for at least one year after the start of vaccination (Terpstra, 1991).

Currently, in case of a CSF outbreak the EU requires the following procedure (Roberts, 1995): All stock on an infected holding has to be slaughtered followed by cleaning and disinfection. At the same time, protection and surveillance zones have to be established with a radius of 3 km and 10 km, respectively. Within these zones movement of pigs is prohibited for a fixed time period (15-30 days) and strictly regulated after this period has elapsed. Epidemiological investigations and serological and clinical surveillance have to be carried out in the restricted zones. Vaccination remains basically prohibited.

Since this policy was adopted, large CSF outbreaks in areas with very high pig densities have occurred within the EU (Roberts, 1995; Kramer *et al.*, 1995). These epidemics have challenged the practicality of the directive (Pittler *et al.*, 1995). The impossibility to move pigs combined with very long movement restriction periods caused overwhelming problems for farmers, who could not sell their animals. This caused illegal animal movements and required expensive additional market support measures from the EU (Roberts, 1995). Therefore, it has been discussed whether either duration or radius of the restriction zone could be reduced (Roberts, 1995; Teuffert *et al.*, 1995). A reduction of the duration does not seem to be epidemiologically justifiable. On the contrary, it was recommended that the restriction zone should be maintained for at least 35 days as most secondary outbreaks are likely to be detected within that time period (Roberts, 1995; Teuffert *et al.*, 1995). Yet, a reduction of the surveillance zone to a 6-km radius seems to be made justifiable by data from recent outbreaks where the maximum distance between primary and secondary outbreaks was <6km.

The key to efficient control of a CSF outbreak is early detection of infected farms. This largely depends on effective forward and backward tracing (Terpstra, 1987) as part of the epidemiological investigation, and on clinical surveillance (Teuffert and Schlüter, 1994). Forward tracing identifies risky contacts to other farms to which the infection might have spread. Backward tracing is targeted at identifying the infection source for an infected farm. The rapid

detection of contact farms depends on the complete registration of pig movements and on a reliable and efficient pig identification scheme. It has been demonstrated that the economic losses due to CSF can be reduced by better animal identification and recording systems (Saatkamp, 1996). Especially electronic identification systems can reduce the economic impact by reducing the time period until secondary outbreaks are discovered.

Besides insufficient animal identification chronic and persistent infections offer a major challenge to any eradication attempt (Carbrey *et al.*, 1980). In order to detect these animals, targeted serological screening has been used (Vannier *et al.*, 1986). However, it has been argued that serology is not necessarily very efficient in the early detection of infected premises (Teuffert and Schlüter, 1994, Kramer *et al.*, 1995). Therefore large-scale use of virus isolation is seems more appropriate for early detection of the infection (Koenen *et al.*, 1996). Antibody screening remains important to demonstrate the absence of endemic infection in an area.

Early detection of secondary outbreaks is particularly important in areas with high pig density where the infection can potentially spread very quickly. In order to avoid the time loss due to laboratory testing, the strategy of pre-emptive slaughter has been introduced. In this case, all pigs on high-risk farms such as direct neighbours and farms that had a high-risk contact with an infected farm will be immediately slaughtered without diagnosis. This strategy has been used in Belgium (Koenen *et al.*, 1996) and in Germany (Roberts, 1995). Experience in Germany showed that depopulation of all holdings within 200m of the infected herd should be practised (Teuffert *et al.*, 1995).

Reviewers of CSF outbreak control activities have identified a series of critical factors for eradication success. The importance of a regional or national crisis centre and the co-ordination and collaboration of veterinary services, producer organisations and the government has been stressed (Pittler *et al.*, 1995). Furthermore, epidemiological investigations and documentation are of utmost importance (Terpstra, 1987; Caporale *et al.*, 1988; Teuffert *et al.*, 1995). For data collection the use of an investigation questionnaire is suggested (Terpstra, 1987). Spatial relationships should also be recorded (Teuffert *et al.*, 1995). Unfortunately, due to hectic conditions during an emergency, detailed epidemiological documentation of CSF outbreaks is still rare.

As wild boars may be involved in CSF transmission in a region, specific strategies targeting this problem have been developed. Infection pressure among wild boar can be reduced through population decimation (Kaden, 1995). Virological and/or serological testing of any shot wild boar should be performed in CSF-infected regions or countries in order to further investigate the epidemiological importance of this species (directive 80/217 EEC). The correct disposal of wild boar carcasses and offal also has to be assured. An additional option is the oral immunisation of wild boars with a live attenuated CSF virus strain. This approach was used in areas with chronically infected wild boar populations in France and Italy. In Germany, vaccination buffers were successfully applied to stop the spread of the disease. It is estimated that vaccination has to be used over 2-3 years in order to eradicate CSF in wildlife (Kaden, 1995). Strict separation of wild boars and domestic pigs seems to be the simplest way to prevent transmission between the two populations in the short term.

2. Epidemiology of African swine fever

2.1 Introduction

African swine fever (ASF) was first reported by Montgomery in 1921 (Wardley *et al.*, 1983). He described outbreaks of an acute swine disease in Kenya characterised by a high mortality and clinical features similar to hog cholera, but possibly related to wild pigs which seemed to act as a virus reservoir. Since this report, ASF has been diagnosed in many African countries and outbreaks also occurred in the Western Hemisphere and some southern European countries. The international spread of the disease was mainly due to feeding of contaminated pork products to domestic pigs. In 1994, ASF cases were reported from Africa, Italy and Spain (OIE, 1995). ASF has never occurred in New Zealand.

2.2 Aetiology

ASF is caused by an enveloped icosahedral DNA virus, tentatively classified as an Iridovirus (Mebus, 1988) and with a diameter of 200 nm. The structure of the virus is complex with more than 100 identified proteins (Sanchez-Vizcaino, 1992). In the pig, ASF virus mainly replicates in cells of the mononuclear phagocytic system. Additionally, ASF virus also replicates in some soft ticks.

Although the virus does not seem to induce the production of neutralising antibodies, the presence of infection-inhibitory antibodies has been demonstrated in recovered pigs (Ruiz Gonzalvo *et al.*, 1986).

The ASF virus is highly resistant to environmental inactivators such as heat and low pH. It is able to survive at room temperature in blood and serum for up to 18 months and even longer when frozen (Sanchez-Vizcaino, 1992). Yet, it can be inactivated when heat-treated at 60°C for 30 minutes (Plowright and Parker, 1967). A number of lipid solvents and most commercially available disinfectants are also reliable inactivators.

2.3 Affected species

Both domestic pigs *(Sus scrofa domestica)* and wild pigs are susceptible to ASF (Mebus, 1988). In Africa, warthogs *(Phacochoerus aethiopicus)* principally and to a lesser degree bush pigs (*Potamochoerus porcus*) and giant forest hogs (*Hylochoerus meinerizhageni*), can act as virus reservoirs (Wardley *et al.*, 1983). They harbour the virus without showing clinical signs. In Europe, wild boars *(Sus scrofa ferus)* are susceptible to the disease in its clinical form (Firinu and Scarano, 1988). The same seems to be true for feral pigs in Florida (McVicar *et al.*, 1981).

ASF virus has also been isolated from soft ticks of the *Ornithodoros* genus, mainly *O. moubata and O. erraticus,* but it is believed that most *Ornithodoros* species are capable vectors of ASF virus (Mebus, 1988). Infected ticks have also been found in Spain (Oleaga-Pérez *et al.*,

1990). It has been suggested that ASF virus may be a tick virus with the pig being an accidental host.

2.4 Sylvatic cycle

In nature, ASF is being maintained as an inapparent infection in mainly warthogs and soft ticks inhabiting their burrows (Wilkinson, 1984). Transovarial, sexual and transstadial transmission occurs in ticks (Wardley *et al.*, 1983). The virus is excreted by the tick in a number of body fluids including saliva and therefore readily transmitted to pigs during blood feeding. Ticks can survive for many years and thus maintain infectivity in an area after pigs have been removed (Oleaga-Pérez *et al.*, 1990). It has been shown that the virus can persist up to 8 years in infected ticks (Sanchez Botija, 1982).

It is likely that most warthogs acquire ASF by a tick bite, because neither horizontal nor vertical transmission seems to occur (Wardley *et al.*, 1983). Especially young warthogs develop viraemia sufficiently high to infect ticks feeding on them (Thomson *et al.*, 1980) but without showing clinical signs. This cycle is considered to be important for the maintenance of the disease in addition to the within-tick transmission and makes it difficult to eradicate the disease.

The epidemiological role of European wild pigs is different from warthogs, because wild pigs are similarly susceptible to the disease as are domestic pigs (McVicar *et al.*, 1981; Sanchez Botija, 1982; Firinu and Scarano, 1988).

2.5 Disease in domestic pigs

The signs of the disease depend on the virus strain involved and the route of infection and may range from a mild, subclinical form to acute outbreaks with loss of appetite, fever, haemorrhages on the skin and high mortality (Sanchez-Vizcaino, 1992). These clinical signs are not specific and make it impossible to discriminate ASF from other acute viral pig diseases, particularly classical swine fever. It has been observed that at the beginning of an outbreak acute forms predominate while at a later stage subacute or chronic forms are more frequent (Wilkinson, 1986b). The latter are characterised by abortions and low mortality. In the Mediterranean, subclinical forms have also been observed where the infection can only be detected by means of serological testing (Sanchez Botija, 1982; Wilkinson, 1984).

The incubation period may vary from 4 to 8 days, but can last up to 19 days in natural infections (Sanchez-Vizcaino, 1992), while incubation periods under experimental conditions are usually shorter. This depends again on the virus virulence and the route of infection.

2.6 Virus transmission

When a pig gets infected with ASF virus the primary site of replication are the tonsils and mandibular lymph nodes. From there the virus spreads and reaches the blood vessels and organs, where secondary replication takes place. Viraemia starts between 6 and 8 days *post infection* and is maintained for considerably long time periods (TABLE 35).

At this stage of the infection, oral and nasal secretions as well as urine, faeces and conjunctival fluid (TABLE 36) spread the virus. The amount of virus shed is usually low, unless blood is shed. The concentration in blood may reach $\log_{10}$ 8-9.25 HAd_{50}/ml (Greig and Plowright, 1970; Wilkinson *et al.*, 1977). Otherwise the concentration seems to be highest in conjunctival fluid (maximal 4.8 HAd_{50}/ml) and faeces (maximal 5.8 HAd_{50}/g) and reaches maximal concentrations 2 - 5 days after the onset of clinical signs (Greig and Plowright, 1970; McVicar, 1984). The infective dose for a pig depends on the route of inoculation. McVicar (1984) reported an ID_{50} for the intranasal-oral route of 18,200 HAd_{50} and one of 0.13 HAd_{50} for intramuscular or intravenous inoculation.

TABLE 35. Incubation period and onset of viraemia of African swine fever

Virus strain	Inoculation	Dose	Time of onset of pyrexia	Time of onset of viraemia[a]	Source
Tengani	intranasally contact	$2*10^7$ HAd_{50} n.a.	2.9 d 3.9 d	0 d -2 d	Greig and Plowright, 1970
Kirawira KWH/12	intranasally contact	$2*10^7$ HAd_{50} n.a.	3.1 d 4.4 d	0 d 0 d	
Dominican Republic DR'79	intranasally/orally	10^5-10^7 HAd_{50}	3-4 d	-	McVicar, 1984
Lisbon L'60	intranasally/orally	10^5-10^7 HAd_{50}	3 d	-	

[a]days relative to onset of pyrexia.

TABLE 36. Earliest onset of African swine fever virus excretion in relation to onset of clinical signs

Virus strain	Inoculation	Pharyngeal swab	Nasal swab	Conjunctival swab	Urine	Faeces	Source
Tengani	contact	- 2 d	- 1 d	1 d	n.s.	0	Greig and Plowright, 1970
Kirawira KWH/12	contact	- 2 d	- 2 d	- 1 d	n.s.	0	
Dominican Republic DR'79	intranasally/orally	2 d	2 d	2 d	1 d	2 d	McVicar, 1984
Lisbon L'60	intranasally/orally	1 d	1 d	2 d	3 d	1 d	

Transmission between individual animals occurs by direct contact. Airborne spread over short distances has also been demonstrated (Wilkinson *et al.*, 1977) by exposing pigs to air drawn through ducting from a pen with infected pigs. Transmission was also possible when pigs were kept on a platform 2.3 m above donor animals which were in a late stage of acute disease (3-5 d after inoculation). The latter technique required a minimal time of exposure of at least 48 h. But ASF virus could not be isolated from the air by either biological (mice) or mechanical sampling techniques. It was concluded that airborne virus spread was only a problem in intensive housing systems.

Between premises, the most efficient way of transmission is movement of infected stock. Additionally, spread occurs through contaminated veterinary equipment, people, vehicles and feed (Mebus, 1988). The analysis of ASF outbreaks in Spain revealed the following distribution amongst the different means of spread: 65% contact between neighbouring farms, 19% introduction of carrier pigs, 5% ticks, 5.8% contact with wild boars (Sanchez Botija, 1982). Introduction of inapparently infected stock was also assumed to be the cause of ASF outbreaks in Africa (Nakel, 1984).

Many primary outbreaks of ASF in formerly unaffected areas were traced back to feeding swine on swill containing infected pork products originating from aeroplanes or ships. Infected pigs in the incubation period, chronic carrier pigs or subclinically infected pigs may get into the pork production chain (Mebus, 1988). Although these pigs may look clinically healthy, the tissue will frequently contain ASF virus which will subsequently be present in pork products. Experiments for the survival of ASF virus in various products have been performed (TABLE 37).

TABLE 37. Persistence of African swine fever virus in various pork products

Pork product	Treatment	Survival	Source
Tinned ham	69-70°C	Inactivated	Sanchez Botija, 1982
Sausage	uncooked	3-6 months	
Filet	uncooked	3-6 months	
Dry ham	uncooked	3-6 months	
Parma ham	processed 300d	not inactivated	McKercher *et al.*, 1987
	processed 400d	no viable virus found	
Iberian ham Iberian shoulder	processed 140 d	no	Mebus *et al.*, 1997
	processed 140 d	no	
Iberian loin Serrano ham	processed 112 d	no	
	processed 140 d	no	

Pigs that survive ASF infections probably remain lifelong virus carriers (Wardley *et al.*, 1983). The viraemia persists after appearance of anti ASF virus antibodies. These carriers may play an important role in the maintenance of the disease in domestic pig populations. While they seem to be protected against infection with a homologous virus, they are susceptible to repeated inoculation with more virulent strains (Mebus, 1988). Transmission between carriers and susceptible pigs is particularly likely in stress situation when virus shedding may be re-activated.

Piglets born of ASF-convalescent sows seem to be free of infection but seroconvert after colostrum ingestion. They may survive a virus challenge (Schlafer and Mebus, 1984) but develop lower antibody titres than control animals.

2.7 Diagnosis

A fast reliable laboratory diagnosis is essential for the control of an ASF outbreak. Because the clinical signs can easily be mistaken for another infectious pig disease, diagnosis relies on laboratory techniques. It can either be based on the detection of viral antigen or specific anti-

bodies. Currently, several tests are available. Indirect immunofluorescence, immunoblotting and ELISA are the most frequently used techniques. They are all highly sensitive and specific tests.

2.8 Prevention and control

No treatment or effective vaccine for ASF is currently available (Sanchez-Vizcaino, 1992). The control of the disease is complicated in those geographical areas where the infection occurs in soft ticks and wild pigs. These reservoirs are unlikely to be eliminated (Wardley *et al.*, 1983), although tick and warthog control may be applied (Sanchez-Vizcaino, 1992). Also husbandry systems that prevent tick and wild pig contact are effective measures. The occurrence of these factors support the persistence of the disease (Wilkinson, 1984). Additionally, pig movement control is crucial in order to impede the introduction of carrier pigs. In Zimbabwe it has been shown, that pigs can be raised ASF-free in an endemic area provided proper isolation and sanitary procedures are applied (Mebus, 1988).

Stamping-out procedures and strict sanitary measures have been adopted as eradication strategies by most countries currently free of ASF. After disease reconfirmation, slaughter of all infected and in-contact pigs, followed by intensive cleaning and disinfecting, has to be performed. This procedure has successfully been applied in Malta, Dominican Republic and Haiti (Wilkinson, 1986b). Prevention of the introduction of the disease into ASF-free regions relies on animal movement control and appropriate treatment of swill containing pork products. Garbage originating from aeroplanes and ships should be incinerated and not used as pig feed at all (Sanchez-Vizcaino, 1992).

Rapid diagnosis of any suspect disease is essential to prevent the virus from getting established and developing chronic, hard to detect forms. In countries, where these forms already occur intensive serological surveillance has to be organised in order to detect and slaughter virus carriers (Sanchez-Vizcaino, 1992).

The eradication of ASF has been proved to be extremely costly, requiring sometimes the complete elimination and re-building of a national pig population (Wilkinson, 1986a,b). Further economic losses occur as a result of trade barriers and loss of international market shares for live pig and pork products.

References

Aubert, M., Picard, M., Fouquet, E., Conde, J., Crucierce, C., Ferry, R., Albina, E., Barrat, J., and Vedeau, F. (1994). La peste porcine classique du sanglier en Europe. Annales de Médecine Vétérinaire 138:239-247.

Caporale, V., Rutili, D., Nannini, D., Francesco, C. di, Ghinato, C., and Di Francesco, C. (1988). Epidemiology of classical swine fever in Italy from 1970 to 1985. Revue Scientifique Et Technique, Office International Des Epizooties 7:599-617.

Carbrey, E.A., Stewart, W.C., Kresse, J.I., and Snyder, M.L. (1980). Persistent hog cholera infection detected during virulence typing of 135 field isolates. American Journal of Veterinary Research 41:946-949.

Carbrey, E.A., Stewart, W.C., and Young, S.H. (1966). The changing picture of hog cholera: case studies. Journal of the American Veterinary Medical Association 149:1720-1724.

Carnero, R., Gayot, G., and Costes, C. (1983). Classical swine fever. Epidemiological study of the disease in

France over the last few years. Bulletin De L'Academie Vétérinaire De France 56:299-311.

Dahle, J., and Liess, B. (1992). A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. Comparative Immunology, Microbiology and Infectious Diseases 15:203-211.

Dahle, J., Patzelt, Th., Schagemann, G., and Liess, B. (1993). Antibody prevalence of hog cholera, bovine viral diarrhoea and Aujeszky's disease virus in wild boars in Northern Germany. Deutsche Tierärztliche Wochenschrift 100:330-333.

Depner, K., Gruber, A., and Liess, B. (1994). Experimental infection of weaner pigs with a field isolate of hog cholera classical swine fever virus derived from a recent outbreak in Lower Saxony. 1. Clinical, virological and serological findings. Wiener Tierärztliche Monatsschrift 81:370-373.

Depner, K. R., Müller, A., Gruber, A., Rodriguez, A., Bickhardt, K., and Liess, B. (1995a). Classical swine fever in wild boar (*sus scrofa*) - experimental infections and viral persistence. Deutsche Tierärztliche Wochenschrift 102:381-384.

Depner, K.R., Müller, A., and Liess, B. (1995b). Epidemiological significance of antibody and antigen detection in classical swine fever - a case report. Tierärztliche Umschau 50:571-573.

Depner, K.R., Rodriquez, A., Pohlenz, J., and Liess, B. (1996). Persistent classical swine fever virus infection in pigs infected after weaning with a virus isolated during the 1995 epidemic in Germany: Clinical, virological, serological and pathological findings . European Journal of Veterinary Pathology 2:61-66.

Depner, K.R., Hinrichs, U., Bickhardt, K., Greiser-Wilke, I., Pohlenz, J., Moennig, V., and Liess, B. (1997a). Influence of breed-related factors on the course of classical swine fever virus infection. Veterinary Record 140:506-507.

Depner, K.R., Moennig, V., and Liess, B. (1997b). Epidemiologische Aspekte der Infektionsbiologie der klassischen Schweinepest. Der praktische Tierarzt, collegium veterinarium XXVII:63-67.

Dulac, G.C., and Mebus, C.A. (1992). Hog cholera. In: Foreign Animal Diseases United States Animal Health Association, Richmond, Virginia, U.S.A. 236-245.

Edwards, S. (1989). Epidemiology and control of classical swine fever. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Exeter, 74-79.

Edwards, S., and Sands, J.J. (1990). Antigenic comparisons of hog cholera virus isolates from Europe, America and Asia using monoclonal antibodies. Deutsche Tierärztliche Wochenschrift 97:79-81.

Ellis, P.R., James, A.D., and Shaw, A.P. (1972). Studies on the epidemiology and economics of swine fever eradication in the EEC. Commission of the European Communities, Luxembourg, Report EUR 5738e.

Firinu, A., and Scarano, C. (1988). African swine fever and classical swine fever (hog cholera) among wild boar in Sardinia. Revue scientifique et technique, Office International des Epizooties 7:901-915.

Greig, A., and Plowright, W. (1970). The excretion of two virulent strains of African swine fever virus by domestic pigs. Journal of Hygiene Cambridge 68:673-682.

Haas, L., Freiser-Wilke, I., Depner, K., and Moennig, V. (1997). Aufbau einer Nukleotidsequenzdatenbank zur molekularen Epidemiologie der klassischen Schweinepest. Der praktische Tierarzt, collegium veterinarium XXVII:60-62.

Helwig, D.M., and Keast, J.C. (1966). Viability of virulent swine fever virus in cooked and uncooked ham and sausage casings. Australian Veterinary Journal 42:131-135.

Hofmann, M.A., Brechtbühl, K., and Stäuber, N. (1994). Rapid characterization of new pestivirus strains by direct sequencing of PCR amplified cDNA from the 5' noncoding region. Archives of Virology 139:217 229.

Hone, J., Pech, R., and Yip, P. (1992). Estimation of the dynamics and rate of transmission of classical swine fever (hog cholera) in wild pigs. Epidemiology and Infection 108:377-386.

Horner, G.W. (1997). Survey of New Zealand pigs for swine fever antibody. Surveillance 24:28.

Hughes, R.W., and Gustafson, D.P. (1960). Some factors that may influence hog cholera transmission. American Journal of Veterinary Research 21:464-471.

Kaden, V. (1995). Möglichkeiten und Grenzen der ESP-Bekämpfung bei Wildschweinen unter besonderer Berücksichtigung der Impfung. Proceedings der Jahrestagung der Fachgruppe Schweinekrankheiten der DVG, Hannover, 16-17 February 1995, 17-19.

Kihm, U. (1994). Final report on hog cholera in Switzerland. Bulletin - Office International Des Epizooties 106:524-525.

Koenen F, Cay A, Lefebvre J, and Desmet A. (1992). Evaluation of the complex trapping blocking ELISA in a serological survey during the Belgian classical swine fever epizootic in 1990. Veterinary Record 131: 396.

Koenen, F., Vancaenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. Veterinary Record 139:367-371.

Kramer, M., Ahl, R., Teuffert, J., Kroschewski, K., Schlüter, H., and Otte, J. (1995). Classical swine fever in Germany - some epidemiological aspects. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Reading, 110-118.

Krassnig, R., Schuller, W., Heinrich, J., Werfring, F., Kalaus, P., and Fruhwirth, M. (1995). Isolation of hog cholera virus (HCV) in imported frozen meat of wildboars. Deutsche Tierärztliche Wochenschrift 102:56.

Küttler, D. (1984). Epidemiological aspects of swine fever. Der praktische Tierarzt 65:1073-1084.

Laddomada, A., Patta, C., Oggiano, A., Caccia, A., Ruiu, A., Cossu, P., and Firinu, A. (1994). Epidemiology of classical swine fever in Sardinia: a serological survey of wild boar and comparison with African swine fever. Veterinary Record 134:183-187.

Liess, B. (1987). Pathogenesis and epidemiology of hog cholera. Annales de Recherche Vétérinaire 18:139-145.

Loepelmann, H., and Dedek, J. (1987). Erfahrungen bei der Bekämpfung der Schweinepest beim Schwarzwild in einem Beobachtungsgebiet der DDR. Monatshefte für Veterinärmedizin 42:313-316.

Lorenz, R J., and Boorberg, I. (1986). Incidence rates of hog cholera infection in various categories of pig herds. Proceedings of the 4th International Symposium on Veterinary Epidemiology and Economics, Singapore, 233.

McKercher, P.D., Morgan, D.O., and McVicar, J.W. (1980). Thermal processing to inactivate viruses in meat products. Proceedings of the US Animal Health Association 84:320-328.

McKercher, P.D., Yedloutschnig, R.J., Callis, J.J., Murphy, R., Panina, G.F., Civardi, Y., Bugnetti, M., Foni, E., Laddomada, A., Scarano, C., and Scatozza, F. (1987). Survival of viruses in "Prosciutto di Parma" (Parma ham). Canadian Institute of Food Science and Technology Journal 20:267-272.

McVicar, J.W. (1984). Quantitative aspects of the transmission of African swine fever. American Journal of Veterinary Research 45:1535-1541.

McVicar, J. ., Mebus, C.A., Belden, R.C., and Gibbs, E.P.J. (1981). Induced African swine fever in feral pigs. Journal of the American Veterinary Medical Association 179:441-446.

Mebus, C.A. (1988). African swine fever. Advances in Virus Research 35:251-269.

Mebus, C.A., Arias, M., Pineda, J.M., Tapiador, J., House, C., and Sánchez-Vizcaíno, J.M. (1997). Survival of several porcine viruses in different Spanish dry-cured meat products. Food Chemistry 59:555-559.

Nakel, V. (1984). Evaluation of outbreaks of African swine fever in Africa and some differences from classical swine fever. Monatshefte für Veterinärmedizin 39:772-775.

OIE. (1995). World animal health in 1994. Paris: Office International des Epizooties.

Oleaga Perez, A., Perez Sanchez, R., and Encinas Grandes, A. (1990). Distribution and biology of Ornithodoros erracticus in parts of Spain affected by African swine fever. Veterinary Record 126:32-37.

Paton, D.J., Sands, J.J., Lowings, J.P., Smith, J.E., Ibata, G., and Edwards, S. (1995). A proposed division of the pestivirus genus using monoclonal antibodies, supported by cross-neutralisation assays and genetic sequencing. Veterinary Research 26:92-109.

Pittler, H., Fiedler, J., Jentsch, D., Hasselbach, P., and Kramer, M. (1995). Der Schweinepest-Seuchenzug

1993/94, Probleme und Konsequenzen. Tierärztliche Umschau 50:522-530.

Plateau, E., Vannier, Ph., and Tillon, J.P. (1980). Experimental study of a mild virulence strain of hog cholera: Individual variations and horizontal transmission. Zentralblatt der Veterinär-Medizin B 27:650-657.

Plowright, W., and Parker, J. (1967). The stability of African swine fever virus with particular reference to heat and pH Inactivation. Archiv für die Virusforschung 21:383-402.

Ressang, A.A. (1973). Studies on the pathogenesis of hog cholera. I. Demonstration of hog cholery virus subsequent to oral exposure. Zentralblatt der Veterinär-Medizin B 20:256-271.

Ressang, A.A., van Bekkum, J.G., and Bool, P.H. (1972). Virus excretion in vaccinated pigs subject to contact infection with virulent hog cholera strains. Zentralblatt der Veterinär-Medizin B 19:739-752.

Roberts, M. (1995). Evaluation of optimal size of restriction zones in disease control with particular reference to classical swine fever. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Reading, 119-130.

Ruiz Gonzalvo, F., Carnero, M.E., and Caballero, C. (1986). Inhibition of African swine fever infection in the presence of immune sera in vivo and in vitro. American Journal of Veterinary Research 47:1249-1252.

Rümenapf, T., König, M., Lengsfeld, T., Stark, R., and Thiel, H.J. (1995). Status of development and outlook for the application of a labeled vaccine against classical swine fever. Der Praktische Tierarzt 76:54-55.

Saatkamp, H.W. (1996). Simulation studies in the potential role of national identification and recording systems in the control of classical swine fever. PhD Thesis, Wageningen Agricultural University, Wageningen.

Sanchez Botija, C. (1982). La peste porcine africaine. Nouveau développements. Revue scientifique et technique Office International des Epizooties 1:1031-1064.

Sanchez-Vizcaino, J.M. (1992). African swine fever. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, and D.J. Taylor (eds.) Diseases of swine 7th Edition. Iowa University Press, Ames, Iowa, U.S.A, 228-236

Schlafer, D.H., and Mebus, C.A. (1984). Abortion in sows experimentally infected with African swine fever virus: Clinical features. American Journal of Veterinary Research 45:1353-1360.

Stalheim, O.H.V. (1988). The hog cholera battle and veterinary professionalism. Agricultural History 62: 116-121.

Terpstra, C. (1987). Epizootiology of swine fever. Veterinary Quarterly 9:50S-60S.

Terpstra, C. (1991). Hog cholera: an update of present knowledge. British Veterinary Journal 147:397-406.

Terpstra, C., and Wensvoort, G. (1988). The protective value of vaccine induced neutralising antibody titres in swine fever. Veterinary Microbiology 16:123-128.

Teuffert, J., and Schlüter, H. (1994). Epidemiologische Aspekte eines Monitoring am Beispiel der europäischen Schweinepest. DVG Tagung der Fachgruppe Epidemiologie und Dokumentation, Berlin 117-134.

Teuffert, J., Schlüter, H., Staubach, C., Otte, J., and Kroschewski, K. (1995). The spread of CSF in the 1 km zone, the protection and the surveillance zone - are changes of the measures applied in these zones justified or necessary? DVG-Tagung der Fachgruppe Tierseuchen, Giessen 59-74.

Thomson, G.R., Gainaru, M.D., and van Dellen, A. F. (1980). Experimental infection of warthog *(Phacochoerus aethiopicus)* with African swine fever virus. Onderstepoort Journal of Veterinary Research 47:19-22.

van Oirschot, J.T. (1992). Hog cholera. In: A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, and D.J. Taylor (eds.) Diseases of swine 7th Edition. Iowa University Press, Ames, Iowa, U.S.A, 274-285

van Oirschot, J.T., and Terpstra, C. (1977). A congenital persistent swine fever infection. I. Clinical and virological observations. Veterinary Microbiology 2:121-132.

Vannier, P., Colcanap, M., Carnero, R., Costes, C., Josse, J., and Tillon, J.P. (1986). Study of the origin of an epizootic of classical swine fever. Journal of Veterinary Medicine, B 33:294-302.

Vilcek, Š., Stadejek, T., Takácsová, I., Strojný, L., and Mojziš, M. (1997). Genetic analysis of classical swine fever virus isolates from a small geographic area. Deutsche tierärztliche Wochenschrift 104:1-40.

Wachendörfer, G., Reinhold, G.E., Dingeldein, W., Berger, J., Lorenz, J., and Frost, J.W. (1978). Analyse der Schweinepest-Epizootie in Hessen in den Jahren 1971-1974. Deutsche tierärztliche Wochenschrift 85: 113-152.

Wardley, R.C., de Andrade, C.M., Black, D., de Castro Portugal, F.L, Enjuanes, L., Hess, W.R., Mebus, C., Ordas, A., Rutili, D., Sanchez Vizcaino, J., Vigario, J.D., and Wilkinson, P.J. (1983). African swine fever virus. Archives of Virology 76:73-90.

Wensvoort, G., and Terpstra, C. (1985). Classical swine fever: a changing clinical picture. Tijdschrift voor Diergeneeskunde 110:263-269.

Wensvoort, G., Terpstra, C., de Kluijver, E.P., Kragten, C., and Warnaar, J.C. (1989). Antigenic differentiation of pestivirus strains with monoclonal antibodies against hog cholera virus. Veterinary Microbiology 21: 9-20.

Wilkinson, J.P., Donaldson, A.I., Greig, A., and Bruce, W. (1977). Transmission studies with African swine fever virus. Infection of pigs by airborne virus. Journal of Comparative Pathology 87:487-495.

Wilkinson, P.J. (1986a). African swine fever in Belgium. State Veterinary Journal 40:123-129.

Wilkinson, P.J. (1986b). Epidemiology of African swine fever. Revue scientifique et technique, Office International des Epizooties 5:487-493.

Wilkinson, P.J. (1984). The persistence of African swine fever in Africa and the Mediterranean. Preventive Veterinary Medicine 2:71-82.

Williams D.R., and Matthews D. (1988). Outbreaks of classical swine fever in Great Britain in 1986. Veterinary Record 122:479-483.

Young, S.H. (1970). The use of supplemental tests in the diagnosis and eradication of hog cholera. Journal of the American Veterinary Medical Association 157:1855-1859.

CHAPTER 2.2

FAILURE TO ISOLATE CLASSICAL SWINE FEVER VIRUS FROM THE AIR OF ROOMS HOUSING EXPERIMENTALLY INFECTED PIGS

Parts of this chapter will be published in the *Proceedings of the 15th International Pig Veterinary Society Congress, Birmingham* in a paper by: Stärk, K.D.C., Frey, J., Nicolet, J., Thür, B, and Morris, R.S. (1998) Assessment of aerosol transmission in the epidemiology of infectious diseases in pigs using air sampling and polymerase chain reaction.

1. Introduction

In recent outbreaks of classical swine fever (CSF) in The Netherlands, Germany and Belgium, the majority of farms in secondary outbreaks was infected through animal trade (Terpstra, 1987; Kramer *et al.*, 1995). Other means of between-farm transmission include contact with contaminated livestock trucks and contact with infected wild boars. Terpstra (1987) introduced a new category of infected properties affected by 'neighborhood spread'. This category was used when a farm contiguous to an infected property broke with CSF and no direct contact involving animals, persons and/or vehicles could be established. The concept of 'neighborhood' or 'local' spread was earlier used in the context of foot-and-mouth disease by Sanson (1993). It represents a conglomerate of possible risk factors effective over a short distance from an infected property. Such factors could be wild birds, rodents and other wild animals with short habitat ranges, unobserved personal contacts (for example children) and airborne spread. Neighborhood spread was considered to have occurred in 11-14% of CSF outbreaks in Germany (Pittler *et al.*, 1995; Teuffert *et al.*, 1997) and a concentration of secondary CSF outbreaks in the close neighbourhood of infected farms was also described in Belgium (Koenen *et al.*, 1996).

The occurrence of airborne spread is however generally considered to be of minor importance, but cannot be completely excluded, as some aerosol transmission trials have been successful (Hughes and Gustafson, 1960). It was suggested that airborne transmission could possibly occur between mechanically ventilated units over short distances (Terpstra, 1987), and the analysis of recent outbreaks has described distinct spatial movements of the disease, which suggests airborne spread (Roberts, 1995).

The objective of this experiment was to isolate CSF virus from the air of rooms housing experimentally infected animals in order to further clarify the possibility of airborne spread of CSFV.

2. Animals and Methods

2.1 Animals

Six 13 week-old Landrace x Large White SPF pigs were used. During the experiment, the behaviour of the pigs was monitored daily. Clinical signs and body temperature were recorded. If pigs developed serious illness or paralysis, they were removed from the room, killed and a post-mortem investigation performed.

The animals were housed in two identical rooms in a high security containment facility. The rooms had a constant temperature of 20°C. The air humidity was generally 50-60%, but reached 85% for approximately 1 hour after cleaning each morning. The rooms had a volume of 110 m^3, and the ventilation rate was 375 m^3 per hour. Three animals were housed in each room.

2.2 Virus

A recombinant Alfort/187 virus strain (vA187-1, Ruggli *et al.*, 1996) and the highly virulent CSF strain Brescia were used in the experiment. Three pigs each were assigned to one room and intranasally/orally infected on day 0 with 10^6 TCID50 of either vA187-1 (Group A) or Brescia (Group B).

2.3 Air sampling

Air was sampled for 5.5 hours (11 a.m. - 4.30 p.m.) using a polyethersulfon membrane filter (Supor200, Gelman Sciences, Ann Arbor, Michigan) with 0.2 µm pore size and a diameter of 47 mm. Air was pumped through the membrane with a vacuum pump (Millipore, Bedford, Massachusetts) at a flow rate of 950 l/h (calibrated with rotameter; Messerli Messtechnik, Riehen, Switzerland). The total volume sampled was therefore 5225 l/sample. Samples were taken on days -1 (negative control), 2, 4, 6, 9, 13, 16 and 20. The filters were stored at -70°C until analysed.

2.4 Sample analysis

A reverse transcription coupled with a polymerase chain reaction (RT-PCR) was used as described by Hofmann *et al.* (1994). In order to establish the test system including filter membranes the following preparatory analyses were performed:

vA178-1 was diluted with dilution medium in order to obtain concentrations of 10^0, 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, $10^{6.4}$, $10^{7.4}$ TCID50 per 100 µl medium. Filter pieces of approximately 3.5 cm^2 (1/5 of total filter) were used for each dilution step. 100 µl of the liquid was transferred onto the filter. The filter was left to dry for 2 hours. An RNA extraction was performed using Trizol®Reagent (Life Technologies Inc., Grand Island, N.Y.) according to the prescriptions of the manufacturer. With this method, CSF virus could be detected at concentrations of $\geq 10^{4.1}$ TCID50 per filter. These results were reproducible with filters that were first stored at -70°C before RT-PCR. When virus dilutions were used directly without filters, the results were identical. This excluded a negative effect of the filters and of storage in the assay.

Before analysis, the filters from the animal rooms were cut into two equal parts, one of which was used for the RT-PCR. The second part was kept as a reserve and was further stored at -70°C.

Blood samples of all pigs were collected on days 0,3,5,7,10,14, and 17. The same RT-PCR was used for antigen detection. Serology was performed using an ELISA test system (Moser *et al.*, 1996).

3. Results

Pigs in Group B showed increased body temperature from day 2 and a fever peak in all animals of above 41°C on days 3 and 4. All pigs showed reduced activity on day 3 and increas-

ingly distinct clinical signs including eye discharge, tremor, ataxia and paralysis. The animals were killed on days 7 (1 animal) and 8 (2 animals).

Pigs in Group A developed fever on day 3 with a peak on day 5 and thereafter decreasing until normal temperature levels below 39°C were reached on day 10. Clinical signs were not very distinct and included reduced appetite and conjunctivitis on days 5 and 6. All pigs were clinically normal on day 13. The pigs were killed on days 13 (1 animal) and 18 (2 animals). Results of serological and virological analyses are shown in TABLE 38

TABLE 38. Serological and virological results of blood samples from pigs infected with classical swine fever virus

Day after infection	Group A Number of animals with positive results/all animals tested		Group B Number of animals with positive results/all animals tested	
	RT-PCR	Serology[a]	RT-PCR	Serology[a]
0	0/3	0/3	0/3	0/3
3	0/3	n.a.[b]	3/3	n.a.
5	3/3	0/3	3/3	0/3
7	3/3	0/3	3/3	0/3
10	3/3	0/3	n.a.[c]	n.a.[c]
14	0/3	2/2	n.a.[c]	n.a.[c]
17	0/1	2/2	n.a.[c]	n.a.[c]

[a]An ELISA activity of >40% was interpreted as a positive result.
[b]n.a.=not analysed.
[c]All animals in this group were dead by day 8.

All air samples from both rooms were negative in the RT-PCR assay. Virus could also not be cultured from filter fragments transferred onto cell culture.

4. Discussion and conclusions

The attempt to isolate airborne CSF virus under the experimental conditions described above was not successful. There are three possible explanations for the failure:

1) *Low sensitivity of test system (air filtration in combination with RNA isolation and RT-PCR)*

If material is used for a RT-PCR that contains factors possibly inhibiting the assay, the RNA has first to be isolated (Ehrlich, 1989). Also if the material is collected on a soluble filter, the isolation procedure has to be performed first. After the additional steps required the amount of RNA available for the test is reduced, as the isolation is not 100% effective. Also, during the time of the RNA isolation, the RNAse in the material has time to start destroying the RNA, which also results in a lower RNA concentration. In the case of air samples, because the test material is attached to a filter membrane and because it is assumed that dust particles in the sample potentially have a negative effect in the assay, the RNA isolation has to be performed. The effective-

ness of RNA extraction from filter-associated CSF virus is not known, but the sensitivity of the test system may be considerably reduced due to this fact. It was shown that the sensitivity without RNA isolation was $10^{1.4}$ TCID50 as opposed to $10^{4.1}$ TCID50 with the isolation step. This is approximately a 500-fold reduction.

2) *Great dilution of airborne virus concentration due to high air volume and high ventilation rate in animal rooms*

In this experiment 3 pigs were housed in a room of 110 m^3 volume. Due to the high ventilation rate, the concentration of possibly excreted virus was further reduced. For the following estimations, an excretion rate of airborne virus particles similar to what is observed with foot-and-mouth (FMD) disease virus in pigs is assumed (best case scenario). With FMD 1 pig is shedding $10^{4.7}$ ID50 per hour (Sellers and Parker, 1969). It is further assumed that 1 ID50 ≈ 1 TCID50. In this situation the total amount of virus excreted under the conditions described in this experiment was $3*10^{4.7}$ TCID50 (3 pigs) / 110 m^3, which results in a concentration of $10^{3.1}$ TCID50 per m^3. With the air filtration system used in this experiment the volume of air sampled per filter was 5.225 m^3. The maximal amount of virus to be expected per filter would thus be $10^{3.8}$ TCID50, only, half of which was used in the analysis, as the second half was stored. This resulting maximal concentration of $10^{3.5}$ TCID50 is well below the sensitivity of the assay used here. Furthermore, the real concentration is likely to be much lower due to the high turnover of air in the animal room.

3) Low excretion rate of airborne CSF virus in infected animals

As points 1 and 2 were predominating in this experimental setting, point 3 cannot be assessed. However, field data does not support the hypothesis that airborne transmission is a major risk factor with CSF. If any airborne virus is shed, the plume concentration is likely to be low and will definitely not be detected under the described experimental conditions. The negative results of the RT-PCR in this experiment are thus caused by a combination of all three factors.

In order to improve the sensitivity of air sampling two options are possible: to increase the sampling volume or to sample under more realistic conditions where the virus concentration is likely to be higher.

In order to increase the sampling volume, it is recommended to use high volume air samplers, for example cyclone samplers (Errington and Powell, 1969) that can draw up to 900 l/min, and/or to sample from a limited air space with low ventilation rate. For example Donaldson *et al.* (1982) and Bourgueuil *et al.* (1992) have successfully used such settings to measure the excretion of virus in infectious aerosols generated by pigs.

For more realistic sampling conditions with higher animal concentrations, lower air exchange rates and higher virus concentrations, air sampling should be performed on acutely infected conventional pig farms during a CSF epidemic. For example farms in the current CSF outbreak in the Netherlands could be used. This outbreak is caused by a medium-to-high virulent CSF strain causing relatively distinct clinical signs (Bouma, personal communication). The use of a portable air sampling device such as the one described in this chapter could easily be used to collect samples before stamping out. Results from such field sampling – no matter

whether the samples are negative or positive - would be more significant than laboratory experiments.

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References

Bourgueuil, E., Hutet, E., Cariolet, R., and Vannier, P. (1992). Experimental infection of pigs with the porcine respiratory coronavirus (PRCV): measure of viral excretion. Veterinary Microbiology 31:11-18.

Donaldson, A.I., Ferris, N.P., and Gloster, J. (1982). Air sampling of pigs infected with foot-and-mouth disease virus: comparison of Litton and cyclone sampler. Research in Veterinary Science 33:384-385.

Ehrlich, H.A. (1989). PCR technology, principles and applications for DNA amplification. Stockton Press, 17-21.

Errington, F.P., and Powell, E.O. (1969). A cyclone sampler for aerosol sampling in the field. Journal of Hygiene Cambridge 67:387-399.

Hofmann M.A., Brechtbühl K., and Stäuber N. (1994). Rapid characterization of new pestivirus strains by direct sequencing of PCR amplified cDNA from the 5' noncoding region. Archives of Virology 139:217 229.

Hughes, R.W. and Gustafson, D.P. (1960). Some factors that may influence hog cholera transmission. American Journal of Veterinary Research 21:464-471.

Koenen, F., Vancaenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. Veterinary Record 139:367-371.

Kramer, M., Ahl, R., Teuffert, J., Kroschewski, K., Schlüter, H., and Otte, J. (1995). Classical swine fever in Germany - some epidemiological aspects. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Reading 110-118.

Moser, C., Ruggli, N., Tratschin, J.D., and Hofmann, M. (1996). Detection of antibodies against classical swine fever virus by indirect ELISA using recombinant envelope protein E2. Veterinary Microbiology 51:41-53.

Pittler, H., Fiedler, J., Jentsch, D., Hasselbach, P., and Kramer, M. (1995). The problems and consequences of the swine fever epidemic during 1993/94 in Germany . Tierärztliche Umschau 50:522-530.

Roberts, M. (1995). Evaluation of optimal size of restriction zones in disease control with particular reference to classical swine fever. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Reading 119-130.

Ruggli, N., Tratschin, J.-D., Mittelholzer, C., and Hofmann, M.A. (1996). Nucleotid sequence of classical swine fever virus strain Alfort/187 and transcription of infectious RNA from stably cloned full-length cDNA. Journal of Virology 70:3478-3487.

Sanson, R.L. (1993). The development of a decision support system for an animal disease emergency. PhD Thesis, Massey University, Palmerston North, New Zealand.

Sellers, R.F. and Parker, J. (1969). Airborne excretion of foot-and-mouth virus. Journal of Hygiene Cambridge 67:671-677.

Terpstra, C. (1987). Epizootiology of swine fever. Veterinary Quarterly 9:50S-60S.

Teuffert, J., Schlüter, H., and Kramer, M. (1997). Europäische Schweinepest. Übersicht zur internationalen (Europa) und nationalen Schweinepestsituation – ermittelte Einschleppungsursachen und Verschleppungsrisiken. Deutsches Tierärzteblatt 45:1078-1080.

CHAPTER 2.3

ELICITATION OF EXPERT KNOWLEDGE ON RISK FACTORS FOR CLASSICAL SWINE FEVER TRANSMISSION IN SWITZERLAND

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1. Abstract

In a situation of scarce or unreliable field data, expert knowledge may be an alternative to provide input estimates for building expert systems and simulation models. Adaptive conjoint analysis (ACA) is a technique that is relatively new to the veterinary field. It has been used for the elicitation of expert knowledge on the importance of risk factors for classical swine fever (CSF) transmission in Switzerland. Experts with a background in CSF research, national animal-health administration or field veterinary services were asked to participate in either of two workshops. Each participant was provided with a personal computer on which to complete a specifically designed, fully computer-supported questionnaire. The participants were asked to estimate the relative importance of risk factors for the farm-to-farm transmission of CSF in Switzerland. A total of 10 possible risk factors was evaluated. The participants were also asked to evaluate the ACA technique from an interviewee's point of view. The most important risk factor according to the experts was animal trade with 26.6% relative importance, followed by swill feeding and livestock trucks. Generally, the ACA technique was well received by the participants and may be quite practical to use for future investigations. The outcome of the workshop was compared with field data from recent classical swine fever outbreaks in Germany. Results showed that there was relatively good agreement between the relative importance of risk factors in the German epidemic and the estimates of Swiss experts. The potential and the implications of the use of expert opinion in future decision support systems is discussed.

2. Introduction

During an exotic disease epidemic, large amounts of data are collected and have to be processed efficiently in order to allow appropriate decision making. Computer programs can facilitate this task to a large extent by the use of powerful data management tools and epidemiological simulation models (Morris *et al.*, 1993). Such models are based on the characteristics of the disease. However, in many countries exotic disease outbreaks are rare, and if they occurred in the past, they were seldom documented in detail. Therefore, necessary inputs for outbreak simulations, such as the relative importance of alternative disease transmission pathways, are often lacking. In a situation of incomplete information, the consultation of experts is a possibility to complete the available data.

Different interview techniques for the elicitation of expert opinions have been used in the field of exotic animal diseases. Recently, Horst *et al.* (1996) introduced computer-based questionnaires using conjoint analysis and adaptive conjoint analysis (ACA) to estimate risk factors for disease introduction in a country. One advantage of these methods is that each expert expresses his/her opinion independently. This prevents psychological and sociological processes that influence a person's opinion in a group situation.

This paper describes the use of ACA in Switzerland for the quantification of risk factors responsible for between farm spread of classical swine fever (CSF).

3. Material and methods

Swiss experts with different backgrounds were invited to participate in either of two identical workshops. Experts were either researchers in veterinary virology (research group), federal veterinary office staff (administration group) or district veterinarians (field group). The directors of the research and administrational institutions involved selected expert candidates from among their staff after having been explained the objective of the experiment. The district veterinarians were invited to participate in the exercise during one of their regular training seminars.

Each workshop started with a short introduction to the subject and some technical explanations. The questionnaire used in the experiment was fully computer-supported. Each participant was provided with a personal computer to work independently from the others. Technical questions were answered individually. The questionnaire was designed using ACA software (Sawtooth Software, Evanston, IL).

The exercise consisted of two parts: 1) ACA task, and 2) evaluation of the ACA technique from the experts' point of view. In the ACA part, the participants were asked to estimate the relative importance of risk factors for the farm-to-farm transmission of CSF in Switzerland. ACA is based on the principles of conjoint analysis, which was originally used in marketing research for the elicitation of consumer preferences (Green and Srinivasan, 1990). The basic assumptions in conjoint analysis in the context of animal diseases are: 1) a risky situation (profile) can be described by the levels of a set of risk factors (attributes), and 2) a person's judgement over a situation is based on the levels of the risk factors (Horst *et al.*, 1996). Risk factors can have two levels: present or absent. The risk factors considered in the experiment were: animal trade between farms, animal trade to slaughter, visitors with pig contact, visitors without pig contact, livestock transport vehicles, swill feeding, airborne transmission, rodents or birds, wild boars.

First, the candidates were asked to rank the levels of each risk factor to assess the preferred level. Then, pairs of profiles each consisting of 2 or 3 risk factors with differences in one or several risk factor levels were compared and the preference of the candidate recorded. The program adapted the selected pairs according to earlier answers given by a candidate in order to maximise information gain while still limiting the number of combinations to be evaluated. All additional scores given by the candidate were used to update the original risk estimate for a risk factor using an iterative algorithm. In the end, the program designed a series of customised profiles for each candidate. These were progressing in preference from highly undesirable to highly desirable based on earlier answers of the candidate. The internal consistency of a candidate could thus be assessed. The relative importance of the different factors (utilities) were then calculated from the program output and re-scaled to add up to 100. For more technical details see Johnson (1993).

In the second part of the workshop, participants were asked to evaluate the ACA session, in which they had just participated, with respect to different criteria, such as technical feasibility of the technique or realism of profiles. A total of 7 questions were asked. Answers were given as scores ranging from 1 to 7.

The results from the ACA workshop were compared with field data from a recent CSF outbreak in Germany. The data included 121 farms infected with CSF during 1993-1995. The

source of virus introduction was assessed at CSF notification. The source of virus for each farm was classified in one of 5 categories. The category 'neighbourhood spread' was used when no specific source of infection could be identified and the farm was close to another infected farm. This category is a conglomerate of risk factors (for example rodents, airborne spread) acting over a short distance.

4. Results

A total of 33 experts participated in the exercise, 8 in the research group, 5 in the administration group and 20 in the field group. The results of one participant contained missing values and had to be excluded. Three participants had correlation coefficients for internal consistency <0.1 and were thus excluded from the analysis. The mean correlation coefficient for internal consistency of the remaining participants was 0.69 (SE=0.06). The results of the ACA are presented in TABLE 39. Animal trade between farms is perceived to be the most important risk factor for CSF transmission between farms by Swiss experts. Swill feeding is also assigned a high risk ranking, while wildlife is considered to be of very low importance. There was no significant difference between the different participant groups (Friedman 2-way ANOVA).

TABLE 39. Relative importance of risk factors for the transmission of classical swine fever within Switzerland as estimated by experts (n=29)[a]

Risk factor	Estimated relative importance	95% Confidence interval
Animal trade between farms	28.2	24.8-31.3
Swill feeding	17.6	11.4-19.1
Livestock transport vehicles	13.5	10.3-15.0
Visitors with animal contact	12.4	7.1-14.0
Animal trade for slaughter	6.6	2.7-9.8
Slurry vehicles	5.3	2.1-8.4
Wild boars	4.9	3.8-7.9
Visitors without animal contact	4.2	1.3-8.0
Rodents, birds	3.8	0.1-6.3
Airborne transmission	3.5	1.0-6.5
	100.00	

[a] 4 persons excluded due to inconsistent answers (correlation coefficient < 0.1)

The results from the ACA experiment were compared with the virus source of 121 farms infected with CSF during 1993-1995 in Germany (TABLE 40). The risk factors for transmission used in the ACA experiment were not identical to the source categories used in the field. This adds difficulty to the comparison. The experts considered animal trade to be the main source for virus introduction, which was consistent with observations in the field. If categories related to visitors and risk factors acting over short distances in the expert experiment are summarised (23.9%), they rank second - as in the field situation. However, swill feeding was

ranked considerably higher by the experts while wild boar infection was perceived to be relatively less important. The risk of livestock transport vehicles was judged similarly.

All experts were able to finish the computer-supported questionnaire, even though some of the participants had hardly any computer skills. The results of the evaluation of the ACA technique by experts are summarised in TABLE 41. Most participants found the workshop technically easy to complete, interesting and even entertaining. However, some had concerns with respect to the realism of the profiles created by the program. It seems to be important to keep the sessions short in order to keep the participants focused and motivated.

TABLE 40. Source of introduction of classical swine fever virus to 121 farms during the 1993-1995 outbreak in Germany

Risk factor	Number of farms	%
Animal movement	36	29.8
Visitors & Neighbourhood	35	28.9
Wild boar	22	18.2
Livestock transport vehicles	19	15.7
Swill feeding	9	7.4
	121[a]	100.0

[a]For 47 additional farms, the source of virus introduction was unknown.

TABLE 41. Evaluation of adaptive conjoint analysis workshop by participants (n=33)

Question asked:	Score categories (%)			
The computer-supported session was ... (Score)	1-2	3-5	6-7	Median
Interesting (1) – not interesting (7)	43.8	43.7	12.5	3.0
Realistic (1) – unrealistic (7)	25.0	50.0	25.0	4.0
Intellectually demanding (1) – not demanding (7)	9.4	59.4	31.2	5.0
Technically complicated (1) - simple (7)	9.4	40.6	50.0	5.5
Boring (1) – entertaining(7)	9.4	56.2	34.4	5.0
Too long (1) – not too long (7)	21.9	43.7	34.4	4.0

5. Discussion

The question of how exactly an expert in a specific field can be identified is difficult to answer. In this experiment, the objective was to include people who were dealing with CSF in their everyday working situation and who would have to make decisions in the case of a CSF outbreak in Switzerland.

The results of the workshops showed that the ACA technique seems to be a time-efficient and technically appealing technique for the elicitation of expert opinions in the field of exotic animal diseases. It was generally well received by the participants, although some experts questioned the realism of the profiles created by the ACA software. This issue should be ad-

dressed in the design of future questionnaires. Inconsistent answers of participants may be due to a lack of understanding of the program principles or due to a lack of motivation. By keeping the sessions short and by providing incentives to the participants, the impact of this problem should be reduced.

The use of expert opinion is very common in the development of expert systems. In the course of knowledge acquisition, usually some sort of structured interview is used. However, it can be difficult to obtain reliable quantitative information. Some interview techniques such as ACA allow the quantification of expert opinion using regression principles. Inconsistent experts can be identified and excluded from the final analysis. However, the outcome of the exercise will still be the assessment of an expert (but subjective) opinion, which by definition is neither true nor false and therefore - strictly speaking - cannot be validated. Nevertheless, we compared the results of the workshop with field data in order to further investigate our results. The field data used came from a region in Germany that did not have exactly the same production structure as the pig industry in Switzerland. Therefore, some risk factors such as visitors or feeding practices may be different. Also, the definition of the virus source categories was not congruent. When discussing expert opinion, a potential bias by recent experience or general policy has also to be considered. However, the ranking of the risk factors according to expert opinion in this experiment was relatively similar to the field results except that the third and the fifth ranks were swapped. Yet, a relatively large proportion of field outbreaks (28%) was not classified with respect to the virus source. Whether these farms would have been evenly distributed over all categories – as currently assumed – is unknown.

In conclusion, the use of quantitative techniques such as ACA for the elicitation of expert opinion is a possible alternative to direct use of field data in a situation where the latter is not available. Technical concerns in terms of the validity of estimates are inherent to the subjective nature of expert opinions and will have to be addressed on a case-by-case basis. The results of the CSF risk factor experiment are considered appropriate for use in a decision support system for the management of CSF outbreaks (EpiMAN-SF; Stärk *et al.*, 1996).

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References

Green, P.E., and Srinivasan, V. (1990). Conjoint analysis in marketing: New developments with implications for research and practice. Journal in Marketing October 1990, 3-19.

Horst, H.S., Dijkhuizen, A.A., Huirne, R.B.M., and Steenkamp, J-B.E.M. (1996). Eliciting expert opinions on risk factors concerning introduction of virus: application of conjoint analysis. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Glasgow 8-17.

Johnson, R.M. (1993). Adaptive Conjoint Analysis - version 4. Technical Paper. Sawtooth Software Inc.

Morris, R.S., Sanson, R.L., McKenzie, J.S., and Marsh W.E. (1993). Decision support systems in animal health. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Exeter 188-199.

Stärk, K.D.C., Morris, R.S., and Sanson, R.L. (1996). EpiMAN-SF – development of a decision support system for managing a swine fever epidemic. Proceedings 14th IPVS Congress, Bologna 534.

CHAPTER 2.4

COMPARISON OF ELECTRONIC AND VISUAL IDENTIFICATION SYSTEMS IN PIGS

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1. Abstract

Internal and external electronic identification devices (EID) were compared with visual ear tags in a series of trials in pigs. In sows (244 animals), external EID tags and visual tags performed comparatively well with loss rates of 1.6% and 3.7%, respectively, over - on average - 338 days (SD=168 days). External EID technical failure rate was 0.4%. In two other trials, 180 piglets were tagged at weaning with external EID, visual tags and implantable EID (type A: 23 mm; type B: 11.5 mm). A location at the right ear base was used for implantation. Only minor migration of < 1 cm occurred. A total of 3.3% of pigs showed signs of infection at the implantation site (all with type A). The loss rate of type A and type B transponders within 4 weeks after implantation was 18.1% and 0%, respectively. Loss rates before slaughter for the different identification systems were: EID ear tags 0%, visual ear tags 1.7%, implant A 19.4%, implant B 0%. At slaughter, 23.4% of the EID ear tags were either lost or damaged. Remaining technical problems related to practical use of EID systems will have to be removed before their value can be fully realised within the pig industry.

2. Introduction

Individual animal identification is an important prerequisite of modern management practices at different levels within the pig production industry. At the farm level, computer-supported applications for breeding and selection, sow management, and in particular sow feeding and weighing of fattening pigs have been developed over recent years (Huiskes, 1991; Blair *et al.*, 1994).

Within the pig industry, individual animal identification is becoming increasingly more important with respect to health and quality assurance systems (Augsburg, 1990). Integrated production chain quality monitoring is expanding from the abattoir towards the farm, forming pre-harvest food quality assurance schemes. These schemes are linking slaughterhouse information back to the farm of origin (Leese, 1993; Klindtworth, 1995).

At a national level, tracing of farms and individual animals during disease emergencies rely on farm and animal identification. Assessing the animals and herds at risk at any point in time requires a powerful identification and registration system on a national basis (Saatkamp *et al.*, 1996). Animal health surveillance in general also requires reliable animal identification in order to improve the value of the collected data (Bridgewater, 1990). Animal identification systems can hence serve individual producers, the industry and the public.

Current systems in use consist of visual ear tags, back or ear tattoos and notching or punching of the auricles (van Houwelingen, 1991). All these systems have serious performance problems. Ear tags can be lost, tattoos fade and ear notches are difficult to read. When identification is lost or misread this can cause severe losses to individual pig farmers or in the case of an exotic disease outbreak for a complete production sector.

For these reasons, new technologies have been developed over recent years. Among these are three types of devices that can be electronically read:

1) Injectable transponders (Baldwin *et al.*, 1974; Dorn, 1987). These systems consist of a subcutaneous bioglass device (passive transponder) containing a data element (chip) for data storage and of a sender/receiver unit (reader) for the activation and transmission of the data. The minimum reading distance depends on the size of the implant and the strength of the electromagnetic field generated by the reader.

2) Transponders integrated in ear tags. The technology is identical to that of the injectable implant.

3) Bar-coded ear tags. This technology is widely used in non-agricultural applications. The information is coded in a series of bars of variable width. With the help of a laser reader the information can be retrieved. Ear tags with bar codes for animal identification are now commercially available.

These new systems have to meet certain standards in order to make them attractive and acceptable to the industry. These requirements are: a) easy to apply; b) reliable and quick reading, resistant to damage or fraud; c) not hazardous to animal health and animal products, safe and quick retrieval, and d) reasonably priced. However, it has proved difficult to me*et al.*l of these requirements in a single device.

The objective of the trials presented in this article were to compare different electronic identification (EID) systems with visual ear tags and to discuss the potential of these systems with respect to the different applications described above.

3. Methods

3.1 Sow trial

This trial was conducted on a commercial pig-breeding herd with an average herd size of 190 sows. A total of 244 sows were tagged between June 21, 1995 and March 14, 1996 (107 Landrace, 137 Large White). Two types of ear tags were used. A conventional visual ear tag was applied into the sows' right ear using a tag applicator provided by the manufacturer. The stem length of the male tag was 15 mm. In the right ear, an EID ear tag was applied. This tag contained a sealed-in transponder device which was read with help of an electronic reader (700 mm antenna, 134.2 kHz). The reading distance according to the manufacturer was 40-50 cm. Two types of male parts with different stem lengths (15 mm and 18 mm) were used for the EID tag. Sows were assigned short and long stem tags in a systematic manner. The first 10 sows were tagged with short stem tags and the following 10 with long stem tags. The sequence of tagging was determined by the sow's location in the building. Then again the short stem tags were used. All tags were applied by the farmer using a commercially available applicator.

The sows were kept in pens and crates and were not moved or restrained for tagging. Regular readings of all tags were performed monthly. The accuracy of electronic readings was recorded. When sows were sold or culled, all ear tags were removed. All tags were visually identifiable in order to make it possible to trace them back to individual sows when lost and

found. When tags were lost or removed the date and the reason for removal or failure was recorded.

3.2 Piglet trials

These trials were conducted at the Massey University Research Piggery, which has an average herd size of 65 sows. One day after weaning, before the piglets were moved from the farrowing room on to the flat decks, the different identification systems used in this trial were applied. Piglets were then between 21 and 28 days old. Pigs were subsequently moved to a wire-floor flatdeck and from there into solid floor grower rooms and eventually to stalls with outdoor pens where they stayed until slaughter at approximately 64 kg body weight. All pigs were fed *ad libitum.*

The tagging scheme was identical to that for the sows described above except that a lightweight version EID ear tag was used (FIGURE 22). Additionally, the piglets were also identified by implantable transponders. Two products were used in the trial. Product A was 23 mm long and applied using a cartridge holding 10 implants stored in iodine gel. The multiple-use needle was not disinfected between injections. This implant was read using the same reader as for the EID ear tags. Product B was 11.5 mm long and implanted using single-use needles and a pen applicator. This implant was read using a hand-held reader (antenna diameter 252 mm, 128 kHz). According to the manufacturer the typical reading distance with this product is 16-20 cm. The implantation site was near the right ear base (FIGURE 23) as suggested by Lambooij (1992). The piglets were picked up and restrained by a technician while tags and implants were applied by the principle investigator who is a trained veterinarian but had no prior experience in implanting electronic transponders.

3.2.1 Sub-trial A

A total of 100 piglets (11 litters) were tagged using visual ear tags, electronic ear tags and implant A. After tagging, regular readings were taken at weekly intervals for the first 8 weeks, and then monthly. If an implant could not be read, the pig was restrained and the assumed location of the implant palpated. Pigs were followed to the slaughterhouse and all implants recovered after scalding. Abattoir staff removed ears and external acoustic canals. If the implant was not removed by these routine cuts, it was located through palpation and removed by a project assistant.

3.2.2 Sub-trial B

A total of 80 piglets were tagged using visual tags and electronic ear tags. Both implants A and B were used. Pigs were assigned to either one of the implant products using systematic sampling with every second pig receiving the same type of implant. The implanting technique and location were the same as described above.

After tagging the location of the dorsal end of the implant was marked by a tattooed dot. During the first 14 days after tagging pigs were checked daily. They were picked up, the tags read and the implantation site monitored for wound healing and migration. The distance between the current location of the dorsal end of the implant and the tattooed initial location was

measured in mm. After 14 days the pigs were checked weekly and all tags retrieved as in Subtrial A.

3.3 Data handling and analysis

Data was stored using a computer software for data management (Microsoft Access V. 7.0, Microsoft Corporation, Redmond, USA). Statistical comparisons between groups were conducted using the χ^2 test (Epi Info V.6.04; Dean *et al.*, 1994). To explore possible risk factors for lesions related to ear tags, Cox proportional hazard analysis was used. As explanatory variables, the age and breed of the sow, the number of days that the tag was in place, and the stem length of the ear tag were included in the model. Survival analysis was performed using the statistical package NCSS V.6.0.21 (Number Cruncher Statistical Systems, Kaysville, USA).

4. Results

4.1 Sow trial

Tags were monitored until November 20, 1996. During this time period, 116 animals were culled or sold. The average duration of a sow in the trial was 338 days (min. 1, max. 524, SD 168 days). The reading of all tags took approximately 45 minutes.

Four EID tags (1.6% [95% C.I. 0.0-3.2]) and 9 visual tags (3.7% [95% C.I. 1.3-6.1]) did get lost during the trial 111-330 days and 120-518 after application, respectively. Two sows lost both tags. One EID tag stopped transmitting 7 days after application. This tag remained *in situ* until the sow was culled. All other EID tags were accurately reading on all instances. Technical failure occurred thus in 0.4% [95% C.I. 0.0-1.2] of EID ear tags.

After the first tags had been applied for approximately 10 months, it was discovered that 18.9% of all sows still in the trial had developed an adverse tissue reaction at the tag application site. This reaction visually consisted of a tissue swelling and proliferation, sometimes accompanied by tissue damage and bleeding (FIGURE 24). As from April 18, 1996, these lesions were monitored monthly. Survival curves were generated for the 158 EID tags and the 137 visual tags that had not caused lesions at the beginning of the monitoring period. The graph showed that with both tags lesions were unlikely to occur before approximately 150 days after application. The mean time until development of the lesions was 318 days (min. 98, may. 454, SD 109) for visual tags and 279 days (min. 63, max. 496, SD 122) for EID tags. Later, visual tags were distinctly more likely to induce lesions, with a survival rate of only 75% at the end of the trial (524 days). Breed seemed to be a risk factor for lesion development in EID tags, but not in visual tags. When comparing Large White vs. Landrace, an OR of 0.337 [95% C.I. 0.156-0.732] was calculated. There was no influence of stem length or age.

FIGURE 22. Piglets with electronic ear tag (left ear) and visual ear tag (right ear)

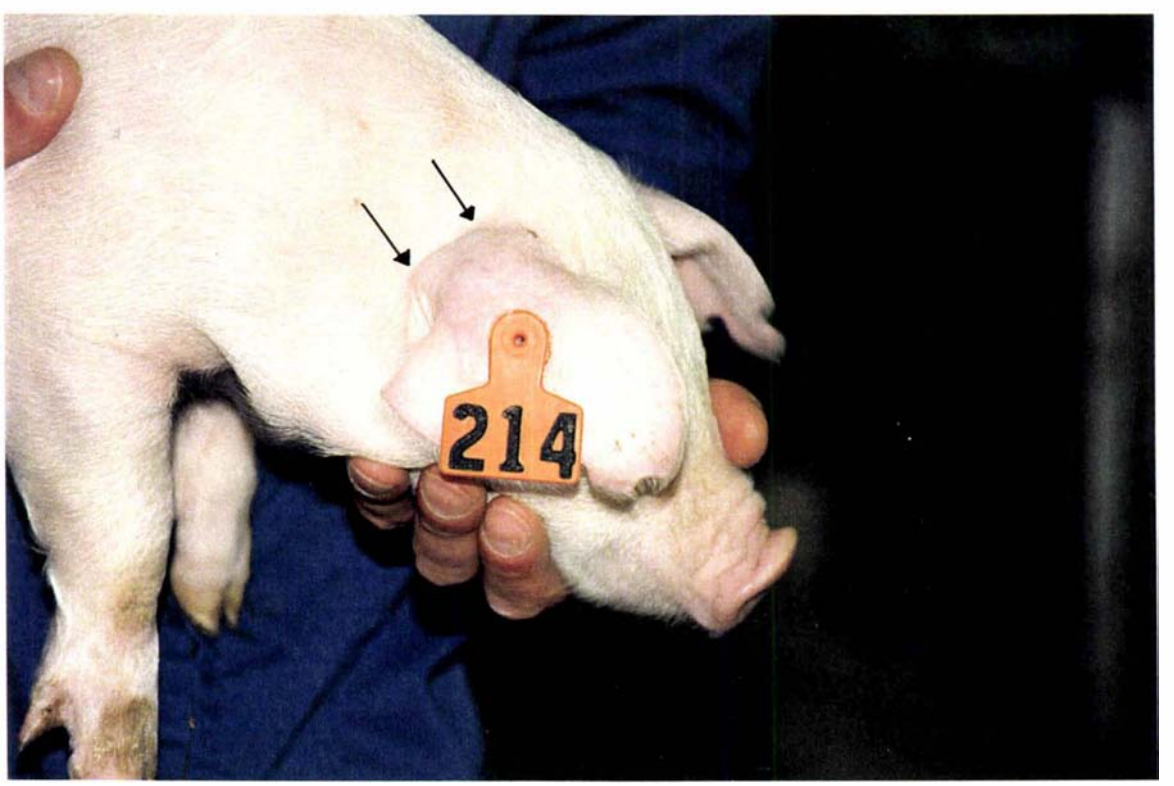

FIGURE 23. Implantation site (arrows) at right ear base

4.2 Piglet trials

Out of 180 piglets 12 were used in feeding trials and killed on the farm (implant A: 3; B: 9). Three pigs died during the trial due to unrelated reasons (implant A: 1, B: 2).

Tag application required 2 persons, one holding the pig and one applying the tags. As for the implants, the application with the single shot application pen was easier as it was smaller and therefore handier to manipulate than the multi-shot pistol. However, the use of the single shot needles was more time consuming. Problems occurring during application included: perforation of the skin (3, applicator A), extraction of chip when removing needle (2, applicator B). These events required a repeated implantation at the same site, but not using the same implantation canal.

4.2.1 Sub-trial A

The average time of pigs in the trial (tagging until death/slaughter) was 109 days per animal (min. 17, max. 145). Signs of infection associated with the implanted chip were observed in 4 animals (4% [95% C.I. 0.2-7.8]), 3 of which subsequently lost their implants. In total, 20 (20% [95% C.I. 12.2-27.8]) implants were lost before slaughter, with the highest risk of loss between week two and four. The probability for an implant to remain in place for at least 4 weeks was 0.80. In 6 animals signs of an exit canal were observed ventrally at the ear base. During the monitoring period, all implants read promptly and accurately.

Out of the 96 pigs killed at the abattoir (4 died on the farm or during transport), 76 (79.2%) still had working implants (TABLE 42). One implant was found crushed after scalding. This results in an overall proportion of 73.4% (58/79) readable implants after slaughter.

None of the EID tags or the visual tags was lost during the rearing and fattening period. All EID tags were read promptly and accurately at all times during the trial. Eight of the 70 tags retrieved at slaughter (11.4%) were lost during scalding and 10 tags (14.3%) were in place but did not work after scalding. Fifty-two EID tags (74.3%) remained in place and functioning after scalding (TABLE 42). No visual tags were lost at slaughter. However, visual tags could not be read on several occasions during the fattening period because the numbers were covered with dried dirt. None of the visual tags was damaged by chewing. The study farm has no history of tail biting.

The comparison of the performance of the different identification systems at several stages of the trial revealed significant differences between external and internal tags before slaughter and visual and electronic systems after slaughter (TABLE 42).

4.2.2 Sub-trial B

The average duration in the trial was 113 days (min. 6, max. 147). Implants were monitored for healing and migration during 10 and 14 days, respectively. Healing was faster when applicator B was used. Also, less swelling and fewer signs of infection were observed when using product B (data not shown).

TABLE 42. Performance of pig identification tags as percentage tags in place and working at different trial stages (actual numbers in brackets).

Tag type	4-week %	[95% C.I.[a]]	Before slaughter %	[95% C.I.]	After slaughter %	[95% C.I.]
Sub-trial A[b]						
Visual tag	100 (98/98)		100 (96/96)		100 (70/70)	
EID tag	100 (98/98)		100 (96/96)		74.3[c] (52/70)	[64.1-84.5]
Implant A	79.6[c] (78/98)	[71.6-87.6]	79.2[c] (76/96)	[71.1-87.3]	73.4[c] (58/79)	[63.7-83.1]
Sub-trial B[d]						
Visual tag	100 (79/79)		96.1 (73/76)	[91.7-100.0]	92.5 (62/67)	[86.2-98.8]
EID tag	100 (79/79)		100 (76/76)		79.1[c] (53/67)	[69.4-88.8]
Implant A	87.5[c] (35/40)	[77.3-97.7]	84.2[c] (32/38)	[72.6-95.8]	84.8[c] (28/34)	[72.5-97.1]
Implant B	100 (39/39)		100 (38/38)		100 (34/34)	
Combined Trials						
Visual tag	100 (177/177)		98.3 (169/172)	[96.3-100.0]	96.4 (132/137)	[91.9-100.0]
EID tag	100 (177/177)		100 (172/172)		76.6[e] (105/137)	[69.5-83.7]
Implant A	81.9[e] (113/138)	[75.3-88.5]	80.6[e] (108/134)	[73.9-87.3]	76.1[e] (86/113)	[68.2-84.0]

[a]C.I.= confidence interval
[b]2 pigs killed on farm, 26 pigs lost at abattoir
[c]$p<0.05$ when compared with 100%
[d]7 pigs killed on farm, 2 pigs lost at abattoir
[e]$p<0.05$ when compared with visual tag

The comparison of the two implanted chips in terms of migration is shown in FIGURE 25. The mean migration distance was larger for the smaller implant (Implant B). After day 5 the location of implant A stabilised until pigs were moved to the flat deck (day 13). Similar to implant B, implant A started to migrate again when the pigs were moved to the flat deck. The larger implants A were easily palpable during the whole monitoring period. Three implants B (7.7%) could not be palpated 14 days after application.

Fewer implants were lost during this trial when compared with sub-trial A. One implant A was lost through the application canal on day 1 after application and another one on day 9 after implantation, both without any signs of infection. However, the exit wound in the second pig was some distance from the scar of the implantation needle. One pig lost the implant on day 12 after having had severe infection of the implantation canal since day 1 after implantation. One other implant A was lost on day 20 with signs of an exit canal ventrally at the ear base. In total 5 implants A were lost, out of which only one was lost after week 4. This pig, that lost implant A during week 7, had had an abscess since week 5 at the implant site. This eventually broke open and the implant was lost. One implant A stopped responding on day 2 after application. It was left in place until slaughter. All other implants could be read accurately at all times. None of the implants B were lost during the trial. None of the implants of either type suffered any damage at slaughter.

None of the EID were lost or damaged during the lifetime of the pigs. At slaughter, 7 tags (10.4%) were lost and 7 (10.4%) did not respond to the reader after scalding. This reduced the percentage of working EID tags after slaughter to 79.1% (TABLE 42). During the trial, 3 visual tags were lost on the farm. The remaining tags were sometimes not readable because of

dirt. This was not recorded in detail. At slaughter 2 more visual tags were lost after scalding. Thus 92.5% of the tags remained in place after slaughter.

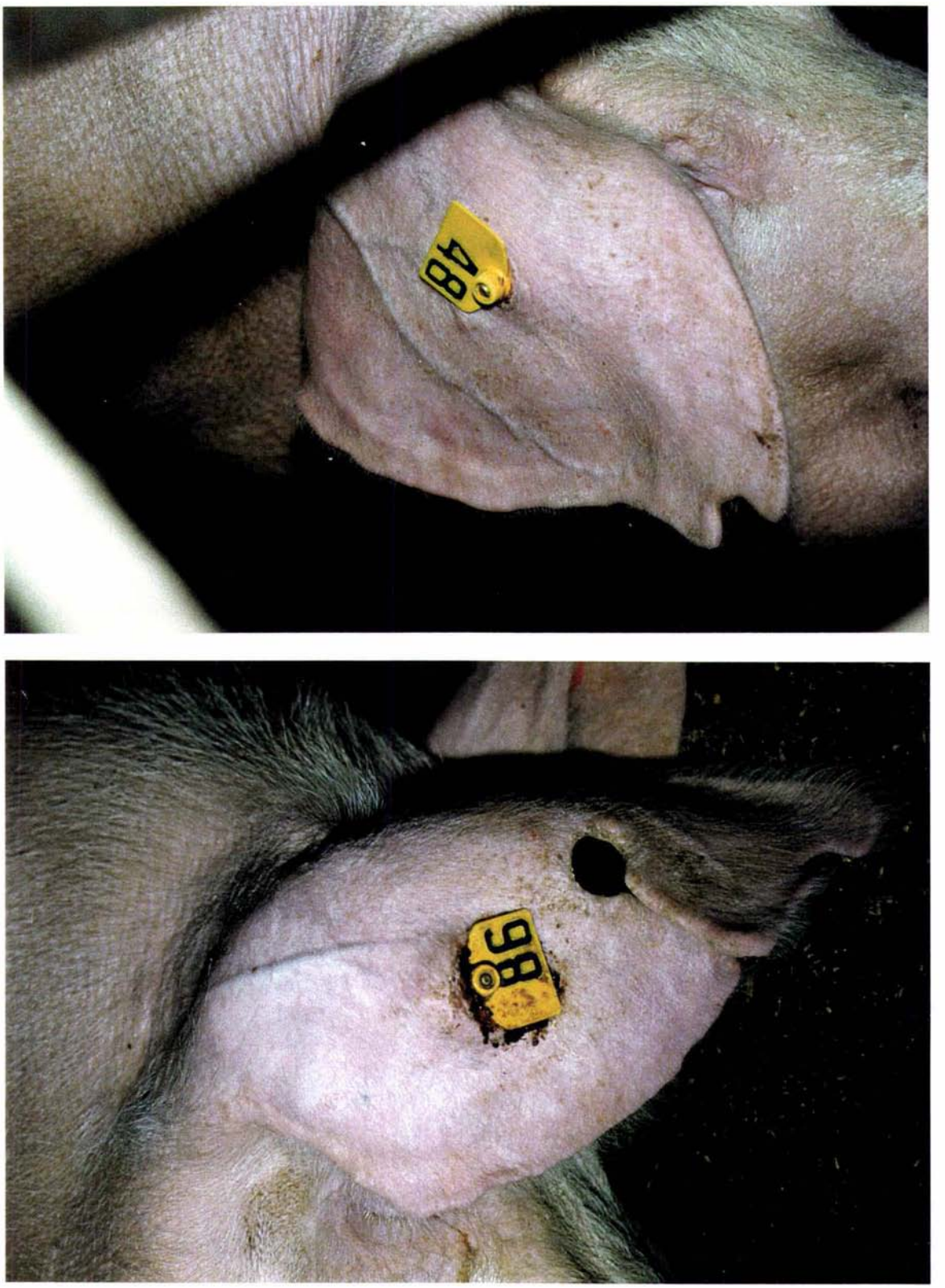

FIGURE 24. Lesions observed with visual ear tags: a) minor lesion, b) major lesion

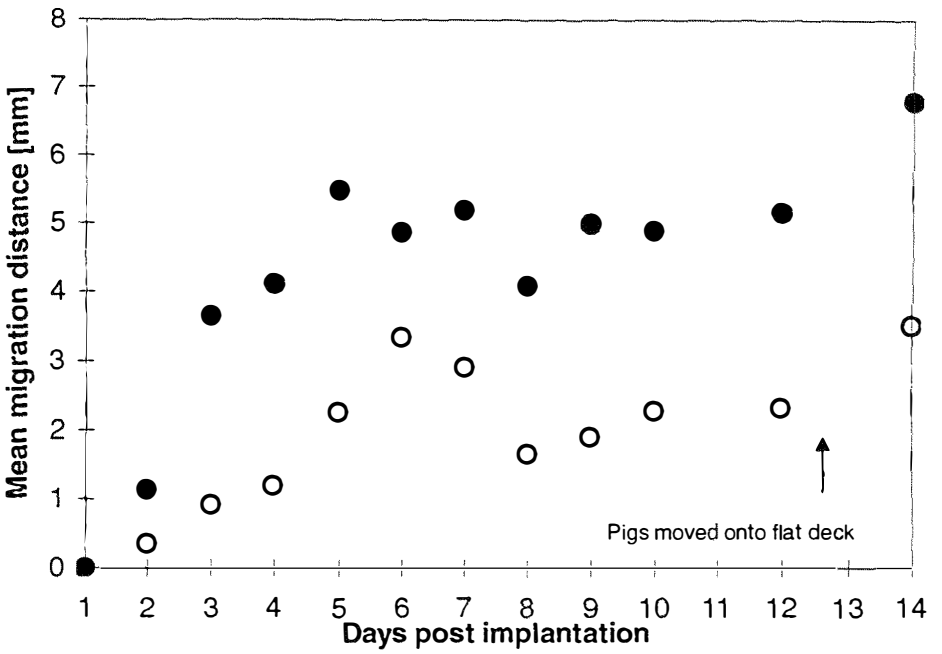

FIGURE 25. Comparison of mean migration distance of two different injectable electronic identification transponders (○ implant A, ● implant B)

The comparison between the tags demonstrates a significant difference between the implant products A and B at all stages of the trial. After slaughter there was also a significant difference between the EID tags and implant B but not between the two external tags and also not between the visual tag and implant B. Results from the two sub-trials were then pooled (TABLE 42).

The influence of the stem length of EID tags on the survival of tags at the abattoir was evaluated by pooling the result from the sub-trials. From 80 short-stem tags, 13 (16.3% [95% C.I. 8.2-24.4]) were either lost (6) or recovered damaged (7) at slaughter. From 57 long-stem tags, there were 19 (33.3% [95% C.I. 21.1-45.5) in this category (9 lost, 10 damaged). Thus the risk of damage or loss at the abattoir was 2.6 times as high for long-stem tags as for short-stem tags (odds ratio 2.6; 95% C.I. 1.1-5.8).

5. Discussion

The results of this study indicate that there are still technical problems associated with the use of both internal and external electronic identification systems. These problems are related to the following areas: 1) application, 2) loss rate and performance on farm, 3) retrieval problems at slaughter.

5.1 Application of internal EID

In this trial, pigs were tagged at weaning because implanting in pigs younger than four weeks is considered difficult (Lammers *et al.*, 1995). Problems encountered during application were similar to other studies (Lammers *et al.*, 1995; Lambooij *et al.*, 1995). The use of a small single-shot applicator was advantageous but more time consuming because a new needle had to be screwed on for each application. Implantation of transponders requires two people, as the animal has to be restrained. Acknowledging these problems, implanting by the farmer may be possible (Niggemeyer, 1994), but training will be necessary in order to increase speed and application accuracy (Ribó *et al.*, 1994).

The risk of inflammation after implantation in this study was smaller than in other reports, where up to 40% of the pigs showed signs of inflammation at one week after implantation (Janssens *et al.*, 1996). Also, a relapse in the occurrence of inflammation at 10-14 days after implantation as described by Lambooij *et al.* (1992) was not observed. Our results were comparable to some trials that reported inflammation in about 1-2% of the pigs (Aarts *et al.*, 1991; Aarts *et al.*, 1992). The size of the implant and the type of applicator used influenced the healing process. The multiple use of an applicator without specific cleaning between applications may lead to contamination of the implantation site with organic material (data not shown; also: Janssens *et al.*, 1996). This may result in the development of abscesses (Lambooij *et al.*, 1992). However, disinfecting the skin and/or of the implanting device does not seem to necessarily reduce the occurrence of infections (Janssens *et al.*, 1996; Lambooij *et al.*, 1995).

5.2 Loss rate and performance on farm

Figures on the proportion of retained transponders from other studies have been collated in TABLE 43. In our trial, the loss rate of implanted transponders was up to 20% in the first 4 weeks after application for implant A. This is different from other trials where most implants were lost soon after implantation. Furthermore, we observed lesions that looked like exit canals located ventrally at the ear base. A possible explanation may be that the larger transponders generate tension on the skin that may lead to necrosis and subsequent loss of the implant. In 5 cases (20%) the loss was likely to be linked with inflammatory processes. An increased loss after infection has been reported in the literature. Janssens *et al.* (1996) calculated a more than 50-fold increase of the risk with increasing severity of infection. The results indicate that smaller implants may be better suited for this site when applied at a young age.

The readability of both internal and external electronic devices was very high in this trial. Out of a total of 604 tags and implants, only two stopped responding on the farm, while all other devices read correctly at all readings. The reading distance was according to the manufacturers' specification. However, the 16-20 mm with implant B (small implant) and reader B were too short for pigs in a group-housing situation. Although the readability of the visual tags was not specifically recorded, it was observed on several instances that visual tags were dirt-covered and could not be read. The reading efficiency was not recorded in this study. Yet clearly, in a group-housing situation, the reading of electronic tags is faster, particularly when a reader with a long antenna can be used.

In sows, problems occurred with both visual ear tags and EID tags after long-term use. The reaction of the tissue seems to be proliferative in its nature and possibly due to trauma or pressure. This is a new finding, which is not yet fully understood.

TABLE 43. Summary of results from different electronic identification trials in pigs

#Animals	Implant size	Age at implantation	Trial duration	% loss	Source
EID Implants					
3125	30 mm	3-5 weeks	until slaughter	10	Aarts *et al.*, 1991
149	18 mm	4 weeks	until slaughter	3.8 (Av.)	Lambooij, 1992
3200 3500 5800	?	at weaning	until slaughter	11.6[a] 4.9[a] 5.7[a]	Aarts *et al.*, 1992
20	11 mm	14-21 days	165 d	5.2	Scheidegger and Leu, 1994
16	11 mm	14-21 days	154 d	12.5	Scheidegger and Leu, 1994
>10.000	28 mm	3-4 weeks	until slaughter	1	Niggemeyer, 1994
300	19 mm	3-4 weeks	until slaughter	2	Niggemeyer, 1994
69 111 24	18 mm	10 days 4 weeks 6 months	until slaughter	4.3 0.9 8.3	Lammers *et al.*, 1995
3483 2954 2946	30mm	4-5 weeks	until slaughter	6.9[a] 6.4[a] 1.6[a]	Lambooij *et al.*, 1995
296	40 mm	25-80 kg	until slaughter	9.12	Janssens *et al.*, 1996
140	23 mm	3-4 weeks	until slaughter	23.9	This study
40	11.5 mm	3-4 weeks	until slaughter	0	
EID Ear tags					
650		3-4 weeks	until slaughter	5	Niggemeyer, 1994
270 128 329 275		20-25 kg pregnant sows 4-9 weeks 4-9 weeks	100 days 183 days[b] before slaughter after slaughter	1.7 4.8 6.4 26.6	Teixidor Pagés *et al.*, 1995
180		3-4 weeks	after slaughter	23.4	This study
244		sows	before slaughter	1.6	

[a] including technical losses
[b] average

5.3 Problems at slaughter

If electronic implants are used for pig identification, they must be removed before the carcass enters the human food chain. Difficulties result from non-specific application sites or from migration. In one of the sub-trials in this study, migration was assessed. The maximum migration distance for small implants was below 1 cm during the first two weeks after implanta-

tion, and implant recovery was not a problem in this study. In earlier studies using the same location of the implant, the migration proportion was 2-16% (Niggemeyer, 1994; Lambooij *et al.*, 1995). When different locations were used, up to 34% of the implants was found outside the target area (Aarts *et al.*, 1991).

Electronic ear tags were lost or damaged in a high percentage of animals through the mechanical and thermal treatment of pig carcasses after stunning. This was also observed in other studies (TABLE 43) and attributed to possible damage by electro-shock at stunning, high humidity and high temperatures during scalding and burning or mechanical damage at de-hairing (Teixidor Pagés *et al.*, 1995). In this study, there appeared to be a higher risk of loss with tags that had a longer stem length, because these possibly get caught between mechanical parts more easily.

6. Conclusions

At this stage technical problems with both internal and external electronic identification devices are not fully resolved. The target value of a maximum non-readability of 1% for The Netherlands (Merks *et al.*, 1990) and 2% for the U.S.A. (Dukas, 1990) are not being achieved. The need for smaller implants in order to prevent loss and yet maintain sufficient reading distance (at least 30 cm) seems difficult to be resolved in the near future. These issues have to be rigorously addressed by the manufacturing industry in order to achieve performance equivalent to conventional ear tags.

Nevertheless, electronic identification systems have great potential for process automation and particularly data management on the farm, at the abattoir and in emergency situations such as epidemics. The option to use the transponder for monitoring additional body variables, such as temperature, through passive telemetry will help justify the significant investment (Baldwin *et al.*, 1974; Petersen *et al.*, 1994; Geers *et al.*, 1997; Saatkamp *et al.*, 1997).

At this stage electronic ear tags have the advantage that they are easier to use and more flexible because they also provide non-electronic identification. Also the option of recycling ear tags may be interesting in order to reduce costs (Niggemeyer, 1994; Teixidor Pagés, 1995).

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References

Aarts, H., Huiskes, J., Langeveld, N., Lambooij, B., and Den Hartog, L. (1991). Injectable transponders for identifying pigs. Praktijkonderzoek Varkenshouderij 5:15-19.

Aarts, H.L M., Langeveld, N.G., Lambooij, E., and Huiskes, J.H. (1992). Injectable transponders in pig production: applications and field trial. Proceedings of the 12th IPVS Congress, The Hague, 562.

Augsburg, J.K. (1990). The benefits of animal identification for food safety. Journal of Animal Science 68: 880-883.

Baldwin, H.A., Depp, S.W., Koelle, A.R., and Freyman, R.W. (1974). Electronic animal identification and temperature monitoring. Proceedings of the 77th Annual Meetting of the US Animal Health Association 77:141-152.

Blair, R.M., Nichols, D.A., and Davis, D.L. (1994). Electronic animal identification for controlling feed delivery and detecting estrus in gilts and sows in outside pens. Journal of Animal Science 72: 891-898.

Bridgewater, D.R. (1990). The need for future animal identification for surveillance. Proceedings of the 94th Annual Meeting of the US Animal Health Association 94:43-46.

Dean, A.G., Dean, J.A., Coulombier, D., Bendel, K.A., Smith, D.C., Burton, A.H., Dicker, R.C., Sullivan, K., Fagan, R.F. and Arner, T.G. (1994). Epi Info: Version 6.04: a word processing, database, and statistics program for epidemiology on microcomputers. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

Dorn, H.-J. (1987). Vorstellung des EURO I.D. Systems zur Kennzeichnung von Tieren mit Mikrochips in Transpondern. Tierärztliche Umschau 42:978-981.

Dukas, P. (1990). Electronic identification guidelines for implantable transponders. Proceedings of the 94th Annual Meeting of the US Animal Health Association 94: 46-52.

Geers, R., Puers, B., Goedseels, V., and Wouters, P. (1997). Electronic identification and tracking in animals. CAB International, Oxon, U.K.

Huiskes, J.H. (1991). The use of electronic identification in breeding and fattening management of pigs. In: E. Lambooij (Editor), Automatic Electronic Identification Systems for Farm Animals, EUR Report No. 13198, 68-72.

Janssens, S., Rocha, L.A., Bosschaerts, L., Barbosa, M.A., Puers, R., Villé, H., and Geers, R. (1996). Implant recovery and tissue reaction in growing pigs following implantation of packaging materials for injectable electronic identification and monitoring devices. Preventive Veterinary Medicine 25:249-258.

Klindtworth, M. (1995). Transponders - injectates in the slaughtering chain. Fleischwirtschaft 75:153-154.

Lambooij, E. (1992). Positioning of identification transponders in the auricle of pigs. Veterinary Record 131:419-420.

Lambooij, E., de Groot, P.H.S., Molenbeek, R.F., and Gruys, E. (1992). Subcutaneous tissue reaction to polyethylene terephtalate covered electronic identification transponders in pigs. Veterinary Quarterly 14:145-147.

Lambooij, E., Langeveld, N.G., Lammers, G.H., and Huiskes, J.H. (1995). Electronic identification with injectable transponders in pig production: Results of a field trial on commercial farms and slaughterhouses concerning injectability and retrievability. Veterinary Quarterly 17:118-123.

Lammers, G.H., Langeveld, N.G., Lambooij, E., and Gruys, E. (1995). Effects of injecting electronic transponders into the auricle of pigs. Veterinary Record 136:606-609.

Leese, W. F. (1993). Animal identification and traceback for residue control programs. Livestock Conservation Institute Proceddings of the Annual Meeting, St. Louis, Missouri, USA 81-84.

Merks, J.W.M., and Lambooij, E. (1990). Injectable electronic identification systems in pig production. Pig News & Information 11:35-36.

Niggemeyer, H. (1994). Injizierte Transponder in der Schweinehaltung. Deutsche Geflügelwirtschaft und Schweineproduktion 27:21-23.

Petersen, B. and Rosendaal, E.A.M. (1994). The latest development on micro-sensors to control animal health and welfare. Fleischwirtschaft 74:1246-1248.

Ribó, O., Caja, G., and Nehring, R. (1994). A note on a field trial implantation comparing trained and untrained operators. In: Commission of the European Communities, General Direction of Agriculture DG VI (Editor), Electronic identification of farm animals using implantable transponders (final report).

Saatkamp, H.W., Dijkhuizen, A.A., Geers, R., Huirne, R.B.M., Noordhuizen, J.P.T.M., and Goedseels, V.

(1996). Simulation studies on the epidemiological impact of national identification and recording systems on the control of classical swine fever in Belgium. Preventive Veterinary Medicine 26:119-132.

Saatkamp, H.W., Dijkhuizen, A.A., Geers, R., Huirne, R.B.M., Noordhuizen, J.P.T.M., and Goedseels, V. (1997). Economic evaluation of national identification and recording systems for pigs in Belgium. Preventive Veterinary Medicine 30:121-135.

Scheidegger, R. and Leu, W. (1994). Versuch Implantation von Datamars-Transponder bei Schweinen 1992-1993. Unpublished Manuscript, Schweiz. Beratungs- und Gesundheitsdienst in der Schweinehaltung, Bern, Switzerland.

Teixidor Pagés, H., Soler Soler, J., and Tibau Font, J. (1995). Test de un sistema electronico de identificacion animal. Anaporc No. 148:131-139.

van Houwelingen, P. (1991). Review of existing identification systems. In: E. Lambooij (ed.), Automatic Electronic Identification Systems for Farm Animals EUR Report No. 13198, 7-12.

CHAPTER 2.5

QUANTIFICATION OF CONTACTS BETWEEN PIG FARMS TO ASSESS THE POTENTIAL RISK OF CLASSICAL SWINE FEVER TRANSMISSION

Parts of this chapter will be published in the *Proceedings of the 15th International Pig Veterinary Society Congress, Birmingham* in a paper by: Stärk, K.D.C., Nielen, M., and Morris, R.S. (1998) Setting priorities for investigating movement traces during a classical swine fever outbreak.

1. Introduction

The most likely way of classical swine fever (CSF) transmission between pig farms is via animal trade, vehicles or people contacts (Terpstra, 1991). The epidemiological investigation of a CSF outbreak therefore includes the recording of all contacts from and to a farm before the point of diagnosis. All contacts are followed and confirmed (traced) in order to 1) identify the origin of the infection (tracing back) and 2) to find farms to which the disease may have spread (tracing on). Experience shows that the number of contacts to be traced during an epidemic may be very large (Roger Morris, personal communication). The larger the number of contacts becomes the more crucial will it be to assign priority to high-risk contacts so that they can be traced first.

An expert system has been developed to assist tracing officers during a foot-and-mouth disease (FMD) epidemic (Sanson, 1993), and a similar system is being designed for swine fever outbreaks (Stärk *et al.*, 1996). These expert systems classify traces in terms of risk of disease transmission according to a set of decision rules. The rules are designed such that they can reliably identify high-risk contacts (high sensitivity) and yet limit the number of priority traces to a workable number (high specificity).

Additionally, the number of contacts having occurred after an index farm became infected but before the disease was diagnosed also influences the size of the epidemic in a region. The more contacts, the higher the number of possible secondary outbreaks may be. When simulating the development of an epidemic, the number of contacts between farms and the risk of spreading disease associated with them is therefore an important input variable. In order to estimate the likely number of contacts between farms for the event of an FMD outbreak, two surveys have been conducted, one in New Zealand (Sanson *et al.*, 1993) and one in the Netherlands (Nielen *et al.*, 1996). The objective of the study presented here was to record contacts specifically on pig farms and to analyse the contacts with respect to their risk for CSF transmission.

2. Material and Methods

Two data sets were analysed, one from Switzerland and one from the Netherlands.

2.1 Dutch data set

The Dutch data set originated from a survey conducted in 1994, when Nielen *et al.* (1996) recorded contacts between farms to quantify the potential risk for FMD transmission in a region with high pig and cattle density. The survey area was similar in size to the minimum restriction zone around an infected premise after a CSF outbreak according to EU legislation (directive 80/217 EEC; minimum radius 3 km). All farmers were contacted by their local veterinarian and visited after they had agreed to participate in the study. During the visit a questionnaire was completed. Farmers were asked to record all movements onto and off their farm for a 2-week period between December 5 and 19, 1994. This time period was used because in the case of an FMD outbreak farmers would be asked to report all contacts during the last 2

weeks before clinical signs were observed. For each movement a set of attributes had to be recorded (TABLE 44). Contacts of the family, such as children going to school or visiting friends were not recorded. For more details on data recording and processing see Nielen *et al.* (1996). From the final data set of this study consisting of 144 farms, all farms with pigs were selected and used in the analysis described here.

2.2 Swiss data set

Farms for the Swiss survey were selected from two groups of pig farms: 1) farms recruited for a national research project on diseases in pigs and 2) farms to be involved in an eradication programme for respiratory diseases in an area in the north-east of Switzerland. All farms were contacted by mail and asked for their co-operation. As an incentive, all participants went into a draw for a subscription to a farmers' journal. Farmers were mailed a general questionnaire (APPENDIX D) to record farm characteristics. Separate forms were provided for visitors from outside the farm and for contacts by family members living on the farm (APPENDIX E). For each contact a set of attributes were recorded (TABLE 44). All movements on and off the farm were recorded from October 26 to November 8, 1996. All farms with complete data were analysed.

TABLE 44. Contact-related attributes recorded by farmers

Dutch data set	Swiss data set
Animal movements: Date, species, number of animals in transport, origin or destination address, type of establishment at origin/destination (farm with pigs, farm with cattle, mixed farms, farm with other livestock, live-animal market, farm of animal trader, animal collection centre for export, slaughterhouse, town or village, other), distance to origin/destination (km). People and vehicles: Date, number of persons, animal contact on farm (yes/no), contact category (subsequently coded as 27 categories, for example: veterinarian, family or friend, extension worker, pig transport), origin and destination address, type of establishment (see above).	Farm contacts: Date, pig contacts on farm, number of persons, type of contact (subsequently coded as 11 categories, for example: veterinarian, neighbour, delivery), type of vehicle (car, truck, bicycle, other), delivered commodities (subsequently coded as: susceptible animals, non-susceptible animals, animal product, slurry, other), type of establishment at origin and destination (farm with pigs, farm without pigs, town or village, slaughterhouse, other), contact with pigs at origin and destination (yes/no), distance to origin and destination (km). Family contacts: Date, name of person, type of contact (subsequently coded as 9 categories, for example: shopping, work off farm, school, delivery), type of vehicle (see above), commodities removed from farm (see above), commodities returned to farm (see above), type of establishment at destination (see above), contact with pigs at destination (yes/no), distance to destination (km).

2.3 Data analysis

Data were coded and entered into a database management programme (Microsoft Access97). Statistical analyses were conducted using the statistical package SPSS for Windows (Version

7.5, SPSS Inc., Chicago, USA). To test for differences between groups the following tests were used: χ^2 test, Fisher's exact test, Kruskal Wallis test.

All contacts with visitors were analysed twice, once in terms of tracing-back and once in terms of tracing-forward, except animal transports in the Dutch data set which were recorded as either contact on or off the farm. However, when the total number of contacts was calculated, each contact was counted only once. Because the Dutch data set distinguished between visitor contacts on and off the farm and therefore contained most contacts twice, only contacts off the farm were used for calculating total contact numbers. The recorded visitor contacts in the Dutch data set were directly comparable with the Swiss farm contacts.

Each contact on and off a farm was classified in terms of its risk of CSF transmission. It is assumed that the farm would have been recently infected and animals are shedding virus, but is still without clinical signs in animals. The classification of the contacts is based on expert rules devised by Sanson (1993) and on the published literature. The following criteria were used: type of contact (for example: animal, person, commodities), origin/destination of contact, contact with pigs on farm, contact with pigs at origin/destination. A set of rules was used to classify all contacts. The risk classification is summarised for visitor contacts in TABLE 45 and for family contacts (Swiss data only) in TABLE 46

TABLE 45. Risk classification rules for visitor contacts on and off farms

Description of contact		Risk
IF transport of life pigs	THEN	Very high
IF person or vehicle *with* animal contact AND origin/destination is establishment *with* pigs	THEN	High
IF person or vehicle *without* animal contact AND origin/destination is establishment *with* pigs	THEN	Medium
IF person or vehicle *with or without* animal contact AND origin/destination is establishment *without* pigs	THEN	Low
IF transport of feed	THEN	Medium
IF transport of manure	THEN	High

TABLE 46. Risk classification rules for family contacts on and off farms

Description of contact		Risk
IF transport of life pigs	THEN	Very high
IF person had contact with pigs outside the farm	THEN	High
IF person was going to other pig farm AND did not have contact with pigs there	THEN	Medium
IF other contact	THEN	Low

Animals are the most likely way of disease transmission between farms as long as no movement control is applied (Terpstra, 1991). As pigs are the only livestock species susceptible to CSF, contacts involving life pigs are the contacts with the highest risk. Indirect disease transmission via people and vehicles is also possible if the conveyors come in direct contact with pigs. All animal handlers and vehicles with pig contact are therefore classified in the high-risk

category provided they also had pig contact at their point of origin/destination. Vehicles and persons without pig contact but coming from/going to other pig farms are considered medium risk, because pig contact may have occurred but was not reported. Medium risk is also assigned to feed transports. The role of liquid manure and particularly slurry tanks has been discussed (Vannier *et al.*, 1986; Terpstra, 1987) and the associated risk is generally considered to be low. If slurry is spread on a pasture an aerosol may be created which is possibly of risk in the close vicinity of susceptible animals. As the risk of such practices is unknown, slurry transports are classified as high-risk contacts. All other contacts fall into the low-risk category.

In the Dutch data set, on-farm contact with pigs was not specifically recorded. What was recorded was the contact with livestock in general. In the analysis of this data set it was assumed that 'contact with livestock' was equal to 'contact with pigs'. Similarly, an undefined transport of manure was assumed to be a transport of pig manure.

When the Dutch and the Swiss data set were compared in terms of number of contacts, only farm contacts were considered in the Swiss data, but not the family contacts, because the latter were not recorded in the Dutch study.

3. Results

3.1 Description of participating farms

Out of 95 (43 and 52 in the two groups) contacted Swiss farms 21 (22.1%) completed the questionnaire and recorded farm contacts over the full time period of 14 days and 19 (20%) also recorded contacts by family members. From 144 Dutch farms, 96 were farms with pigs and 94 of these had also provided questionnaire information. The overall response rate in the Dutch study was 70%.

The pig and dairy cow inventory for all farms is given in TABLE 47 and FIGURE 26. The Dutch farms housed significantly more pigs. Out of the 19 Swiss farms, 61.9% were breeding farms, 14.3% were fattening farms and 23.8% were mixed farms, while out of the 94 Dutch farms, 13.5% were breeding farms, 60.4% fattening farms and 24.0% mixed. With respect to other livestock production, 66.7% (14) of the Swiss farms also had dairy cattle, while only 47.8% (45) of the Dutch farms had dairy cows. Consequently, less Swiss farms (33.3%) than Dutch farms (52.2%) were exclusively producing pigs.

Sixty-two % of the Swiss farms were members of the Swiss Pig Health Service and following the biosafety standards of this organisation. This includes restricted access of visitors to pig houses, use of farm-owned overalls and rubber boots by visitors, and boot disinfecting before entering pig houses. Of the Dutch farms, 67% had a comparable level of biosafety.

TABLE 47. Pig and dairy cow inventory for 21 Swiss and 94 Dutch farms

Animal	Pig farms			Mixed farms[a]			Total		
	Median	N	Min-Max	Median	N	Min-Max	Median	N	Min-Max
Swiss farms									
Piglets	40	7	0-400	30	13	0-230	31	20	0-400
Sows	30	7	0-100	14	14	0-42	16	21	0-100
Fattening pigs	15	7	0-670	14	14	0-110	15	21	0-670
Dairy cows	NA[b]	NA	NA	16	14	8-38	11	21	0-38
Dutch farms									
Piglets	0[c]	49	0-1500	0[c]	45	0-500	0[c]	94	0-1500
Sows	0[c]	49	0-735	0[c]	45	0-126	0[c]	94	0-735
Fattening pigs	300	49	0-1500	150	45	0-1100	192	94	0-1500
Dairy cows	NA	NA	NA	20	45	20-80	0	94	0-80

[a]Mixed farms had other livestock besides pigs, mostly dairy cows.
[b]NA = not applicable
[c]Due to a very high proportion of finishing-only farms in the Dutch data set, the median of sows and piglets is 0.

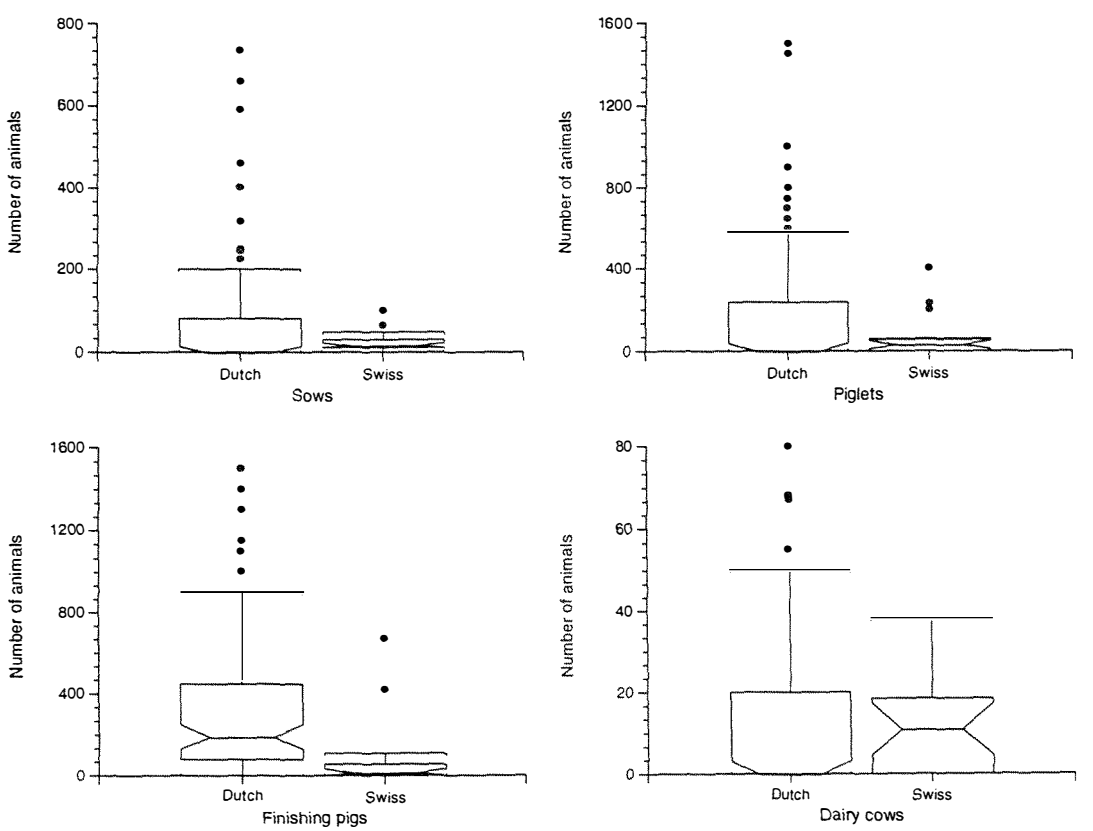

FIGURE 26. Boxplots of animal numbers on 21 Swiss and 94 Dutch pig farms

3.2 Number of contacts

During the 2-week period the total number of recorded contacts in the Swiss and the Dutch study groups were 1654 (counting each contact as 1 event) and 2661 (counting only contacts off farm), respectively. In the Swiss group 64.7% of the contacts were by family members, the rest by visitors from outside the farm. In the Swiss group, 2 farmers were also running a creamery to which farmers delivered milk twice a day and from which customers purchased dairy products. All contacts related to this function of the farmer were excluded as all activities were taking place in a physically separated building and none of the clients did enter the farm.

In the Swiss group 15 contacts were related to transports of life pigs (2 on, 13 off), while in the Dutch group 135 contacts (35 on, 100 off) were pig transports. The total numbers of contacts per farm are listed in TABLE 48.

TABLE 48. Number of contacts per farm for 21 Swiss and 96 Dutch farms in a 2-week period

	Min	25%	Median	75%	Max	Mean	Median per day
Swiss farms							
Number of people visiting	8.0	22.5	31.0	47.5	111.0	40.0	2.2
Farm contacts[a]	8.0	19.5	24.0	81.0	72.0	28.7	1.7
Family contacts[b]	22.0	31.0	43.0	32.0	127.0	56.4	3.1
Total contacts	30.0	59.0	72.0	112.0	153.0	85.7	5.1
Dutch farms[c]							
Number of people visiting	2.0	22.3	42.5	60.0	161.0	42.5	3.0
Farm contacts[d]	2.0	14.3	25.0	38.0	81.0	27.7	1.8

[a]Coming-on and going-off farm counted as one event
[b]19 farms only, including contacts by farm owner
[c]Family contacts not recorded
[d]103 contacts off-farm by farm owner included (3.9% of all off-farm contacts)

No statistically significant differences were detected between pig farm types (breeding, fattening, mixed). However, in the Dutch group, farms with pigs and dairy cows had significantly more contacts than farms with only pigs. For the comparison between the Swiss and the Dutch group, the number of farm contacts and the number of persons visiting was used in the Swiss group and compared with the number of farm contacts and the number of persons visiting in the Dutch group. No statistically significant differences were detected.

3.3 Distance of contacts

The distance between the origin/destination of a contact and the farm under consideration are shown for the Siwss data in TABLE 49 and for both data sets in FIGURE 27. In the Dutch

data set all contacts within the study area were recorded as having a distance of 0.0 km because the exact distance was not recorded. The proportion of missing values was between 14-37%. It was higher for the distance to the destination than for the distance to the origin of a contact.

TABLE 49. Distance (km) between origin/destination of contacts and farm for 21 Swiss farms

Direction (n)	Missing	Min	25%	Median	75%	Max	Mean
On farm (583)	151	0.0	2.0	4.0	10.0	680.0	12.4
Off farm (583)	221	0.0	1.5	4.0	10.0	680.0	12.2

When the results from the two groups were compared, a significant difference in the distribution of the distances was found for both on- and off-farm contacts, although visually the trend in the data does not appear to be much different.

Also, within the data sets of both countries significant differences for the distance to the point of origin/destination were recorded between risk levels. Very-high-risk contacts (pig transports) involved larger distances than other contacts both on and off farm. In Switzerland, pigs were on average transported 18.0 km and in the Netherlands over 20.9 km. However, due to missing values, the number of transports used to calculate this distance in the Swiss data is low (10 contacts only).

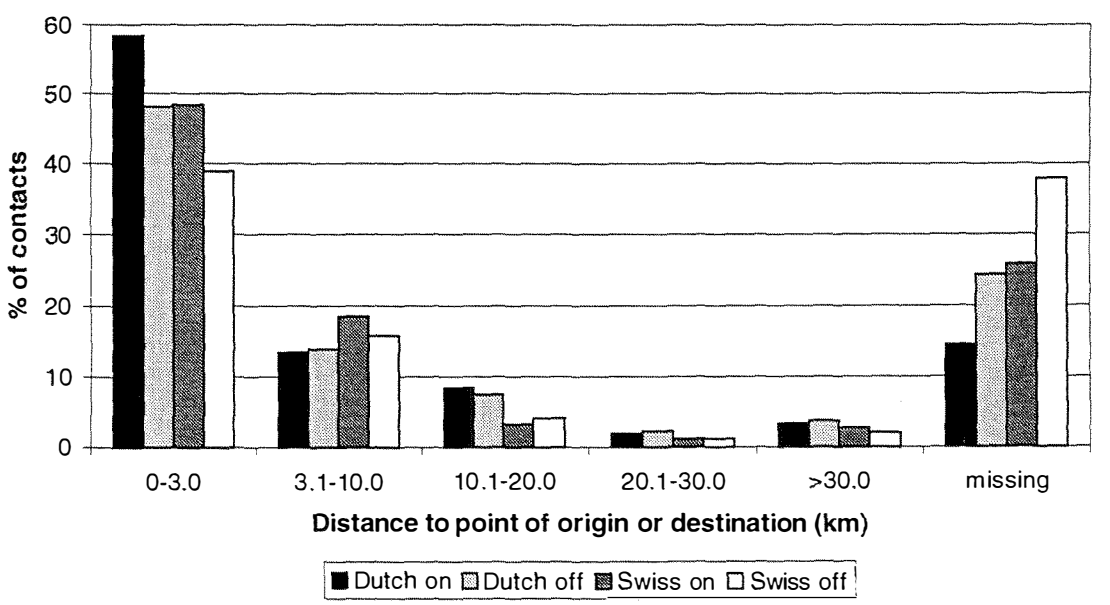

FIGURE 27. Distance to point of origin/destination for contacts on and off 21 Swiss and 96 Dutch pig farms during a 2-week period

3.4 Risk of contacts

The numbers of contacts associated with the different risk levels for CSF spread are listed in TABLE 50. Family contacts recorded on Swiss farms were almost entirely low in risk for disease transmission. When the distribution of contacts over the risk categories was compared between the two countries a significant difference was observed for both on- and off-farm contacts. The Dutch data set contained a larger proportion of very-high-risk and high-risk contacts and the Swiss data contained more medium-risk contacts. The proportion of low-risk contacts was similar in both studies.

TABLE 50. Number of contacts associated with different risk levels for spread of classical swine fever in 21 Swiss and 96 Dutch pig farms during a 2-week period

Risk level	On farm		Off farm	
	Family	Farm	Family	Farm
Swiss farms				
Very high	0	3 (0.5%)	3 (0.3%)	17 (2.9%)
High	4 (0.4%)	15 (2.5%)	1 (0.1%)	7 (1.2%)
Medium	3 (0.3%)	119 (20.4%)	3 (0.3%)	148 (25.4%)
Low	1064 (99.3%)	446 (76.5%)	1064 (99.3%)	411 (70.5%)
Total	1071	583	1071	583
Dutch farms				
Very high	NR[a]	35 (1.4%)	NR	100 (3.7%)
High	NR	214 (8.3%)	NR	193 (7.3%)
Medium	NR	358 (13.8%)	NR	304 (11.4%)
Low	NR	1980 (76.5%)	NR	2064 (77.6%)
Total	NR	2587	NR	2661

[a]NR = not recorded

In a second step the number of contacts per farm were calculated for both data sets (TABLE 51). The results show that when looking at the average values, Dutch farms had more high-risk contacts onto and off the farm. This difference was not statistically significant at the farm level.

TABLE 51. Descriptive statistics for the number of contacts per farm for 21 Swiss and 96 Dutch farms during a 2-week period

Risk level	Min	25%	Median	75%	Max	Mean
Swiss farms						
Contacts on						
Very high	0	0	0	0	2	0.1
High	0	0	0	1.5	3	0.7
Medium	0	0	3	9.5	36	5.7
Low	1	15	18	25.5	67	21.2
Contacts off						
Very high	0	0	1	1	3	0.8
High	0	0	0	1	2	0.3
Medium	0	0	2	11	52	7.0
Low	0	14.5	19	23.5	67	19.6
Dutch farms						
Contacts on						
Very high	0	0	0	1	4	0.4
High	0	0	1	3	15	2.2
Medium	0	0	2	5	21	3.7
Low	0	11	17	29	79	20.6
Contacts off						
Very high	0	0	0	1	14	1.0
High	0	0	1	3	16	2.0
Medium	0	0	2	4	17	3.2
Low	0	11.3	19	29	79	21.5

4. Discussion

4.1 Data quality

In both countries, participation in the study was voluntary for the farmers. While the response rate in the Dutch study was reasonably high and the results were considered valid for other areas with high livestock density in the Netherlands (Nielen *et al.*, 1996), the response by Swiss farmers was poor. Switzerland has not suffered from major CSF outbreaks for many years and farmers did probably not fully appreciate the importance of the study. Also, due to negative experiences with personal data files being illegally maintained by federal investigators in the past, Swiss citizens are generally very reluctant to have personal data recorded even when confidentiality is assured. On the other hand, in the Dutch study farmers were contacted by their herd veterinarian to recruit them for the survey. It was to be expected that the personal relationship between farmer and veterinarian and the level of trust already established would positively influence the response rate.

The Dutch survey included means for checking the accuracy of data recorded by farmers (Nielen *et al.*, 1996). For example, recorded veterinary visits were compared with the records maintained by the veterinarian. It was shown that farmers tended to under-report contacts. Particularly frequently occurring contacts may not be remembered well. This will cause problems in an outbreak situation, when all contacts on and off the farm will have to be remembered. In the case of a low virulent CSF the time period of recording will have to be longer than with FMD due to the longer incubation period and the slow development of clinical signs (Terpstra, 1991). Recording periods of up to 60-90 days have been suggested (Schimansky and Seidler, 1996). With the large number of contacts occurring every day, this will be a fairly difficult task. Any formalised recordings such as registers for animal transports are therefore very helpful provided they are either farm-based or fully computerised and readily accessible.

The pig production systems in Switzerland and the Netherlands are quite different in terms of herd size and production intensity. The majority of the farms in the Dutch survey was large fattening farms and highly specialised with no other livestock production, while the Swiss group consisted mainly of smaller farms with breeding pigs and a majority also had dairy cows as a second production sector. However, in terms of restriction of access of visitors to pig houses, the two groups were comparable.

4.2 Classification of contacts

The objective of the classification of farm contacts is to correctly discriminate between high to medium-risk contacts, which need to be assigned priority for tracing and low-risk contacts. The 4 risk categories used here (very high, high, medium, low) may be misleading as they suggest a linear increase in risk between the categories which in reality is not the case. A logarithmic scale would probably be more appropriate.

In order to achieve optimal discrimination one pursues two conflicting aims: while trying to reduce the number of contacts requiring immediate tracing, one needs to make sure that no risky contacts are missed. In general, the classification rules used in this study are rather conservative, i.e. a contact would rather be assigned too high a risk than too low. The only exception is perhaps that all contacts with persons and vehicles not coming from/going to pig farms are classified as low risk, because these contacts are numerous and no susceptible animals are involved on any end. One might argue that if pig contact occurred on the infected farm, these contacts should still be traced. In reality, the origin and/or destination of contacts will often not be known to or not remembered by the farmer from whom the information is collected. In this case all visitors and vehicles with pig contact will have to be traced.

4.3 Analysis of contacts

Although the type and size of farms surveyed in this study were significantly different in the two countries, the total number of persons visiting the farms and the number of total visits was surprisingly similar. In an outbreak situation 1-2 contacts per day including 2-3 persons will have to be expected as long as no contact restrictions are in place. This figure does not include family contacts. The results of the Swiss survey show that family contacts are very

frequent and almost entirely low risk contacts. It seems justifiable that these contacts receive lower priority if a CSF outbreak occurs.

The distance over which a contact takes place is significant in the context of the restricted zones that will be established around a CSF outbreak. Currently the European Union legislation (directive 80/217 EEC) requires a protection zone and a surveillance zone with a minimum radius of 3 km and 10 km, respectively. In both countries in this study, the largest group of recorded contacts remained within the protection zone (distance <3.0 km), and only a minority of contacts went beyond the surveillance zone. However, this situation is not necessarily representative for an entire country because it depends on the location of major business centres, abattoirs etc. Also, the amount of missing values of up to 37%, particularly for off-farm contacts, was considerable. This may result from the fact that farmers did not know where visitors went after they left the farm and also did not ask them. Data from recent CSF outbreaks in Belgium and in Germany confirmed that secondary outbreaks are most likely to occur within approximately 6 km from the primary outbreak (Roberts, 1995). Yet, this study also showed that the distance over which pigs are transported (very-high-risk contacts) is significantly longer than the distance of other contacts. On average, these contacts are likely to go beyond the border of the protection and possibly also the surveillance zone. In an outbreak situation it is therefore most important that animal contacts are accurately recorded and promptly traced in order to stop the disease from spreading.

Of all farm contacts, less than 4% were pig transports and therefore very-high-risk contacts in this study. In Switzerland this proportion was even smaller because finishing-only operations are rare. Most of these contacts will be trace-forward contacts because generally more pigs will leave a farm than be delivered to a farm. High-risk contacts and very-high risk contacts will get highest tracing priority in a CSF outbreak situation. Together they accounted for 3.1-15.2% of the contacts in this study. This number was significantly higher in the Dutch data set. This may partially be due to classification bias because it was assumed that a visitor with a recorded livestock contact would have had pig contact as well. Yet, some of these visitors may have had contact with cattle only. They would then fall into the medium or low risk category depending on the origin/destination of the visitor. This may diminish the current differences between the Swiss and the Dutch results.

Because CSF virus is also excreted in urine and faeces (Ressang, 1973; Depner *et al.*, 1994), manure from an infected farm has the potential to infect another pig farm. In this study, <1% of all transports were manure transports. Again, the Dutch data did not differentiate between cattle and pig manure and some transports may have been misclassified.

Another way to spread CSF virus is the feeding of contaminated swill. Swill feeding to pigs is prohibited in the Netherlands and therefore such transports were not recorded in the Dutch survey. In the Swiss data set, swill transports were also not specifically recorded and this criterion was therefore not used for risk classification. However, an expert system designed to manage a real CSF outbreak will need to classify all transports of swill to be fed to pigs as high-risk contacts.

In 1997, during a CSF outbreak in the Netherlands the disease infected an artificial insemination (AI) centre from which it possibly could have been spread via contaminated or infectious semen. This means of transmission had not been described before. To date, the risk of semen for CSF spread has not yet been conclusively quantified. In this study, contacts with AI tech-

nicians were recorded in the group of visitors with animal contact. In the Dutch data set, 12 (42.9%) of 28 breeding or mixed farms had a total of 104 AI contacts over the 14 day recording period (Farms with dairy cows were excluded because it was not specifically recorded whether the AI technician was servicing cows or sows). This shows that if a farm uses AI regularly, this type of contact will occur frequently and will have to be considered according to its risk level.

One point that was not covered by this study, is the influence of frequency of contacts. While a particular type of contact may as such have a low risk, this could be changed if the contact occurred very frequently. It is envisaged that the expert system assisting in the management of a CSF epidemic will use a mechanism to calculate the cumulative risk of contacts. The latter is then used to classify the entire farm in terms of risk rather than just individual contacts.

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References

Depner, K., Gruber, A., and Liess, B. (1994). Experimental infection of weaner pigs with a field isolate of hog cholera classical swine fever virus derived from a recent outbreak in lower saxony. 1. clinical, virological and serological findings. Wiener Tierärztliche Monatsschrift 81:370-373.

Nielen, M., Jalvingh, A.W., Horst, H.S., Dijkhuizen, A.A, Maurice, H., Schut, B.H., van Wuijckhuise, L.A., and de Jong, M.F. (1996). Quantification of contacts between Dutch farms to assess the potential risk of foot-and-mouth disease spread. Preventive Veterinary Medicine 28:143-158.

Ressang, A.A. (1973). Studies on the pathogenesis of hog cholera. I. Demonstration of hog cholery virus subsequent to oral exposure. Zentralblatt der Veterinär-Medizin B 20:256-271.

Roberts, M. (1995). Evaluation of optimal size of restriction zones in disease control with particular reference to classical swine fever. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Reading, 119-130.

Sanson, R.L., Struthers, G., King, P., Weston, J.F., and Morris, R.S. (1993). The potential extent of transmission of foot-and-mouth disease: A study of the movement of animals and materials in Southland, New Zealand. New Zealand Veterinary Journal 41:21-28.

Sanson, R.L. (1993). The development of a decision support system for animal disease emergency. Unpublished PhD Thesis, Massey University, Palmerston North, New Zealand.

Schimansky, K. and Seidler, M.-J. (1996). Epidemiologische Ermittlungen bei Ausbruch der KSP; ein Erfahrungsbericht. Amtstierärztlicher Dienst und Lebensmittelkontrolle 3:166-172.

Stärk, K.D.C., Morris, R.S., and Sanson, R.L. (1996). EpiMAN-SF - development of a decision support system for managing a swine fever epidemic. Proceedings of the 14th IPVS Congress, Bologna, 534.

Terpstra, C. (1987). Epizootiology of swine fever. Veterinary Quarterly 9 (Suppl.1):50S-60S.

Terpstra, C. (1991). Hog cholera: an update of present knowledge. British Veterinary Journal 147:397-406.

Vannier, P., Colcanap, M., Carnero, R., Costes, C., Josse, J., Tillon, J.P. (1986). Study of the origin of an epizootic of classical swine fever. Journal of Veterinary Medicine B 33:294-302.

CHAPTER 2.6

EpiMAN-SF – DESIGNING A DECISION-SUPPORT SYSTEM FOR THE MANAGEMENT OF SWINE FEVER[1] EPIDEMICS

[1] The system will be capable of dealing with both African and classical swine fever. In order to keep the text simple and easy to read both diseases are summarised under the term 'swine fever'.

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1. Introduction

Exotic disease outbreaks require fast and efficient decision making which needs to be based on all available information on the epidemiology of the disease and the current stage of the epidemic. The following problems that are likely to occur during an epidemic make it difficult to comply with this principle (Morris *et al.*, 1993).

1. The amount of data accumulating during an epidemic is considerable, and if the epidemic is escalating, can become overwhelming. An efficient system is needed for data storage and management during the epidemic to allow continuous analysis of the situation.
2. Field personnel may not be familiar with exotic diseases that have not occurred in the country at all, or for a considerable time. This may make it difficult for them to make appropriate decisions under emergency circumstances.

In order to deal with the problem described under point 1, the first step is to design a computer-based data management system. In a recent outbreak of CSF in the Netherlands for example, a database was developed within the first 4 weeks of the epidemic (Benard, 1997). The second point however is much more difficult to tackle because it requires the development of a knowledge-based system, which is capable of compensating for the lack of human expertise.

In an attempt to solve both types of problems an **epi**demiological **i**nformation **man**agement system (EpiMAN) for exotic disease outbreaks was developed (Sanson, 1993). It consists of a core database for data management, expert systems and a set of further tools to analyse the collected information (FIGURE 28). The system was initially designed for the control of vesicular diseases (EpiMAN-FMD), but has since been adapted to deal with other diseases, for example tuberculosis (McKenzie *et al.*, 1997). This chapter describes the design features of EpiMAN-SF, a new product in the EpiMAN suite, which is designed to deal with classical and African swine fever.

The following terms will be used in order to identify different farm types within EpiMAN-SF:

- Restricted property (RP): a restricted property is known or presumed to be infected and is therefore subjected to defined restrictions. This category includes the following three subcategories.
- Infected property (IP): an infected property has got a laboratory-confirmed infection status.
- Suspect property (SP): a suspect property is presumed to be infected but has not yet received a laboratory confirmation
- Pre-emptively slaughtered property (PE): on a pre-emptively slaughtered property all stock is removed without confirmation of the infection status.

2. Problem domain definition

EpiMAN-SF is designed to manage the large amount of data that is typically accumulated during a classical swine fever epidemic in a region with medium to high pig density. It provides accurate and timely summaries of the epidemic in both textual and graphical form. In

addition, it provides tools for the epidemiological analysis of the emergency and for the assessment of the current and alternative control strategies. EpiMAN-SF is based on the concepts and elements of EpiMAN-FMD (Morris *et al.*, 1993; Sanson, 1993).

3. Users

EpiMAN-SF is used by disease control officers, epidemiologists and managers, all of whom are involved in the control of the epidemic in the field. As in EpiMAN-FMD, different modules are designed for different types of users and their specific tasks, for example a tracing module or an epidemiology module. Reasonable computer literacy is expected from all users. Users are not necessarily at the same physical location. Therefore the system needs strong networking capabilities.

4. Tasks

A series of primary tasks have been identified, a short description of which follows. Most of the tasks are similar if not identical to what was considered for EpiMAN-FMD (Morris *et al.*, 1995). In all tasks, the farm is the basic reference unit. The elements needed to perform the tasks and the data flow through the system is shown in FIGURE 28 This is analogous to the structure of EpiMAN-FMD.

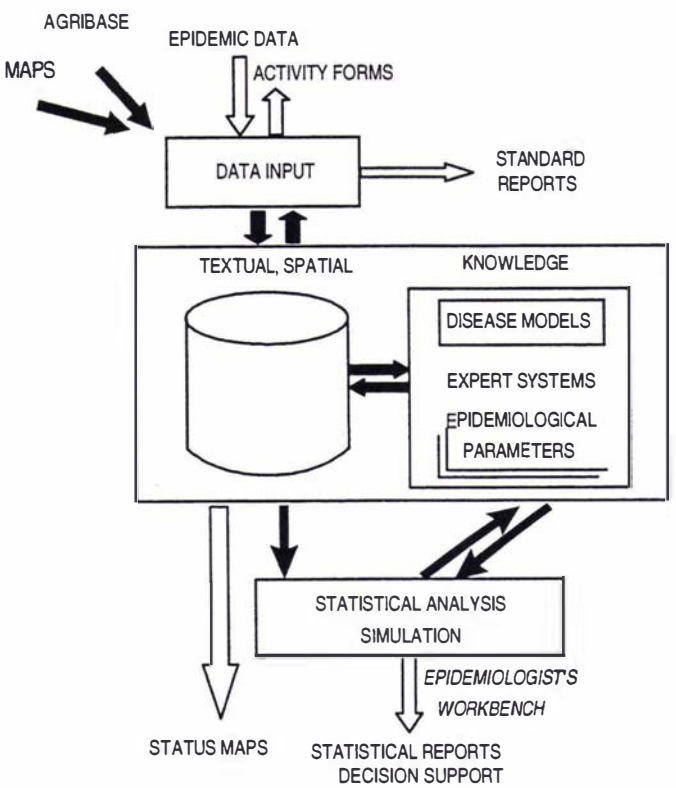

FIGURE 28. Components of EpiMAN-SF

4.1 Virus strain identification

Several components of EpiMAN-SF use virus characteristics such as the incubation period as input for further calculations. Due to the large variability between swine fever virus strains, at least two options have to be made available by the system: 1) low-moderate virulence, and 2) high virulence. It is unlikely that the virus strain will be known early in the epidemic. EpiMAN-SF therefore first uses low-moderate virulence as a default value because it appears to be the more cautious assumption, which reduces the risk of seriously erroneous decisions (see CHAPTER 2.7). The virus strain information is used by the expert system and the simulation modules.

At a later stage, the user (the epidemiologist) enters strain information and the system re-evaluates earlier decisions, for example classification of traces, and produces a summary report for the user.

4.2 Farm data management

Farm data consists of two types of data: 1) emergency-independent data such as farm location, ownership and livestock numbers, and 2) emergency-related data, for example farm infection status and data from epidemiological investigations.

The first category of data is stored in national farm databases. EpiMAN-SF needs an interface to access and (optionally) update these data.

The emergency-related data is entered continuously during the epidemic. All these data are linked to individual farms. According to the current status of a farm, different types of data will need to be entered. Possible categories of infection status are outlined below in a state-transition flowchart (FIGURE 29). Initially, all farms are in the 'nil risk' category. After the first outbreak farm has been identified, some farms will become 'at risk'. Different risk categories will be used for at-risk farms according to the likelihood of exposure to virus (see CHAPTER 2.7). These farms will be visited according to their risk rating and surveillance data will become available. The further classification of the farm depends on the outcome of the farm visit. According to directive 80/217 EEC a farm can also have the status 'suspect farm'. These farms are subject to special restrictions. In EpiMAN these farms are labelled SP. These farms are RPs (restricted properties), but specifically identifiable as farms with an undetermined infection status.

Data accumulated during an epidemic:

- **Episodes**: An episode is a significant event occurring during an epidemic, associated with potential virus introduction to a farm. Possible episode categories are: tracing event (animal, people, semen or vehicle contact), swill feeding, contiguous property, location in protection zone, feral pig contact, and location in surveillance zone (as well as 'user defined'). As a consequence of an episode, a farm will be put at risk with the possibility of becoming a restricted property (SP, IP or PE). In contrast to EpiMAN-FMD, airborne disease transmission is not considered. A farm risk rating is applied for each episode type by an expert system (CHAPTER 2.7). The time period when clinical signs are to be expected if virus transmission has occurred is derived and stored for use by the surveillance team.

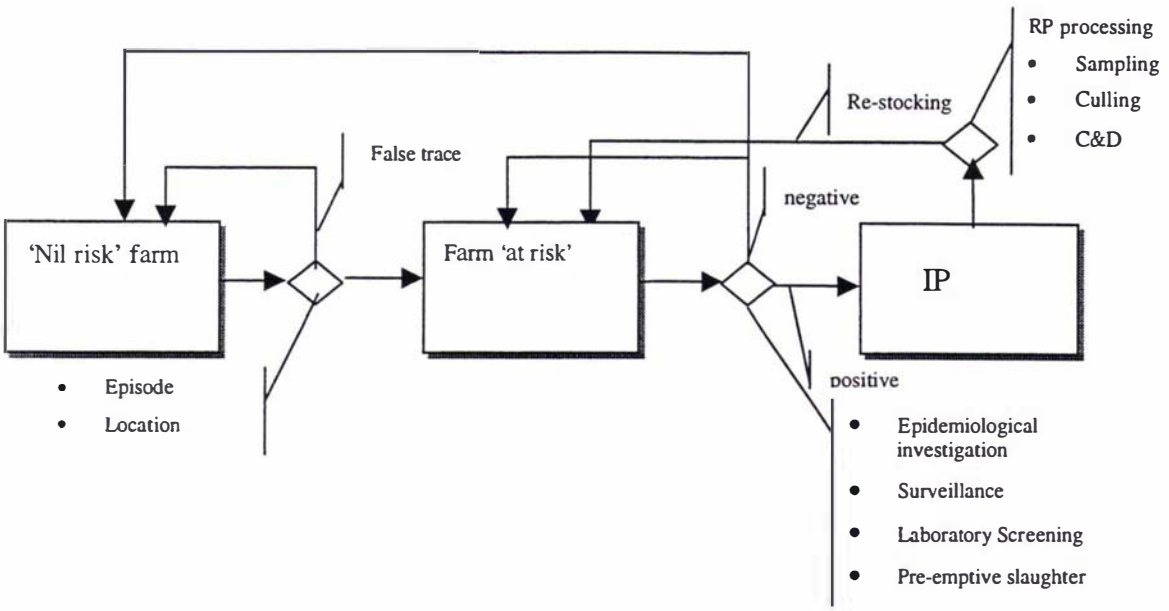

FIGURE 29. State-transition flowchart for farm status during a swine fever epidemic

- **IP information**: After a farm is declared an infected property (IP), it will be visited to perform an epidemiological investigation. For each IP the following data will be entered: Number of management groups[1], updated pig numbers by management group, likely source of infection, likely date of infection, tracing information, history of swill feeding, distance to next pig farm, possible contact with feral pigs (for example if outdoor housing is used or if there is a history of CSF in wild boar in the region). As part of the IP processing, all pigs are examined according to the protocol, samples collected, all pigs are killed and the farm is cleaned and disinfected (C&D) according to the contingency plan (see 4.5 IP and PE management).
- **Surveillance data**: Farms will be visited for epidemiological investigation. Data related to farm visits, collected samples and laboratory results will need to be processed and stored (see 4.7 Surveillance and laboratory management).

4.3 Spatial data management

Once an IP has been identified, a map with the farm boundaries and the contiguous properties is required for the control form. Then, two types of restriction zones will have to be declared:

- **Protection zone**: all farms within a radius of 3 km of the IP[2] fall into the protection zone and are immediately put at risk. These farms are visited according to a special protocol.

[1] A management group is defined as a group of pigs of approximately the same age and production status which are kept physically separate from similar groups, for example in different buildings.

[2] All specifications are currently according to directive 80/217 EEC.

All livestock movements are prohibited onto or off these farms. The protection zone is maintained for at least 30 days.

Within the protection zone, the contiguous properties with pigs around each IP have to be flagged, as these are at very high risk. If pre-emptive slaughter is applied, these farms will have to be considered first. Alternatively, all farms within a certain radius, for example 1 km can be selected.

- **Surveillance zone**: all farms between 3 km and 10 km from the IP fall into the surveillance zone. These farms are visited according to the protocol. Livestock movements are restricted. The zone is maintained for a minimum of 15 days.

The boundaries of the restricted zones will normally follow either administrative boundaries (e.g. municipality) or natural boundaries (e.g. river). The user will need to be able to edit the boundaries using a digitiser table or on-screen digitising.

EpiMAN-SF has to identify all farms within the restricted zones immediately after an IP is declared. The number of farms and pig numbers have to be extracted and stored and the total number of pigs on these farms calculated. Maps displaying IPs, SPs, PEs, restriction zones, abattoirs and rendering plants will be needed.

If spatial information on wild boar populations and their infection status is available (for example test results from hunted wild boar with location of shooting), EpiMAN-SF will use these data to create an additional layer in the GIS. This will allow the identification of farms at risk of wildlife infection.

4.4 Management of traces

As in EpiMAN-FMD, traces are managed in a separate module. An expert system is used to support the tracing officers in identifying high-risk traces and thus in setting priorities (CHAPTER 2.7). The user can overrule the risk category assigned by the expert system. Traces are recorded for IPs, RPs and Pes.

4.5 IP and PE management

The processing of each IP and PE includes the following activities: farm visit and pig census, securing of property, rodent control, sampling of pigs for laboratory tests according to the protocol, killing of all pigs and safe transport to rendering plant, cleaning and disinfecting (C&D) of vehicles, C&D of pig houses. C&D is performed according to the contingency plan and will take at least 8 days. Earliest re-stocking date is 30 days after C&D. Slurry has to be stored for at least 42 days. In case of pre-emptive slaughter, the farms will be treated in an identical fashion.

4.6 Movement control management

Farms within the restricted zones need permits to be allowed to move livestock or vehicles. The owners have to apply for the permits and the latter will be granted if - under the current

control policy – the type of movement under consideration is allowed. For example, according to EU legislation, pigs must not be moved within 21 days after C&D of the related source IP. After 21 days permission may be obtained to transport pigs to the abattoir provided all pigs are clinically healthy. In the surveillance zone the total standstill lasts for 7 days, afterwards movements directly to an abattoir can be allowed once a farm visit has occurred. All applications for movements are processed by an expert system within EpiMAN-SF, as movement permission largely depends on the location and the status of a farm, all of which is stored in the EpiMAN database. C&D orders are also issued and managed within this module. All pigs that are moved within a restricted zone need to be identified by ear tags.

In recent CSF outbreaks in Europe, movements of pigs off farms in the protection zone were allowed when the animals became too large for their accommodation and needed to be destroyed for animal welfare reasons (Benard, 1997). These pigs were bought by the European Union and transported to special killing facilities. Such movements were only allowed after the farm had been visited and no clinical signs were detected.

4.7 Surveillance and laboratory management

During a CSF epidemic, farms will be visited and intensive laboratory investigations will take place. In order to be able to manage all this information, a new module will be developed: the surveillance and laboratory management module. As this module was not part of the original EpiMAN-FMD it is described in more detail. This module has the following tasks:

1. Scheduling visits and assigning field teams
2. Printing questionnaires for farm visit
3. Printing laboratory submission form
4. Capture and storage of visit-related data

Visits are scheduled according to a protocol. This defines when a farm needs to be visited and what examinations need to be performed. As an example the visit and scheduling requirements according to EU legislation are shown in TABLE 52.

TABLE 52. Visit schedules and task protocol according to European Union legislation (directive 80/217 EEC)

Type of visit	Description and timing	Tasks
A. Epidemiological investigation of IP	Each infected farm needs to be visited immediately (within 24 hours).	Perform clinical examination. Complete farm inventory. Collect contact information, interview. (Can be combined with visit G).
B. SP	Each farm that is a suspected IP (very-high risk farms) needs to be visited immediately (today).	Perform clinical examination. Complete farm inventory. Take blood samples from ill animals. Submit animals for post-mortem.
C. Visit of at-risk farm	Each farm in the protection zone and farms at-risk due to movements or another episode need to be visited according to their risk level but no later than 7 days after the related IP is declared.	Perform clinical examination. Complete farm inventory. Take blood samples from ill animals. Submit animals for post-mortem.

Type of visit	Description and timing	Tasks
D. Lifting of protection zone	Before the protection zone can be lifted, all farms must have negative clinical and serological results. Earliest date: 30 days after C&D of related IP.	Perform clinical examination. Complete farm inventory. Take blood samples according to the following protocol: - Individually housed pigs (eg. Sows in crates): <20 all 10-100 20 + 20% of rest >100 20 + 10% of rest - Group-housed pigs: <20 in group 2 >20 in group 2 + 5% of rest
E. Surveillance zone visit	Each farm in the surveillance zone (not at risk) needs to be visited no earlier than 15 days after C&D of related IP	Perform clinical examination of all farms. Complete farm inventory. Take blood samples of a representative number of farms according to the following protocol under D.
F. Transport visit	Each farm in the restricted zones requesting transport of pigs to the abattoir needs to be visited (such a request cannot be made before 7 days (surveillance zone) or 21 days (protection zone) after C&D of related IP	Perform clinical examination Apply ear tags to each animal leaving the farm.
G. Stamping out	Before all animals are killed on an IP or PE, samples are collected for laboratory analysis	Take serum samples of at least 10% of randomly selected animals and heparin samples from sick animals (virus isolation).
H. Follow up to inconclusive serology results	If after a visit of type B,C,D, or E some samples test serologically positive and none is antigen-positive, resample within 7 days.	Perform clinical examination. Take blood samples according to protocol.
I. Follow up to inconclusive antigen results	If after a visit of type B,C,D, or E infection cannot be excluded, then resample immediately.	Perform clinical examination. Take samples according to laboratory specifications.
J. Follow up to SPs	If no samples are collected and no clinical signs are observed, very-high risk farms need to be re-visited at specified intervals (e.g. twice per week) until the maximum incubation period has elapsed.	Perform clinical examination. Complete farm inventory. Take blood samples from ill animals. Submit animals for post-mortem.
K. User-defined visit		

During an epidemic, the protocol for the different visits and the visit frequencies may change. In order to keep the module as generic as possible, all specifications with respect to sampling protocols are stored separately. Protocol sheets will be provided to the sampling groups. It should also be possible to add new visit types and to change the time requirements for scheduling the visits. Area or time-specific visits should be kept separate from risk factor-dependent visits (CHAPTER 2.7).

It is assumed that only farms at risk are visited. This is not necessarily true. According to directive 80/217 EEC a contact farm does not necessarily have to be put under restriction, but

can be under surveillance only. However, it is still visited and the animal inventory is done. The farm can be put under restriction at any time. Conversely, farms in the surveillance zone are not at risk but still visited.

EpiMAN-SF will produce updated lists of farms to be visited and help assign field teams. If a large number of farms are at risk, priorities will be set by the system according to the risk level of the farm. It is possible that not all visit types will be managed by the same team within the crisis centre. For example, type F visits may be initiated by the Movement Control Team while type G visits could be rostered by IP and PE Support and all the other visits by the Disease Investigation Group. In this case it has to be made sure that generally only one visit should be scheduled per day, and that type F visits receive lowest priority.

For each farm visit, a form with the following information is printed: Farm number (used as key), name of farmer, address, driving instructions, date of visit (used as key), responsible visiting veterinarian or team, type of visit (A-H, see TABLE 52), area map (with neighbours). If more than one visit occurred on a day, the combination of date and visit type is used as an identifier of the visit (key). The form will also display the number of the related IP and the reason why the farm is visited (episode). If the farm is visited because of a contact, the details of that contact should also be listed. The field veterinarian will record the following data on the farm: visit time (this is important in determining the sequence of visits later), updated inventory by management group, mortality and morbidity by management group, description of clinical signs. There is also room for free text comments.

If laboratory samples are collected, the veterinarian uses the laboratory submission form already printed and provided together with the visit form. The submission form already contains the following information: Farm number (used as key), visit type, name of farmer, address, name of visiting vet or team, name of laboratory to which samples are submitted, date of visit (used as key). The visit type is important for the laboratory to set priorities for processing. The field veterinarian enters the following data: Date of submission, number and type of samples submitted and tests required.

The results from the laboratory can either be entered from hard copies of laboratory reports or preferably the laboratory will provide the results in an electronic form (either by file or by entering data directly using a remote terminal). The laboratory will report the following: Farm number (key), date of farm visit (key), date of laboratory analysis, tests performed (indirect immunofluorescence test (IFT), direct IFT, serology, virus isolation, pathology), number of samples analysed with each test, number of positive results with each test, number of negative results with each test, number of not interpretable results with each test, description of pathology results (free text), diagnosis (antigen negative or positive, serology negative or positive, CSF infection cannot be excluded). There is also a free-text field for the laboratory to enter specific requirements for follow-up sampling.

With help of the laboratory results, the rules of TABLE 53 can be applied.

In order to illustrate the relationship between events related to the IP and the control zones on the one hand and procedures performed by EpiMAN-SF, an attempt to list them in a chronological manner is shown in TABLE 54.

TABLE 53. Rules for scheduling farm visits based on laboratory test results

Rule 1		
	IF	farm is not IP AND antigen positive
	THEN	farm is declared IP AND follow-up visit (type A) is required immediately. (This will probably be done manually).
Rule 2		
	IF	farm is not IP AND more than x samples serologically positive
	THEN	farm is declared IP AND follow-up visit (type A) is required immediately. (This will probably be done manually).
Rule 3		
	IF	farm is not IP AND less than x samples serologically positive
	THEN	farm is declared SP AND follow-up visit (type H) is required within 7 days.
Rule 4		
	IF	farm is not IP AND CSF infection cannot be excluded
	THEN	farm is declared SP AND follow-up visit (type I) is required immediately.

TABLE 54. Chronology of events during a classical swine fever outbreak on different levels with respect to procedures performed in EpiMAN-SF

IP level	Protection zone level	Surveillance zone level	EpiMAN-SF procedure
Suspicion			Enter episode
			Declare SP
			Schedule visit B, print forms
Farm visit, sampling			Enter data
Declaration of IP1			Declare IP
			Schedule visit A, print forms, print map
Farm visit, data collection, sampling			Enter data
	Declaration of protection zone	Declaration of surveillance zone	Identify control zones and tag farms
	Farms 'at risk'	Farms 'under surveillance'	Put farms in protection zone 'at risk'
			Tracing contacts of IP
	Identify contact farms	Identify contact farms	Enter episodes for traces
			Enter episodes for swill feeding, contiguous property.
			Declare SPs if necessary
			Declare PEs if required
			Schedule farm visits B

IP level	Protection zone level	Surveillance zone level	EpiMAN-SF procedure
			or C, print forms
	Visit farms, sampling	Visit farms, sampling	Enter data
			Declare IP if necessary
			Schedule visit H or I if necessary, print forms
IP and PE processing			IP processing, initiate stamping out, schedule visit G, print forms
Stamping out, sampling, C&D			Enter data
	Movement requests	Movement requests	Schedule visit F, print forms
Declaration of new SPs and IPs or PEs			
			Schedule visits D and E, print forms
	Farm visits, sampling	Farm visits, sampling	Enter data
			Schedule visit H or I if necessary, print forms
			Declare IP if necessary
	Farm visits, sampling	Farm visits, sampling	
	...	...	
	Lifting of restrictions	Lifting of restrictions	All farms back to 'nil risk'

4.8 Epidemic analysis

4.8.1 Epidemiologist's workbench

As in EpiMAN-FMD a set of tools will be needed by the epidemiologist to further analyse the epidemic, for example graphical display of epidemic network, epidemic curve, estimation of disease dissemination rate, proportion of farms infected by the different episode types, and survival analysis. For an example of the output of these tools see CHAPTER 2.9.

4.8.2 Date calculator

As an addition, EpiMAN-SF will also offer the user a date calculator (working name DAX) with which calculations with dates can be performed. For example the user will be able to add the current incubation date, say 12 days, to a given date of possible infection, say 27 February, and the calculator will provide the accurate date of likely onset of clinical signs. Or the difference between two dates in days can be calculated. This simple tool will be very useful and help prevent mistakes as experience has shown that field personnel have a lot of difficulties performing such calculations. This tool is accessible from EpiMAN-SF modules.

4.8.3 Virus production model

A virus production model will be used to estimate the probability of infection in the different management groups on a specific IP at a given time. This is needed to assess the risk of virus transmission related to contacts during the time period between the end of the incubation period and the declaration of the IP. This model needs a series of input parameters with regard to the transmission dynamics of CSF within an infected farm. These need to be measured in animal experiments, most of which have not yet been performed (see CHAPTER 2.8). It is therefore at this stage not possible to develop the virus production model. It will be reconsidered as soon as the necessary information becomes available and will be integrated in a later version of EpiMAN-SF.

4.8.4 INTERSPREAD-SF

In a separate model, disease transmission is simulated between farms. This model uses the same principles as INTERSPREAD (Stern, 1994; Jalvingh *et al.*, 1997). INTERSPREAD-SF is used to predict the likely development of the epidemic given the current or an alternative control strategy. The model will evaluate 'what-if' scenarios and thus provide decision support in the selection of the most efficient control strategy. Ideally, this model will be integrated in EpiMAN-SF. It will then use information on the current situation of the epidemic as a starting point for the simulation. It will also provide input to economic prediction models, which will be developed in a later version of EpiMAN-SF.

The following types of spread will be considered in INTERSPREAD-SF: local spread, contact-related spread, swill feeding-related spread and recrudescence. As an additional option, the presence of SF-infected wild boars in a region can be integrated into the simulation. Note that airborne spread is not being considered (see also CHAPTER 2.9).

The user will be able to make policy alterations such as introducing pre-emptive slaughter and/or vaccination buffers. A list of farms to be considered for these interventions can be obtained together with the total number of farms and their livestock details.

4.9 Documentation and reporting

The principal outlines of the reports available in EpiMAN-FMD can be used. For special activities such as intensive laboratory testing, new reports will have to be designed.

5. Technical principles and requirements of EpiMAN-SF

EpiMAN-SF can be run as a stand-alone application on a PC running Windows 3.1 or higher (or Windows NT) and an ODBC-complient database, or it can be set up in a client-server structure (FIGURE 30). The network situation is more realistic as a number of people will be entering data during an outbreak. The front end of EpiMAN-SF is based on Microsoft Access (Microsoft Corporation, Redmond, U.S.A.). Most other functionalities are based on SQL queries.

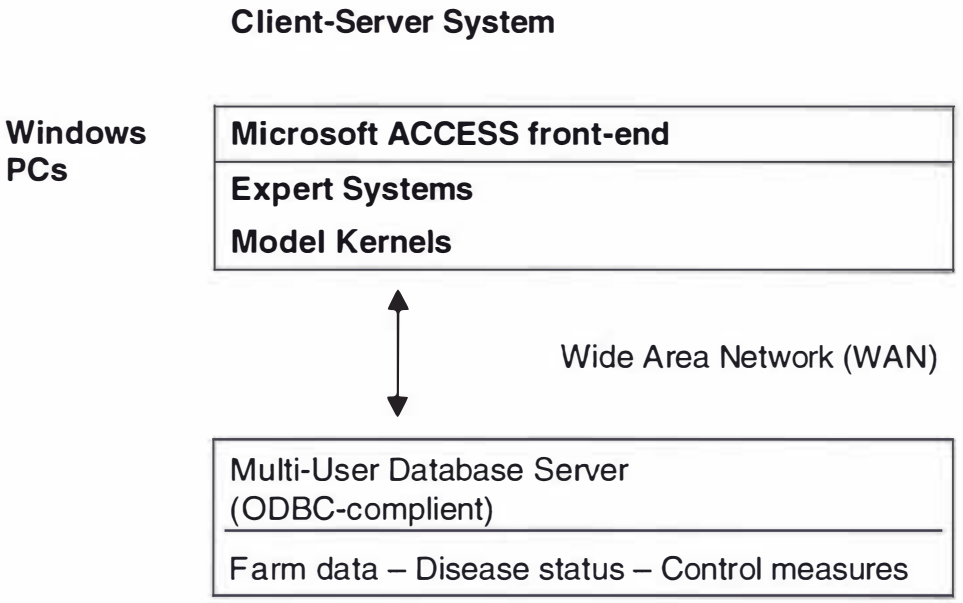

FIGURE 30. System architecture of EpiMAN-SF

Users can be given access at different data entering and processing levels. After logon a person will only 'see' the options available to him/her. For example, a tracing officer will only have access to the tracing module, while the epidemiologist can also use the analytical tools. Data security and integrity is guaranteed by internal locking mechanisms.

In a network setup using a fileserver, each client machine requires 2.5 Mb of space, and 7 Mb are shared client software. The back-end database server's requirements are entirely dependent on the amount of spatial data stored in the database. At least 16 Mb of RAM are recommended.

6. Discussion

An epidemic information system should provide information support for decision-making at all levels within an emergency response organisation. This means any computer-based system has to fit in with the structure of the organisation it is intending to support (Lippeveld *et al.*, 1997).

The basis of exotic disease control in a country is the veterinary infrastructure, the contingency plan and the related legislation. Any computer-based disease control system must also use these specifications and respect the specific organisational structures for disease control in a country. This means that EpiMAN cannot necessarily be readily applied in countries with different disease control principles. A series of adaptations of the system will always be necessary. Points to be considered are:

- National farm database. Is such a database available in a country and which fields does it contain? The development of a suitable national database is likely to be time-consuming and technical as well as privacy issues have to be addressed (Sanson and Pearson, 1997).
- Spatial data. Are the locations of farms geo-referenced and if yes, is it polygon or point information. In the case of point locations, which point of the property was used (pig

house vs. farm house vs. farm gate)? For a discussion of this issue see Nielen *et al.* (1996) and Jalvingh *et al.* (1996).

- Disease control strategy. Most countries where CSF does not occur endemically would be applying the stamping-out strategy (O.I.E., 1997). However, how exactly the stamping out is performed, how large the control zones are and what additional strategies are used may differ considerably. In the CSF outbreak in the Netherlands of 1997 for example, a whole series of additional strategies such as pre-emptive slaughter, a ban of artificial insemination and a breeding ban were introduced in specific areas (Benard, 1997).
- Sampling protocols and visit scheduling. The sampling protocol can also differ considerably between countries. For example the EU legislation defines a minimum protocol for farm visits (directive 80/218/EEC), but when an outbreak occurs in one of the member countries, additional specifications are likely to be defined. This happened during the 1997 outbreak in the Netherlands, where for example in addition to the blood samples also temperature readings were performed on suspect farms and additional visit types were introduced (Benard, 1997).

EpiMAN-SF is specifically designed to be able to incorporate these country-specific demands. By keeping sampling specifications separate, the laboratory and surveillance management module can be kept generic and thus flexible. Additional outbreak specifications such as the current control measures and information on the virus strain can be modified interactively using an entry form in EpiMAN-SF. The access to this window however, is restricted to the controller of the emergency headquarters.

If a country decides to adopt EpiMAN, the process of reviewing the current contingency plan and organisational structures needs to be initiated. A way to harmonise the current situation with the EpiMAN structure needs to be found. Disease control officers of all hierarchic levels should be involved. This entire process including possible changes of EpiMAN-SF may take up to several years as was the case with EpiMAN-FMD.

However, after EpiMAN-SF has been adapted, drastic changes during an epidemic may not be as easy to incorporate. For example in a German outbreak 1993-1996 a large number of changes were made both in federal and EU legislation during the outbreak (Teuffert and Schlüter, 1994). Strong computer support is required to keep the system flexible enough to deal with this challenging situation.

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References

Benard, H.J. (1997). Report of classical swine fever field experience, April and May 1997, the Netherlands. Unpublished report, MAF Quality Management, Palmerston North, New Zealand.

Jalvingh, A.W., Nielen, M., Dijkhuizen, A.A., Crauwels, P., and Smak, J. (1996). Use of EpiMAN in the Netherlands: recent developments and planned activities. In: Graat, E.A.M., and Frankena, K. (eds.) Proceedings of the Annual Meeting of the Dutch Society for Veterinary Epidemiology and Economics (VEEC),

Wageningen, 25-31.

Jalvingh, A.W., Nielen, M., Meuwissen, M.P.M., Dijkhuizen, A.A., and Morris, R.S. (1997). Economic evaluation of foot-and-mouth disease control strategies using spatial and stochastic simulation. Epidémiologie et Santé Animale 31-32:10.22.1-10.22.3.

Lippeveld, T, Sauerborn, R., and Sapirie, S. (1997). Health information systems – making them work. World Health Forum 18:176-184.

McKenzie, J.S., Morris, R.S., Tutty, C.J., and Pfeiffer, D.U. (1997). EpiMAN-TB, a decision support system using spatial information for the management of tuberculosis in cattle and deer in New Zealand. In: Pascoe, R.T. (ed.) Proceedings of the 2nd Annual Conference Geocomputation, University of Otago, Dunedin, New Zealand, 271-277.

Morris, R.S., Sanson, R.L., McKenzie, J.S., and Marsh, W.E. (1993). Decision support systems in animal health programmes. Proceedings of the Annual Meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Exeter, 188-199.

Morris, R.S., Sanson, R.L., and Stern, M.W. (1995). EpiMAN – a decision support system for managing a foot-and-mouth disease epidemic. In: Stern, M.W. *et al.*.: EpiMAN for vesicular diseases – an integrated decision support system. A workshop tutorial demonstrating use of the system. Unpublished document. Department of Veterinary Clinical Sciences, Massey University, Palmerston North, New Zealand.

Nielen, M., Jalvingh, A.W., Dijkhuizen, A.A., and Lattuada, R. (1996). Requirements for a geographical information system to be used during a foot-and-mouth disease outbreak. Proceedings of the Annual Meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Glasgow, 178-184.

O.I.E. (1997). Animal health status and disease control methods in member countries in 1996. Office International dés Epizooties, Paris.

Sanson, R.L. (1993). The development of a decision support system for an animal disease emergency. Unpublished PhD Thesis, Massey University, Palmerston North.

Sanson, R.L., and Pearson, A. (1997). Agribase – a national spatial farm database. Epidémiologie et Santé Animale 31-32:12.16.1-12.16.2

Stern, M.W. (1994). INTERSPREAD user manual. Unpublished document. Department of Veterinary Clinical Sciences, Massey University, Palmerston North, New Zealand.

Teuffert, J., and Schlüter, H. (1994). Epidemiologische Aspekte eines Monitoring am Beispiel der europäischen Schweinepest. DVG Tagung der Fachgruppe Epidemiologie und Dokumentation, Berlin, 117-134.

CHAPTER 2.7

EXPERT SYSTEM COMPONENTS OF EpiMAN-SF

Parts of this chapter will be published in the *Proceedings of the 15th International Pig Veterinary Society Congress, Birmingham* in a paper by: Stärk, K.D.C., Nielen, M., and Morris, R.S. (1998) Setting priorities for investigating movement traces during a classical swine fever outbreak.

1. Introduction

Expert systems (ES) are computer programs that use knowledge to solve complex problems in specific problem areas or domains (Feigenbaum, 1992, see also introduction to CHAPTER 1.6). The knowledge can be obtained from human domain experts by a knowledge engineer using structured interviews (Scott *et al.*, 1991; Meyer and Booker, 1991), from the literature or empirically from data (Mani *et al.*, 1997). The knowledge base consists of heuristics or 'rules of thumb' in the form of 'IF ... THEN' statements. The knowledge base is used for reasoning by a part of the programme called the 'inference engine'.

The ES described in this chapter is designed for the use in the domain of exotic disease management in the livestock industry. It is an integral part of the decision-support system EpiMAN-SF (see CHAPTER 2.6) and is used for different aspects during the control of a classical swine fever (CSF) outbreak, specifically to identify high-risk periods for the transmission of the disease, for risk classification of conveyors and for risk classification of farms. The ES is used by tracing officers and for rostering farms for farm visits. Typically, the ES is run in the background with no necessary interventions by the user.

The output of the ES is mainly the risk classification of conveyors and farms. These risk categories are linked to recorded observations in the database (contact events, farms) and displayed together with selected characteristics of these observations. The user utilises the additional information to make decisions on priorities among competing tasks when he/she is under time pressure. More specifically, the ES helps to assure that high-risk traces are processed before lower-risk traces and that high-risk farms are visited before lower-risk farms.

2. Rule base of EpiMAN-SF

The rule base used by the ES components of EpiMAN-SF is described in the following paragraphs in this chapter. The principles for risk classification are based on EpiMAN-FMD, the first programme in the EpiMAN suite which was originally designed for the control of vesicular diseases (Sanson, 1993).

EpiMAN-SF uses heuristic rules for the following procedures:

- calculation of time periods (incubation time, time until onset of clinical signs) based on the virus strain
- risk classification of contacts between farms (conveyors)
- risk classification of farms 'at risk' according to their contacts and other episodes
- scheduling of farm visits

2.1 Rules for calculating time periods

For the purpose of classifying farm contacts in terms of the risk of the conveyors involved and their potential for disease transmission, it is important to know at which stage of the disease they have occurred. For the calculation of the so-called 'period of infection' data related to the

restricted property (RP) such as date of infection and date of occurrence of contact, are needed as well as information on the virus strain.

Information on virus strain is unlikely to be available early in an epidemic. Therefore, the system will have to make a decision about how to handle calculations in the absence of knowledge of strain characteristics such as for example incubation period. In principle, a conservative approach should be chosen. Three possibilities are to be considered: 1) use low-moderate virulence characteristics, 2) use high virulence characteristics, or 3) use some third value. The consequences of choosing a low-moderate virulence strain instead of a high virulence strain will now briefly be discussed (TABLE 55).

TABLE 55. Differences between low-moderate virulence strains and high-virulence strains and effect of assumption on EpiMAN-SF decisions

Characteristics of low-moderate virulence when compared with high virulence strain	Effect
Longer incubation period	Estimated time of infection earlier.
	This will lead to additional forward traces because the incubation period has been extended. These contacts would not have been traced if the strain was high virulence because they then would have happened before the estimated time of infection. Traces falling into the estimated time of infection period under the high virulence assumption now are in the incubation period and thus reduced in risk for forward traces, but still have to be traced.
	The period for tracing back is moved towards an earlier date because the time of infection is expected earlier. The period will also be longer and more contacts will need to be traced. Later contacts will not be traced back. This may result in the source of the outbreak not being found.
Virus spread within farm slower	The consequence of this is that the probability of infected pigs leaving the farm is decreased and occurs later. Yet, forward traces of live pigs will always be very-high-risk traces with the probability of infection being additional information only. Therefore, no changes in risk categories are expected. However, there will be an increase in the number of forward traces as the expected time of infection is now earlier.
Secondary outbreaks occur later and at a lower incidence rate	Because of this the protection zone should be maintained for a longer time period.

In summary, the assumption of a low-moderate virulence strain results in a more cautious approach to tracing. Although a higher number of traces will have to be processed, the increased sensitivity is necessary in order to detect secondary outbreaks under all circumstances. Movement control will tend to be maintained longer in order to prevent further spread. The assumption of low-moderate virulence should therefore be used as default in a situation where there is uncertainty about the virulence of the virus. However, this may result in not back-

tracing some contacts if the virus turns out to be of high virulence. Once the virus has been characterised these traces will have to be processed immediately. In the initial phase called 'undetermined strain' the settings are equal to 'low virulence'.

If the source of infection for a farm is not known, then also the exact date of infection will be missing. Because this date is very important in order to accurately classify contacts into risk categories (FIGURE 31), it will have to be estimated. This can be done by calculating backwards from the onset of clinical signs subtracting one or several incubation period(s) according to the virulence of the virus. Obviously, this system will be very inaccurate due to the biological variability of the incubation period, even more so if the time of onset of clinical signs is also missing and the only available date is the date of diagnosis. It is also extremely difficult to determine how many generation times have already passed on a farm before the disease is detected.

In order to obtain a more accurate way to estimate the day of infection, attempts have been made to develop a system based on the laboratory results of a herd at the time of diagnosis or culling. Laevens *et al.* (1997) collected data on antibody occurrence in an experimental setting. With the help of a logistic regression equation they then calculated the time of infection for three herds with a known infection date. These herds had been naturally infected during the 1993-1994 epidemic in Belgium. In these three cases the calculated day of infection fell within 1-2 days of the true infection date. Although this approach seems to be very promising, it needs more validation before it can be generally applied. Also, because it is based on pen prevalence, it requires sampling of every pig on an infected herd. This will hardly be possible in a large outbreak or when large herds are involved. Furthermore, the serology results will not become available until some time after the herd is culled.

Another simpler idea is to use the occurrence of antibodies as an indicator of 'old' infections. It is known that it takes at least 3-4 weeks until antibodies can be detected in a herd (Terpstra, 1987). This appears to be more practical, but again one would need to wait until all samples are analysed. As back logs of up to 4 weeks have been observed in large outbreaks (Benard, 1997), this type of information may not be available for tracing, which takes place immediately after a farm has been diagnosed as infected.

Until more research results in this area become available, EpiMAN-SF will have to use inaccurate estimation rules based on 'flat' incubation periods and date of onset of clinical signs (TABLE 56). The maximum incubation period is 21 days for low-moderate virulence (18 days reported by Dahle and Liess, 1995) and 7 days for high virulence (Depner *et al.*, 1994). These rules are only applicable as long as the source of infection of a farm is not known. It is possible that a farmer is unable to identify a specific date of onset of clinical signs but he/she is able to make a statement similar to one of the following examples: 1. *There were definitely no clinical signs before date x.* or 2. *I am sure that there were clinical signs after date x.* The system will therefore have to provide the possibility of entering a range for the onset of clinical signs rather than one single date. The following entries should be considered: no clinical signs before date x, most likely onset of clinical signs on date y, definitely clinical signs after date z. Only one or several entries may be available for a specific farm.

These calculations are run by the ES in the background after a property has been declared to be an RP and after the data from the epidemiological investigation performed on that farm is entered. The process is initiated if in the farm field 'source farm' is left empty and if the field

'infection date' is left empty. The ES then automatically puts its calculated time period in that field and the field 'estimated by ES' is ticked (other values: 'estimated by epidemiologist', 'confirmed').

TABLE 56. Rules for estimating the period when a farm became infected

RULE 1.		Period of introduction of infection
	If	Infection date and source farm not known and virus strain = (undetermined or moderate-low virulence) and date of earliest clinical signs known
	Then	Estimated period of infection = date of earliest clinical signs – (14-21 days)
RULE 2.		
	If	Infection date and source farm not known and virus strain = high virulence and date of earliest clinical signs known
	Then	Estimated period of infection = date of earliest clinical signs – (4-7 days)
RULE 3.		
	If	Infection date and source farm not known and virus strain = (not known or moderate-low virulence) and date of earliest clinical signs not known
	Then	Estimated period of infection = between (date of diagnosis – 21 days) and (date of diagnosis –35 days)
RULE 4.		
	If	Infection date and source farm not known and virus strain = high virulence and date of earliest clinical signs not known
	Then	Estimated date of infection = between (date of diagnosis – 14 days) and (date of diagnosis – 21 days)

2.2 Rules for classification of conveyors

As part of the epidemiological investigation of a restricted property (RP), a list of all contacts before and after the likely date of infection is obtained. Because the time of infection may be some time back, the period to be covered may be considerable. During a CSF outbreak in Germany, it was recommended that all contacts occurring 60-70 days prior to diagnosis should be recorded (Schimansky and Seidler, 1996). The problem of recall bias is obvious in such a situation. Once again, if the time of infection can be reliably estimated, the relevant time period can be reduced and more specific questions can be asked.

The list of contacts is processed by the tracing team in the emergency headquarter. First, the conveyors involved in an event are identified and treated separately (split tracing), for example a transport of pigs consists of the conveyors pigs, truck and driver. The conveyors may end up in different places and are thus listed individually. Each conveyor is assigned a risk

category by the ES. The details of a conveyor are confirmed by the tracing officer over the phone. If a trace results in a secondary trace, the latter have to be processed similarly. For example if pigs went to a market, other pigs leaving that market need to be traced as well.

Rules for risk classification of conveyors are based on:

1. Contact on or off the farm (direction)
2. Conveyor type: Susceptible animal (= pig), non-susceptible animal (= all other species), swill (garbage feed), slurry (from pigs), semen (for artificial insemination of pigs), other product, vehicle, person
3. Pig contact on farm (for persons and vehicles only)
4. Origin/destination of conveyor (pig farm yes/no)
5. Time of encounter (FIGURE 31).

When tracing back, the highest risk occurs during the period around the expected infection date. Before and after, the risk is reduced. If a contact occurred more than the maximum incubation period before the onset of clinical signs (before the likely period of infection), the risk is virtually nil, assuming that the onset of clinical signs is correctly recorded. For tracing forward, the high-risk period starts with the onset of clinical signs. During the incubation period, the risk is reduced (except for transports of live pigs) and it is negligible before the expected time of infection. The risk is nil after the diagnosis has been made because the farm will then be under movement restriction.

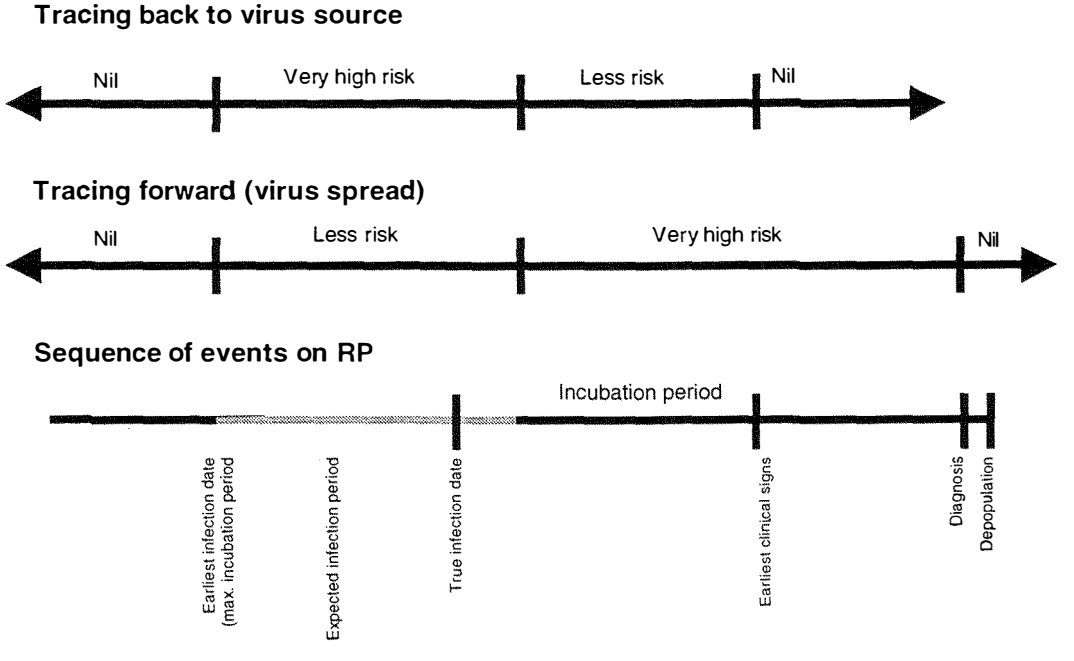

FIGURE 31. Time frames for forward and backward tracing and relationship to risk classification of traces

The following rules assume that the source of the movement is an RP and the direction is either 'on' (tracing back, TABLE 57) or 'off' (tracing forward, TABLE 59). If the source of a

conveyor is not an RP but another farm, for example a neighbouring property of a pre-emptively slaughtered farm then the rules for tracing back and forward are listed in TABLE 58 and TABLE 60. The latter two tables also contain the rules for secondary traces. For an overview of the risk classes also see TABLE 61.

TABLE 57. Rules for classifying conveyors when tracing back to identify the source of infection and the source of the conveyor is an RP

RULE 5.		Eliminate movements before confirmed infection date
	If	Infection date and source farm known and movement date before infection date
	Then	Risk = nil
RULE 6.		Conveyor is source of infection
	If	Infection date and farm source known and conveyor is causing infection on to farm
	Then	Risk = very high and trace conveyor
RULE 7.		Eliminate contacts before maximum incubation period
	If	Infection date and source farm not known and contact before period of infection and conveyor type NOT susceptible animal
	Then	Risk = nil
RULE 8.		Pig transport 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = susceptible animal (pig)
	Then	Possible source and risk = very high and trace back until disease is found
RULE 9.		Pig transport 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = susceptible animal (pig)
	Then	Possible source and risk = high and trace back until disease is found
RULE 10.		Pig transport 3
	If	Infection date and source farm not known and encounter before period of infection and conveyor type = susceptible animal (pig)
	Then	Possible source and risk = medium

		and trace back until disease is found
RULE 11.		Vehicle with pig contact 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = vehicle with pig contact
	Then	Possible source and risk = high and trace back to last C&D
RULE 12.		Vehicle with pig contact 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = vehicle with pig contact
	Then	Possible source and risk = medium and trace back to last C&D
RULE 13.		Vehicle without pig contact 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = vehicle without pig contact
	Then	Possible source and risk = low and trace back to last C&D
RULE 14.		Vehicle without pig contact 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = vehicle without pig contact
	Then	Possible source and risk = very low and trace back to last C&D
RULE 15.		Person with pig contact or from pig farm 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = person with pig contact or conveyor type = person coming from pig farm
	Then	Possible source and risk = high and trace back x days
RULE 16.		Person with pig contact or from pig farm 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = person with pig contact or conveyor type = person coming from pig farm
	Then	Possible source and risk = medium and trace back x days

RULE 17.	Person without pig contact and not from pig farm 1	
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = person without pig contact and not coming from pig farm
	Then	Possible source and risk = low and trace back x days
RULE 18.	Person without pig contact and not coming from pig farm 2	
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = person with pig contact or conveyor type = person coming from pig farm
	Then	Possible source and risk = very low and trace back x days
RULE 19.	Swill 1	
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = swill (garbage feed)
	Then	Possible source and risk = high and trace back to origin
RULE 20.	Swill 2	
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = swill (garbage feed)
	Then	Possible source and risk = medium and trace back to origin
RULE 21.	Semen 1	
	If	Infection date and source farm not known and encounter in period of infection[1] and conveyor type = porcine semen
	Then	Possible source and risk = high and trace back to origin
RULE 22.	Semen 2	
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = porcine semen

[1] According to observations made during an outbreak in the Netherlands in 1997, farms infected by semen were detected on average 14 days later than farms infected by other conveyors. This is possibly due to the slow spread of virus among pigs housed in individual stalls (dry sow housing). Therefore the period of infection may be different or longer for all rules involving semen.

	Then	Possible source and risk = medium and trace back to origin
RULE 23.		Non-susceptible animal 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = non-susceptible animal
	Then	Possible source and risk = low and trace back to origin
RULE 24.		Non-susceptible animal 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = non-susceptible animal
	Then	Possible source and risk = very low and trace back to origin
RULE 25.		Slurry 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = slurry
	Then	Possible source and risk = medium and trace back to origin
RULE 26.		Slurry 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = slurry
	Then	Possible source and risk = low and trace back to origin
RULE 27.		Other product 1
	If	Infection date and source farm not known and encounter in period of infection and conveyor type = other product
	Then	Possible source and risk = low and trace back to origin
RULE 28.		Other product 2
	If	Infection date and source farm not known and encounter after period of infection and before onset of clinical signs and conveyor type = other product
	Then	Possible source and risk = very low and trace back to origin

TABLE 58. Rules for classifying conveyors when tracing back to identify source of infection and source of conveyor is not an RP

RULE 29.	Source is other farm (not RP)	
	If	Source = other farm (e.g. pre-emptive slaughter, neighbour)
	Then	Risk = one level less than if from RP (risk – 1)
RULE 30.	Source is other conveyor	
	If	Animal contact
	Then	Risk = risk of source conveyor
	Else	Risk = risk of source conveyor – 1 (or = nil if source risk = nil)

TABLE 59. Rules for classifying conveyors when tracing forward to identify secondary outbreaks and source of conveyor is an RP

RULE 31.	Eliminate contacts before infection 1	
	If	Infection date and source farm known and movement before confirmed infection date
	Then	Risk = nil
RULE 32.	Eliminate contacts before infection 2	
	If	Infection date and source farm not known and encounter before period of infection and conveyor type NOT susceptible animal
	Then	Risk = nil
RULE 33.	Pigs 1	
	If	Movement in period of infection and conveyor type = susceptible animals
	Then	Risk = high trace forward to final destination
RULE 34.	Pigs 2	
	If	Movement after period of infection and conveyor type = susceptible animals
	Then	Risk = very high trace forward to final destination
RULE 35.	Pigs 3	
	If	Movement before infection period and conveyor type = susceptible animals

	Then	Risk = low trace forward to final destination
RULE 36.		Person with pig contact or going to pig farm 1
	If	movement date in period of infection and conveyor type = person with pig contact or going to pig farm
	Then	Risk = medium and trace forward for x days
RULE 37.		Person with pig contact or going to pig farm 2
	If	movement date after period of infection and conveyor type = person with pig contact or going to pig farm
	Then	Risk = high and trace forward for x days
RULE 38.		Person without pig contact and not going to pig farm 1
	If	Movement date in period of infection and conveyor type = person without pig contact and not going to pig farm
	Then	Risk = very low and trace forward for x days
RULE 39.		Person without pig contact and not going to pig farm 2
	If	Movement date after period of infection and conveyor type = person without pig contact and not going to pig farm
	Then	Risk = low and trace forward for x days
RULE 40.		Swill 1
	If	Movement date in period of infection and conveyor type = swill
	Then	Risk = medium and trace forward until considered no risk
RULE 41.		Swill 2
	If	Movement date after period of infection and conveyor type = swill
	Then	Risk = high and trace forward until considered no risk
RULE 42.		Semen 1
	If	Movement date in period of infection and conveyor type = porcine semen
	Then	Risk = medium and trace forward until final destination
RULE 43.		Semen 2
	If	Movement date after period of infection and conveyor type = porcine semen

	Then	Risk = high and trace forward until final destination
RULE 44.		Non-susceptible animal 1
	If	Movement date in period of infection and conveyor type = non-susceptible animal
	Then	Risk = very low and trace forward x days
RULE 45.		Non-susceptible animal 2
	If	Movement date after period of infection and conveyor type = non-susceptible animal
	Then	Risk = low and trace forward x days
RULE 46.		Vehicle with pig contact 1
	If	Movement date in period of infection and conveyor type = vehicle with pig contact
	Then	Risk = medium and trace forward until C&D and C&D not essential
RULE 47.		Vehicle with pig contact 2
	If	Movement date after period of infection and conveyor type = vehicle with pig contact
	Then	Risk = high and trace forward until C&D and C&D not essential
RULE 48.		Vehicle without pig contact 1
	If	Movement date in period of infection and conveyor type = vehicle with no pig contact
	Then	Risk = very low and trace forward until C&D and C&D not essential
RULE 49.		Vehicle without pig contact 2
	If	Movement date after period of infection and conveyor type = vehicle with no pig contact
	Then	Risk = low and trace forward until C&D and C&D not essential
RULE 50.		Other product 1
	If	Movement date in period of infection and conveyor type = other product
	Then	Risk = very low and trace forward until safely rendered

RULE 51.		Other product 2
	If	Movement date after period of infection and conveyor type = other product
	Then	Risk = low and trace forward until safely rendered
RULE 52.		Slurry 1
	If	Movement date in period of infection and conveyor type = slurry
	Then	Risk =low and trace forward to destination
RULE 53.		Slurry 2
	If	Movement date after period of infection and conveyor type = slurry
	Then	Risk = medium and trace forward to destination

TABLE 60. Rules for classifying conveyors when tracing forward to identify secondary outbreaks and the source of conveyor is not an RP

RULE 54.		for all movements off farm
	If	Farm infection status = suspect or pre-emptive slaughter
	Then	Give (risk level –1) to conveyor
RULE 55.		for all conveyor-conveyor encounters
	If	Direct animal contact
	Then	Give same risk rating to secondary conveyor
RULE 56.		for all conveyor-conveyor encounters
	If	No direct animal contact
	Then	Give (risk level-1) to secondary conveyor

2.3 Rules for re-classification of conveyors after the virus strain becomes known

After the virus strain in an epidemic has been identified not to be of low-moderate virulence as assumed by default, the ES will go back and re-assess all classifications of contacts made earlier but not yet finalised. It will add the updated classifications to a table and produce a report for the user. If the classification of the virus is low-moderate virulence, no changes will be necessary.

TABLE 61. Risk classification table for conveyors (source = RP)

		SUSCEP-TIBLE ANIMALS[1]	VEHICLE With pig contact	PERSON With pig contact or contact to pig farm	PORCINE SEMEN	SWILL	SLURRY	NON-SUSCEP-TIBLE ANIMALS	PERSON Without pig contact and no contact with pig farm	VEHICLE Without pig contact	OTHER PRODUCTS
TRACE BACK	Date in period of infection	VERY HIGH	HIGH	HIGH	HIGH	HIGH	MEDIUM	LOW	LOW	LOW	LOW
	Date after period of infection and before clinical signs	HIGH	MEDIUM	MEDIUM	MEDIUM	MEDIUM	LOW	VERY LOW	VERY LOW	VERY LOW	VERY LOW
TRACE FORWARD	Date after period of infection	VERY HIGH	HIGH	HIGH	HIGH	HIGH	MEDIUM	LOW	LOW	LOW	LOW
	Date in period of infection	HIGH	MEDIUM	MEDIUM	MEDIUM	MEDIUM	LOW	VERY LOW	VERY LOW	VERY LOW	VERY LOW

[1] Pigs

2.4 Validation of rules for the risk classification of conveyors

2.4.1 Material and Methods

Ten experienced tracing officers who had been actively involved in a CSF outbreak within the last 5 years, completed a questionnaire (APPENDIX F) describing 31 fabricated contacts on and off an imaginary RP. For each movement the following information was provided: date of contact, number of people, type of vehicle(s), contact with pigs (yes / no), origin and destination of contact (farm, village, etc.). The history of the RP included an estimated date of infection, the date of first clinical signs and the date of diagnosis. It was also mentioned that it was unlikely that the virus was a very high virulent strain. Each contact was classified in terms of tracing forward and tracing back using a scale from 0 (nil risk) to 5 (very-high risk).

For each question, the median risk score was calculated (NCSS97, Number Cruncher Statistical Systems, Kaysville, Utah) using the answers given by all experts. This value was then subtracted from the actual score given in order to measure the difference between individual answers and the group median. This returned the difference in score categories. A negative value indicates that the expert was putting a higher risk rating than the median (cautious expert), a positive value indicates a more daring expert. These results were graphically analysed using Box plots.

The comparison of expert and ES classifications was performed by subtracting the score given by the ES from the scores given by the experts. A negative value indicates that the ES was putting a higher risk rating than the human experts (cautious), a positive value indicates a more daring assessment by the ES.

2.4.2 Results

There was generally good agreement among experts. The risk ratings differed from the median by ≤1 category in 86% of back-traces and 77% of forward-traces (FIGURE 32).

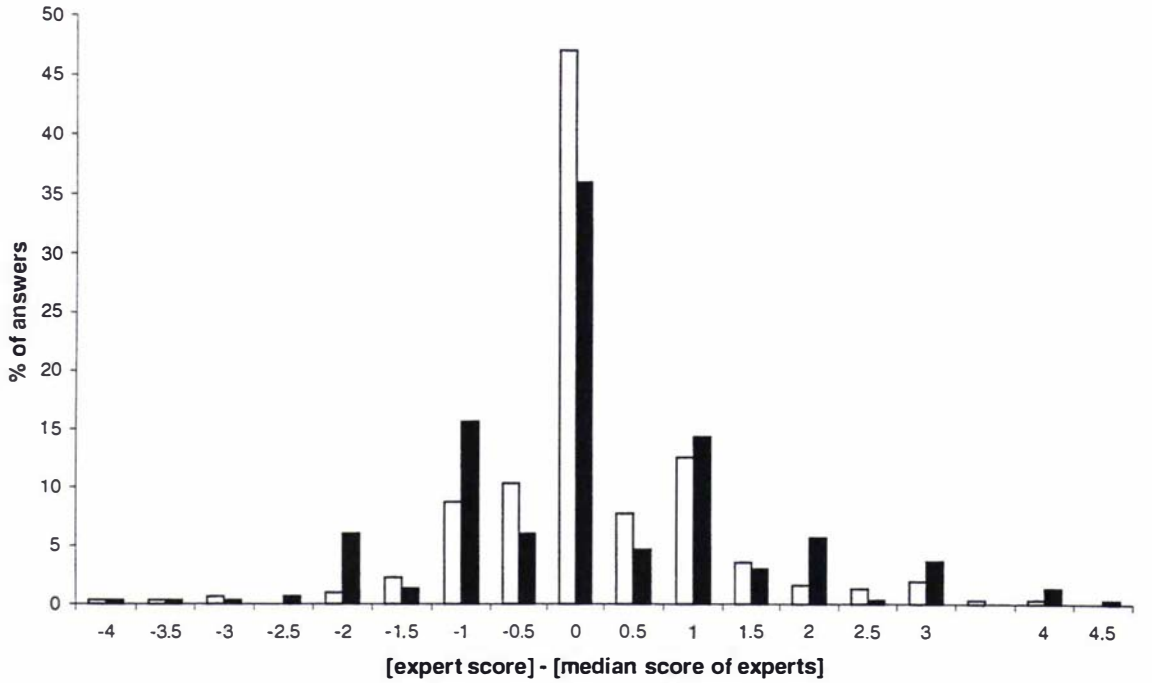

FIGURE 32. Agreement among experts when classifying contacts in an imaginary classical swine fever outbreak expressed as difference from the median (n = 310; ❑ = tracing back, ■ = tracing forward).

However, when the results were analysed by expert and by question, experts with a generally more cautious or daring attitude could be identified (FIGURE 33) as well as questions with significant disagreement among the participants (FIGURE 34). In FIGURE 33, experts with a cautious attitude are, for example, Expert 7 and Expert 8 for forward traces. Disagreement among experts was mostly related to the risk of swill feeding (Questions 14, 18 and 25), and semen for artificial insemination (Question 9) as well as rendering trucks (Question 6).

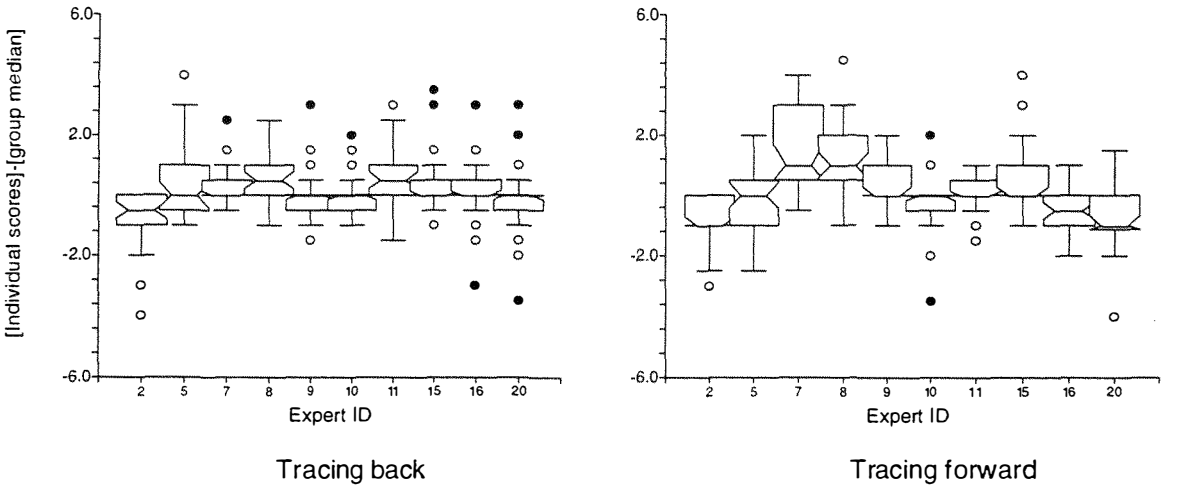

FIGURE 33. Differences between median risk ratings and individual ratings for 10 tracing officers (n = 31 for each expert).

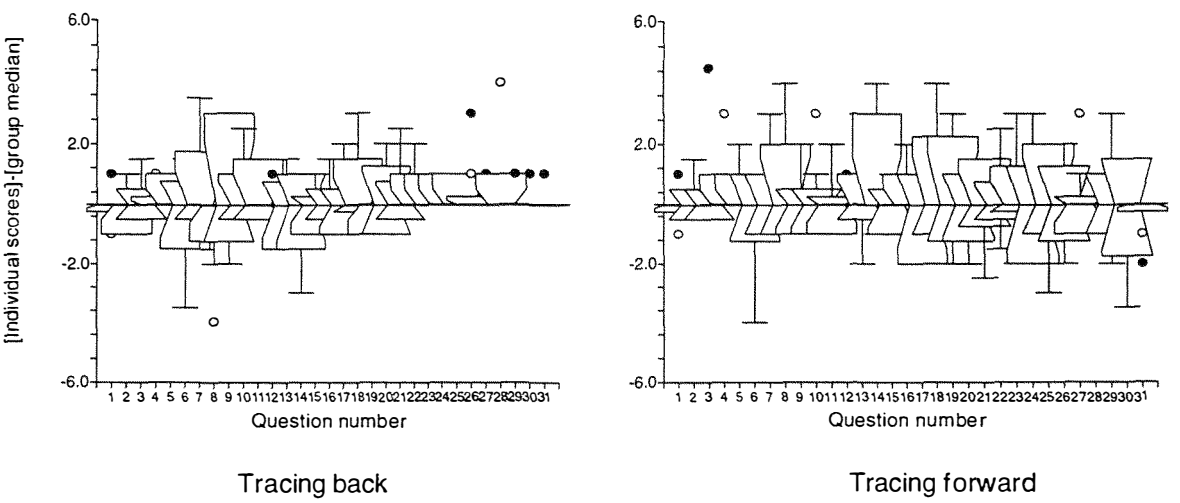

FIGURE 34. Differences between median risk ratings and individual ratings for 31 imaginary traces classified by human experts (n=10 for each question).

The risk ratings given by the experts were then compared with the classifications made using the ES. The two classifications agreed or differed by no more than one category in 62% (tracing forward) and 67% (tracing back), respectively (FIGURE 35). In the majority of the remaining cases, the ES made a more conservative classification. However, in 5% of the forward traces the human experts assigned a risk level that was at least 2 levels higher than the ES rating. This difference was mostly due to disagreement with respect to the risk of rendering trucks and swill feeding (data not shown).

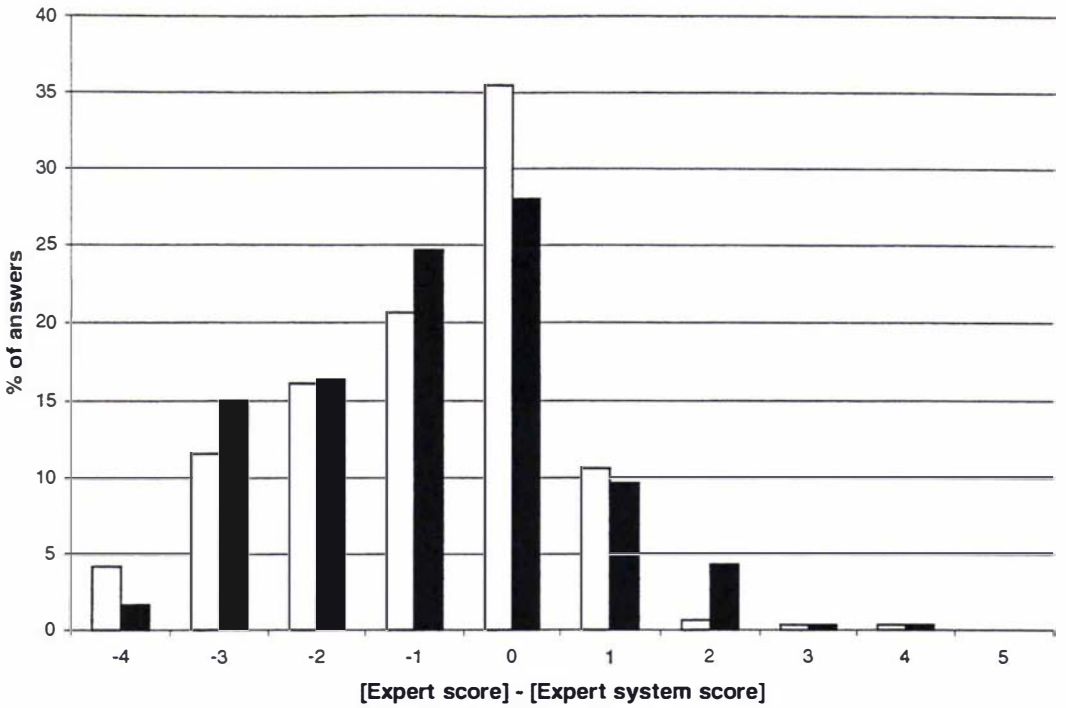

FIGURE 35. Agreement between human experts and an expert system when classifying contacts in an imaginary classical swine fever outbreak expressed as difference from the human rating (n = 310; ❑ = tracing back, ■ = tracing forward

The cautious ratings assigned by the ES resulted in an increased number of medium and high-risk traces, both for tracing back and forward (TABLE 62).

TABLE 62. Frequency (%) of tracing classifications by human experts and expert system

Risk category	Human experts		Expert system	
	Tracing back	Tracing forward	Tracing back	Tracing forward
Very-high	5.5	16.0	3.2	9.7
High	3.9	7.7	16.1	25.8
Medium	5.5.	12.0	22.6	32.3
Low	7.4	13.3	12.9	6.5
Very-low	29.0	24.7	19.4	22.5
Nil	48.7	26.3	25.8	3.2

2.5 Rules for the classification of farms

During an epidemic all farms 'at risk' of being infected with swine fever need to be visited in order to assess the infection status. A farm becomes 'at risk' if it is either located in the con-

trol zone or if an episode is entered (TABLE 63). An episode is an event that has the potential to transmit CSF (Sanson, 1993).

Multiple episodes may be applicable to one farm. During an outbreak situation, when resources are limited, the field teams should be assigned to high-risk properties first. Therefore, some kind of a summary indicator of risk is desirable and has been developed in EpiMAN-FMD (Sanson, 1993). It currently works as follows: Episodes are recorded for each farm in an episode table. For each episode, a risk rating is applied and a summary entry for the farm recorded in the 'at risk' file. Field teams are assigned to visit farms according to this list.

One difference with CSF is that in addition to the farms put 'at risk' by episodes, all farms within the protection zone have to be visited once within 7 days after the RP has been declared and again no earlier than 30 days later. These farms have to be included in the roster list. All farms in the surveillance zone have to be visited as well. The different visit types required have been described in CHAPTER 2.6. The 'at risk' list will contain three types of farms:

1. Farms 'at risk' by episodes with risk rating.
2. Farms 'at risk' due to location in protection zone with risk rating.
3. Farms 'under surveillance' due to location in surveillance zone.

If more than one episode is recorded for one farm the summary risk could be set to be equivalent to the highest composite risk level. The summary then needs to be updated whenever an episode is added. However, it has been argued that this may not be the most appropriate way to calculate a summary risk, because a low-risk conveyor could become significant if the contact occurs frequently (see CHAPTER 2.4). Also the categories used for the different risk levels may create the impression of a linear increase in risk while in reality this is probably not the case.

In order to develop a more realistic system to calculate the summary risk for a farm, the following assumptions were made:

1. a transmission probability can be defined for each conveyor category (TABLE 63). As the exact transmission risk for each conveyor is not known, the expected value of a probability distribution is used.

2. a transmission probability can be defined for each episode type that is not trace-related (TABLE 63). Again, because the exact transmission risk for each conveyor is not known, the expected value of a probability distribution for each event is used.

3. For each conveyor and episode with transmission probability p_i, the survival probability q_i is calculated as $q_i=1-p_i$.

4. If several conveyors and/or episodes are recorded for one farm, the over-all survival probability Q is the product of all composite survival probabilities q_i. FIGURE 36 illustrates how the frequency of a conveyor contact contributes to the decrease of the survival probability.

5. The farms are scheduled for visiting according to their probability to be infected P, which is calculated as $P=1-Q$.

At present, neither field nor research data are available for obtaining estimates of the transmission probabilities for movements and episodes. Therefore, the values listed in TABLE 63 have to be used cautiously. They should be interpreted as weighting factors expressing the urgency of follow-up rather than as transmission probabilities in the true sense of the meaning.

TABLE 63. Transmission probabilities for conveyors and episodes

Episode type	Transmission probability p_i	Survival probability q_i	Distribution of p_i if stochastically simulated (minimum; most likely; mean; maximum)[a]
Farm involved in trace			
Conveyor:			
Very-high risk	0.95	0.05	BetaSubj (0.9;0.95;0.95;1)
High risk	0.75	0.25	BetaSubj (0.6;0.75;0.79;1)
Medium risk	0.50	0.50	BetaSubj (0.4;0.5;0.57;0.75)
Low risk	0.20	0.80	BetaSubj (0.1;0.2;0.29;0.5)
Very-low risk	0.02	0.98	BetaSubj (0.01;0.02;0.03;0.1)
Clinical signs reported by veterinarian or farmer	0.99	0.01	BetaSubj (0.85;0.99;0.98;1)
Neighbouring farm	0.90	0.10	BetaSubj (0.5;0.9;0.85;1)
Swill-feeding farm	0.30	0.70	BetaSubj (0.2;0.3;0.33;1)
Farm in protection zone	0.40	0.60	BetaSubj (0.1;0.5;0.4;0.6)
User defined	User defined		

[a]Because in a real outbreak all contacts and episodes of the same category need to receive equal priority, probability distributions can only be used for research simulations.

When using the system described above, EpiMAN-SF will produce a list of farms 'at risk' and their respective probabilities to be infected. For farms without episodes, which are located in the surveillance zone, the probability to be infected is 0, although some of them may actually be at higher risk due to a contact that remained non-reported. Farms are selected from high-risk to low-risk.

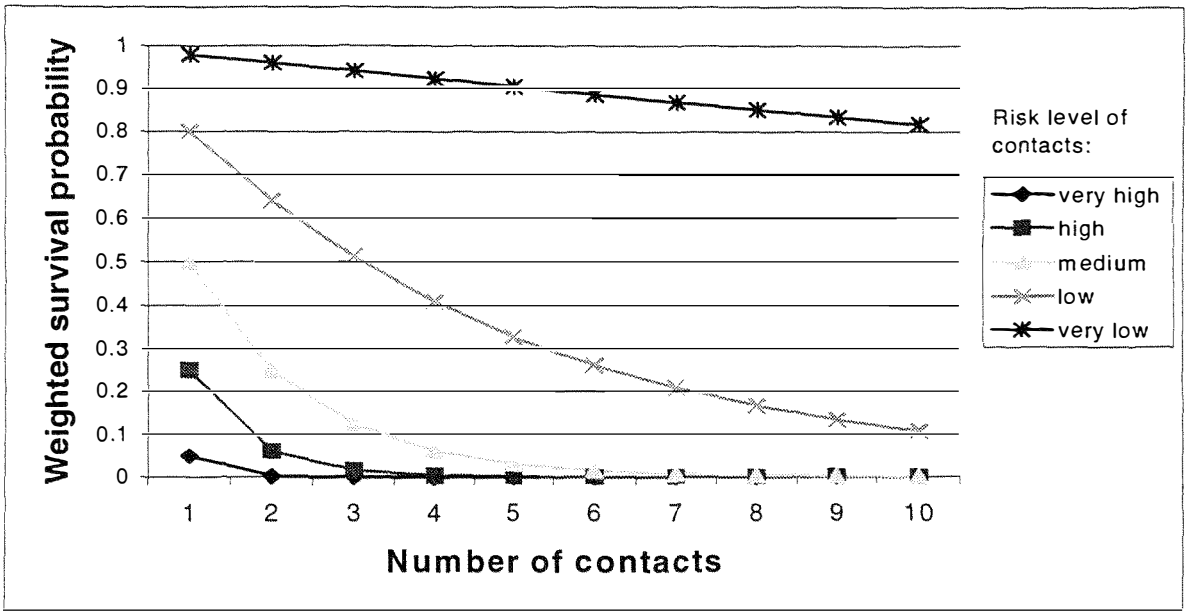

FIGURE 36. Influence of number of conveyor contacts with different risk categories on the survival probability of a farm to remain free of swine fever

In order to evaluate the impact of the above assumptions, a set of scenarios of different farms with different conveyor and episode combinations was created. The risk classification of each scenario was simulated using the software @RISK (v. 3.5e, Palisade Corporation, Newfield, USA). The detailed description of the scenarios is given in TABLE 64. Each scenario defines a farm with a given combination of episodes and a number of different recorded conveyors (movements). All farms are assumed to be in the protection zone, therefore the episode 'Protection zone' is listed for all of them. Scenarios A-D are farms with no contacts with the related RP. Consequently the frequency of all conveyor categories is 0. In scenarios E-I, contacts with the related RP have occurred. In Scenarios J-M, all farms are direct neighbours of the related RP, but they had different levels of contacts. In scenario J a professional pig production-related contact was assumed including pig transport (very-high risk), and at the opposite end of the range in scenario M only social contacts were assumed but at a high frequency.

Each scenario was simulated with 300 iterations. As a result of the uncertainty related to the event-specific transmission probabilities, the result is a distribution of survival probabilities for which the mean and the 5% and 95% percentiles were calculated and plotted in FIGURE 37. As the computed figures are not true survival probabilities they should rather be interpreted as an estimate of the urgency to visit a farm. The lower the calculated value, the higher the priority for a visit of a farm is.

TABLE 64. Characteristics of scenarios used to explore farm risk classifications

Scenario	Episode				Conveyor frequencies				
	Protection zone	Clinical signs	Swill feeding	Neighbouring farm	Very-high risk	High risk	Medium risk	Low risk	Very-low risk
A	1	0	0	0	0	0	0	0	0
B	1	1	0	0	0	0	0	0	0
C	1	0	1	0	0	0	0	0	0
D	1	0	0	1	0	0	0	0	0
E	1	0	0	0	1	0	0	0	0
F	1	0	0	0	0	1	0	0	0
G	1	0	0	0	0	0	1	0	0
H	1	0	0	0	0	0	0	2	0
I	1	0	0	0	0	0	0	0	10
J	1	0	0	1	1	1	0	0	0
K	1	0	0	1	0	0	1	0	0
L	1	0	0	1	0	0	0	2	0
M	1	0	0	1	0	0	0	0	10

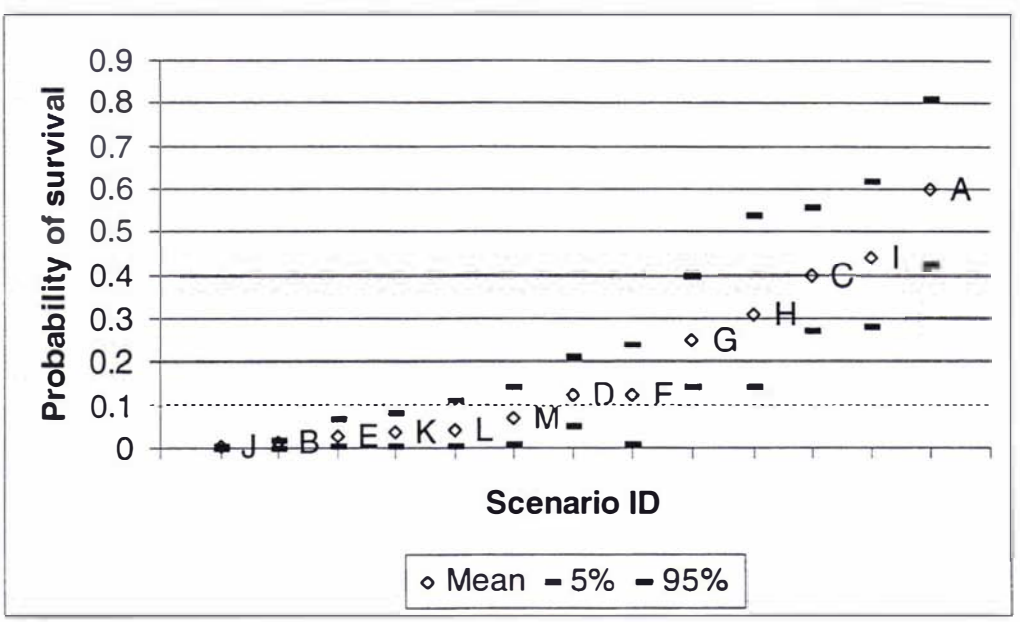

FIGURE 37. Mean ranked survival probability and 5% and 95% percentiles of 13 farms with different episode and conveyor scenarios. It is assumed that farms with a survival probability of <0.1 are to be given highest priority for epidemiological investigation.

This figure shows that scenario J (combination of high-risk contact and neighbouring location), scenario B (clinical signs) and E (very-high risk contacts) have high priority values with

narrow distributions below 0.1. In scenario D (neighbouring farm) and F (high risk contact) the 45th percentile lies above 0.1. Medium risk contacts (Scenario G) will receive lower prioriteies, yet if a medium-risk conveyor is recorded in a neighbouring farm (Scenario K) the farm moves up in the priority list. The same is true for neighbours with more than 1 low-risk contact (Scenario L) and a relatively large proportion of farms with very-low risk but frequent contacts with the related RP (Scenario M). The distance between the 5% and 95% percentile reflects the uncertainty related to the classification of this type of farm, which is predetermined by the distribution parameters (TABLE 63). For example, there is more uncertainty with respect to the transmission probability of a farm located in the protection zone and no other episodes (Scenario A) than for a farm where clinical signs are already observed (Scenario B).

In an outbreak situation where only limited resources are available, the tracing manager may chose to use some threshold value to discriminate between farms that need to be visited immediately (today) and those that may be postponed. For example, all farms with a value of <0.1 could qualify for immediate visit. By plotting the cumulative probability of survival for a scenario, one can now explore the probability of this type of farm not being visited on the same day. As an example, the cumulative probability of survival for scenario L is shown in FIGURE 38. The probability of a survival value of <0.1 (threshold for farms to be immediately visited) is approximately 0.9. Consequently, the probability of such a farm not being visited on the same day is 10%.

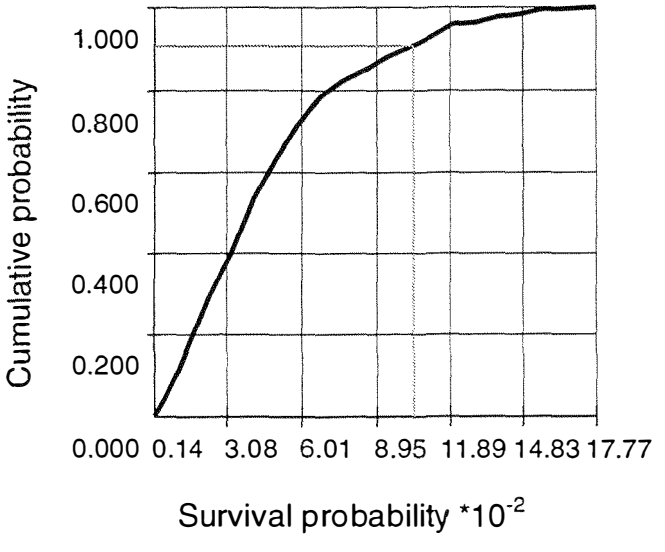

FIGURE 38. Cumulative probability of survival for a neighbouring farm with one medium risk conveyor

Finally, simulation analysis can also be used to identify influential variables using sensitivity analysis. This method uses the results of all simulation iterations in combination with linear regression to calculate coefficients for each uncertain variable (variable value drawn randomly according to distribution characteristics). The coefficients are interpreted as the influence of the uncertainty in the variable on the variability of the outcome, in this case the survival probability of the farm. To illustrate this approach, the following four scenarios were

analysed: farms with 1, 2, 3, and 4 low risk conveyors, respectively, one each of the other conveyor types and no other recorded episodes. Again, the simulation was performed using @RISK and running 300 iterations each.

The results can be displayed as so-called tornado diagrams (FIGURE 39). They display the input distributions, which are most influential on the variability of the simulation result. The longer the bar for an input is, the more critical that input is in determining the simulation output. The coefficients displayed are the standardised beta coefficients taken from the regression analysis results. @RISK uses a multivariate stepwise regression analysis with an inclusion level of p=0.05.

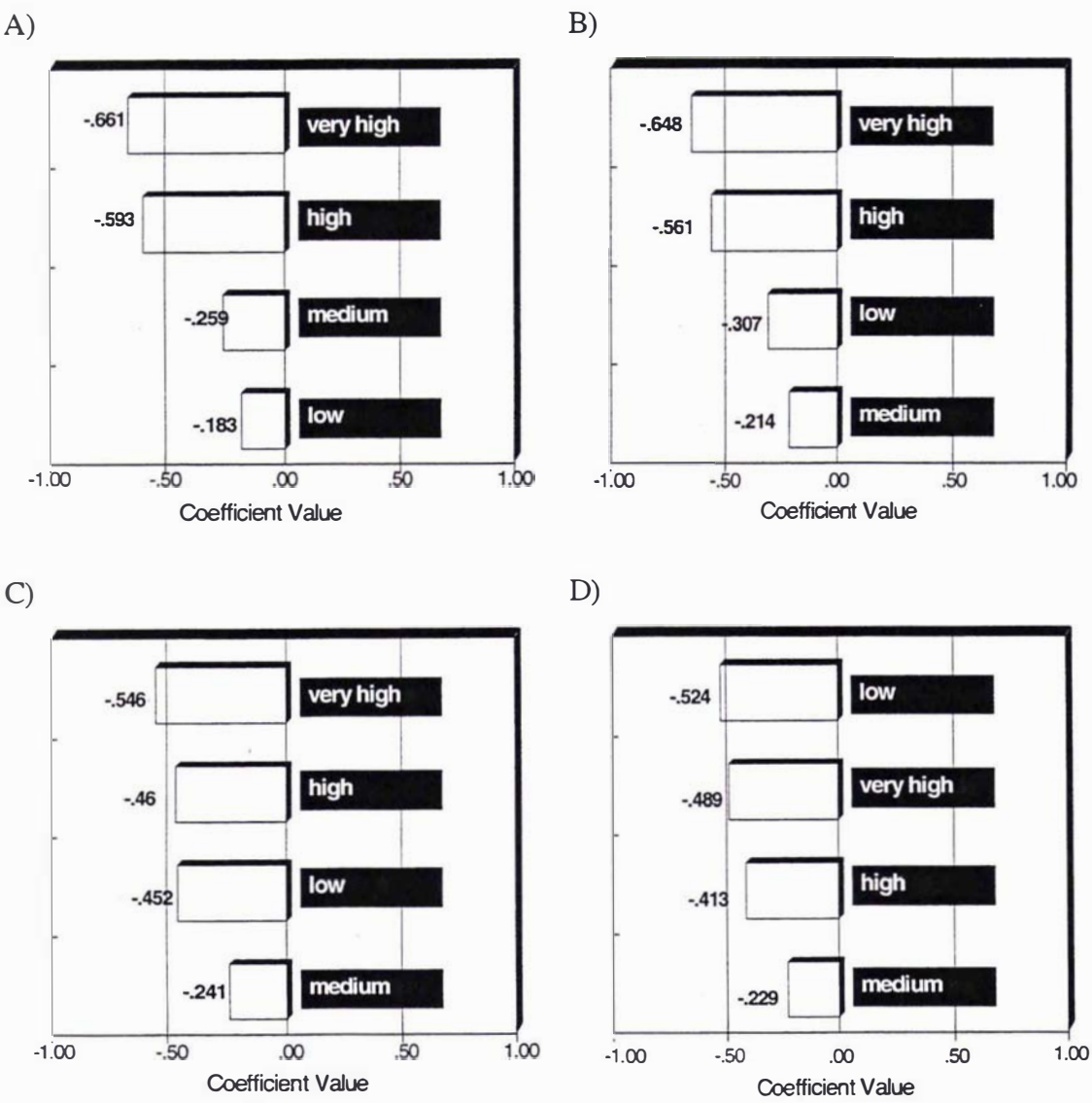

FIGURE 39. Tornado diagrams for farms with A) 1, B) 2, C) 3, or D) 4 very-low risk contacts, respectively

FIGURE 39 illustrates how the outcome of the farm classification becomes more sensitive towards the input used for low-risk conveyors with an increasing frequency of such contacts.

If there is only one 1 low-risk conveyor recorded (A), the uncertainty related to the transmission risk has a small influence on the variability of the over-all outcome, because the latter is more sensitive towards other contact categories. However, if the number of low risk contacts increases (B, C, D) the precision of the risk estimation becomes more influential. By the time the number of low-risk conveyors has increased to 4, the uncertainty of this contact has become most influential. This means that a reduction of the uncertainty of the transmission risk of this conveyor only has a positive effect if a farm is likely to have several of these events. Otherwise, it is preferable to try reducing the uncertainty of other contact categories. Note that the uncertainty of the very-low risk conveyor is not significantly related with the outcome variability in any of the four scenarios. A similar analysis for the significance of the uncertainty related to very-low risk conveyors showed that it became more influential than medium-risk and high risk contacts if >20 and >50 very-low-risk conveyors were recorded, respectively. Other combinations of episodes and conveyors could be explored in a similar way to determine the sensitivity of the outcome with respect to the different input distributions.

In addition to the risk-based scheduling there is also a set of time rules that have to be respected when scheduling visits. For example all farms in the protection zone have to be visited within 7 days after the related RP has been declared according to directive 80/217 EEC (for more details see CHAPTER 2.6). Therefore the risk-based scheduling has to be combined with time-related rules which depend on the current control measures and visit protocols (TABLE 65). An episode can be discounted if the pigs on the farm have been shown to be seronegative no sooner than 30 days after the event (or last occurrence of the event if it occurred repeatedly).

TABLE 65. Rules for scheduling farms for farm visits based on directive 80/217 EEC

RULE 57.		RP
	If	Farm is RP
	Then	Visit type A and visit type G and visit immediately (today)
RULE 58.		Very-high risk farms
	If	Farm probability to be infected ≥x or farm put 'at very high risk' by user
	Then	Farm at very-high risk and additional restrictions apply and visit type B and visit immediately (today)
RULE 59.		Other at risk farms
	If	Farm probability to be infected >x (probability to be not infected >x)
	Then	Farm at risk and visit type C and no later than (RP.date + 7 days)

RULE 60.		Discounting of at risk farms
	If	Farm at risk and visit type B or C performed
	Then	Visit type D and no sooner than (RP_C&D.date + 30 days)
RULE 61.		Farms under surveillance
	If	Farm in surveillance zone
	Then	Farm status = under surveillance and visit type E and no sooner than (RP_C&D.date + 15 days)
RULE 62.		Follow-up visits to antibody test result
	If	Visit type B,C,D or E with diagnosis 'inconclusive serology'
	Then	Visit type H and no later than (laboratory.date + 7 days)
RULE 63.		Follow-up visits to inconclusive antigen test result
	If	Visit type B,C,D or E with diagnosis 'SF infection cannot be excluded'
	Then	Visit type I and no later than (laboratory.date + 1 days)
RULE 64.		Follow-up visits to inconclusive antigen test result
	If	Farm has requested transport
	Then	Visit type F and no sooner than (RP_C&D.date + 7 days in surveillance zone) and no sooner than RP_C&D.date + 21 days in protection zone) and report 'not urgent'

2.6 Validation of rules for the risk classification of farms

2.6.1 Material and methods

The same 10 experts who performed the risk ratings of contacts described above were also asked to classify farms in terms of their likelihood to have been infected with CSF. A list of 21 farms with different histories of contacts was used in this experiment (APPENDIX G). All farms were affected by an imaginary CSF outbreak.

The first task for the tracing officers was to assess the risk of infection for each farm by assigning a score between 0 (nil risk) and 10 (infection certain). For each farm the following information was listed: reason for visit (clinical signs, location in protection zone, neighbouring farm, contact farm, swill feeding), contact details (number and risk level of contacts).

Second, the farms were to be ranked to determine their priority in being visited by a field team (rank 1 = farm needs to be visited first, rank 20 = farm needs to be visited last). The objective was to visit high-risk farms first. Although it was not explicitly stated, it was assumed that the experts would use the scores as a basis for the ranking, i.e. farms with a high score (high probability of being infected) would get a low rank to be visited early on. In order to explore the use of scores as a basis for ranking farms it was counted on how many occasions a farm with a higher score (higher probability of being infected) was scheduled to be visited after a farm with a lower score.

The scores and ranks were compared within experts and between experts and ES as explained in paragraph *2.4.1*. Note that a negative value for scores indicates that the expert was being more daring than the reference group (group median or ES) and a positive value indicates a more cautious assessment. When comparing ranks however, a negative value indicates that the expert would have visited the farm sooner than the reference group (cautious assessment) and a positive value indicates a daring assessment.

In the comparison of ranks given by human experts with the results from the ES the following indicators were used. If the median was below (or above) 0 it was concluded that the experts would visit a certain farm sooner (or later) than the ES suggested. If the entire box of the Box plot was below (or above) 0 it was interpreted that the experts agreed to visit the farm sooner (or later) than the ES advised. If the median was 0, it was concluded that experts and ESs agreed.

2.6.2 Results

When comparing the results within the expert group, relatively good agreement was observed despite high variability both with respect to the scores and the ranking (FIGURE 40).

Six out of ten human experts appeared to consistently use the scores as a basis for their ranking of farms with 0 or 1 contradictory rankings. Four experts however, assigned 6-12 farms with ranks that were inconsistent with the assigned scores.

The ranking performed by experts was then compared with the ES ranking. Strong differences were observed when the results were analysed by farm (FIGURE 41). Five types of farms could be visually identified based on the location of the median and the box of the Box plot:

1) Experts agree to visit farm sooner than ES: Farm 35, 43, 45, 48
2) Experts agree to visit farm later: Farms 36, 37, 38, 40, 49, 50
3) Experts disagree but tend to visit farm sooner: Farms 32, 34, 52
4) Experts disagree but tend to visit farm later: Farms 44, 46
5) Experts agree with ES: Farms 33, 39, 41, 42, 47, 51

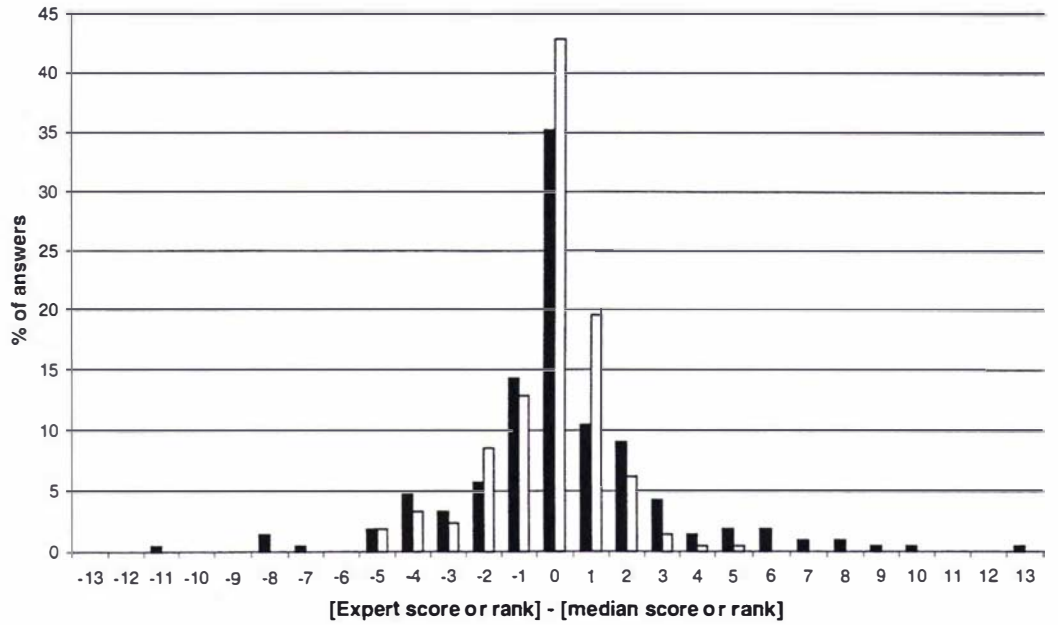

FIGURE 40. Agreement among experts when ranking farms in an imaginary classical swine fever outbreak expressed as difference from the group median (n = 210; ❑ = scores for probability of being infected, ■ = ranking for field visits).

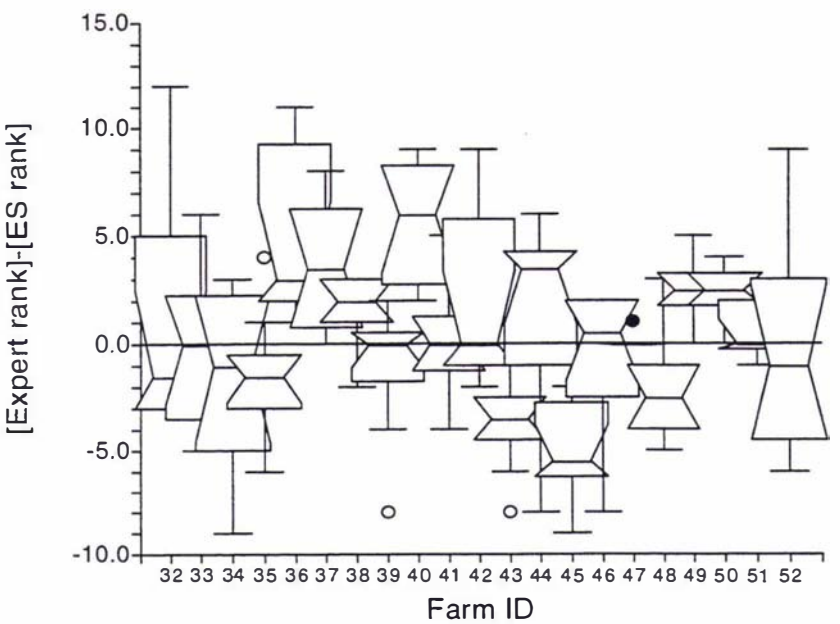

FIGURE 41. Differences between farm rankings performed during an imaginary classical swine fever outbreak by human experts (n=10) and an expert system. A negative value indicates that the experts would have visited the farm sooner, a positive value indicates a later visit.

Due to the large number of factors characterising a farm and the low number of farms it was not possible to establish a pattern for the experts' behaviour. However, it appeared that experts were uncertain about farms with swill feeding and tended to visit them later than the ES would have recommended (Farms 44, 46). On the other hand, experts and ES agreed mostly if a farm was a neighbouring property (Farms 33, 42, 47, 51). Experts also seemed to distinguish between clinical signs reported by a farmer as opposed to clinical signs reported by a veterinarian, particularly if the former were observed on a farm outside the protection zone. The farms with clinical signs reported by a farmer (Farms 32 and 37) would be visited later than farms reported by a veterinarian (Farm 47). In terms of contacts, experts tended to visit farms with a medium-risk contact sooner than the ES (Farms 35, 45) while they would have went later to farms with several low-risk contacts (Farms 40 and 49). Surprisingly, the experts also agreed to visit a farm later that was in the surveillance zone but had had a very-high risk contact with an RP (Farm 36).

In summary, the ranking by human experts agreed or did not differ by more than 3 ranks in 68.1% of the cases (n=210). If the median rank given by the experts for each farm was assumed to be a representative compilation of the human experts, there was agreement or disagreement by less than 3 categories for 17 of the 21 farms (81.0%). The maximum disagreement was 6 categories. This maximum value was observed for a neighbouring property with 4 low-risk contacts (Farm No. 40) that would have been scheduled for a visit much earlier by the ES (rank 4) than by the experts (rank 10) and equal disagreement was observed for Farm No. 45 outside the restricted zones with a medium risk contact. The latter farm would have been visited earlier by the human experts (rank 12) than by the ES (rank 18).

3. Discussion

The knowledge base described in this chapter is based on rules developed for EpiMAN-FMD (Sanson, 1993). EpiMAN-FMD has been constantly improved and refined since it was first published and it is now used as the principal disease information management system in the case of foot-and-mouth disease (FMD) in New Zealand and possibly in Europe in the near future. The basic concepts of tracing and farm classification are not disease-specific but rather a consequence of the level of control as defined in the contingency plan of a country. It seems therefore appropriate to use a similar approach in the case of CSF where the stamping-out strategy is applied as with FMD.

However, in contrast to FMD, CSF is a less contagious disease and the clinical signs are non-specific and sometimes hard to observe. This leads to great uncertainty with respect to the time of disease onset. There is also large variability in the incubation period if a low-virulence virus strain is involved. The estimation of the likely date of infection of a farm will consequently be much more difficult than with FMD. Therefore, a different way of estimating the date of infection is needed, as this information is crucial for the risk classification of contacts. It is standard practice to collect blood samples for laboratory analysis at the time of stamping out a CSF outbreak. If the results from laboratory analyses become available quickly after sample collection, they could be used to determine the state of infection the farm was in and to estimate the likely day of infection. Promising results based on this idea have recently been published (Laevens *et al.*, 1997), but much more research is needed in this area to develop a

practical and reliable algorithm. In the meantime, crude estimates based on the average incubation period will have to be used.

Another element that has been subject to debate is the influence of the frequency of low and very-low-risk contacts. Because the incubation time may be long and the delay between infection of the farm and detection of the disease considerable, a large number of contacts will have occurred. In order to account for the possibility of a large number of low-risk contacts a new approach has been described. It is based on transmission probabilities for each contact. These probabilities are not known and at this stage are our 'best guesses'. The level of uncertainty can be acknowledged by using probability distributions instead of a point estimate. When modelling expert opinion, the BetaPERT distribution based on the minimum, most likely and maximum value is the distribution of choice as it is more robust than alternative distributions (Vose, 1996). When better information on transmission probabilities become available the current values should be adjusted.

Although the use of a probability distribution may scientifically be the best approach to deal with uncertainty, it cannot be used during a CSF outbreak in the field. Because of legal reasons, all contacts of the same nature need to receive equal attention. Therefore, the expected value of the probability distribution should be used instead. With this approach it is still possible to calculate the cumulative survival probability for a farm based on the type and number of contacts that are recorded. Similarly, if a farm has experienced more than one episode, a summary survival probability can be calculated. These comments on the uncertainty related to transmission probabilities also apply to the estimates of episode risk.

When building an ES, validation is a key step in the development process, particularly in the medical domain where confidence in the system should not be eroded (Nykänen, 1991; Clarke *et al.*, 1994). Unfortunately, there is incomplete knowledge about the real risk of transmission related to specific conveyors. The classification results of the ES component of EpiMAN-FMD have been compared with classifications by human experts (Sanson, 1993). We used a similar approach to validate the results of the ES components of EpiMAN-SF. The results demonstrated that this technique has to be applied carefully because there may be considerable disagreement among experts and individual attitudes towards risk are likely to influence the decisions (Bazerman, 1994).

By adopting a worst-case approach, the ES presented here is likely to detect all significant contacts (high sensitivity). However, the percentage of medium and high-risk traces was increased when compared with expert judgement, which possibly indicates a reduced specificity. Alternatively, the human experts may have underestimated the real risk of some contacts. Further evaluation would be necessary to test this hypothesis.

The classification of conveyors is a substantial component of the classification of farms in terms of their likelihood of being infected and the priority of field visits. Although, some of the human experts in our experiment appeared to use this type of reasoning, a substantial proportion used other 'rules' not related to the probability of a farm being infected. More data would need to be collected to further investigate their reasoning process. Techniques for expert knowledge elicitation (as described in CHAPTER 2.3) and decision analysis could be applied. There is probably also substantial interaction between certain factors, which would be worth investigating.

Interestingly, the disagreement between ES and human experts in setting priorities for field visits was smaller than the differences in estimating the likelihood of infection by a contact. This is probably due to the fact that the ranking measures the relative importance rather than the absolute.

When adopting the probability distribution-based approach to classifying farms, the impact of the input assumptions can be explored using Monte Carlo simulation techniques and its related features. The results can be used to validate the classifications of the system. For example, the charts could be shown to experts and the validity of the results could be discussed. The results could also help initiate the discussion on acceptable risk of misclassification of farms.

Despite the incomplete knowledge related to transmission probabilities of classical swine fever virus, the ES components of EpiMAN-SF appear to produce reasonable results. Classifications performed by the ES are advantageous to human ratings in that they are applied consistently. The influence of personal attitudes and lack of experience can be completely excluded. It is also noteworthy that the user can always overrule ratings performed by the ES.

In conclusion, the structure and parameters used by the ES appear satisfactory. However, there is potential for improving the algorithms for estimating the time of infection once more data become available. It is also acknowledged that the probabilities used for classifying conveyors are based on current knowledge and should be constantly adapted, as our understanding of the disease dynamics becomes more complete.

Acknowledgements

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References

Bazerman, M.H. (1994). Judgement in managerial decisions. 3rd Edition. John Wiley and Sons, New York.

Benard, H.J. (1997). Report of classical swine fever field experience, April and May 1997, the Netherlands. Unpublished report, MAF Quality Management, Palmerston North, New Zealand.

Clarke, K., O'Moore, R., Smeets, R., Talmon, J., Brender, J., McNair, P., Nykanen, P., Grimson, J., and Barber, B. (1994). A methodology for evaluation of knowledge-based systems in medicine. Artificial Intelligence in Medicine 6:107-121.

Dahle, J., and Liess, B. (1995). Comparative study with cloned classical swine fever virus strains ALFORT and GLENTORF: clinical, pathological, virological and serological findings in weaner pigs. Wiener Tierärztliche Monatsschrift 82:232-238.

Depner, K., Gruber, A., and Liess, B. (1994). Experimental infection of weaner pigs with a field isolate of hog cholera classical swine fever virus derived from a recent outbreak in Lower Saxony .1. Clinical, virological and serological findings. Wiener Tierarztliche Monatsschrift 81:370-373.

Feigenbaum, E.A. (1992). Expert systems: Principles and practice. In: The Encyclopedia of Computer Science and Engineering, 3rd Edition, Van Nostrand Reinhold Co., New York.

Laevens, H., Deluyker, H., van Caenegem, G., Vermeersch, J.-P., and Koenen, F. (1997). An experimental in-

fection with a classical swine fever virus on weaner pigs. Can serological data be used to estimate the day of virus introduction in natural outbreaks? Epiémiologie et Santé Animale 31-32:12.21.1-12.21.3.

Mani, S., Pazzani, M.J., and West, J. (1997). Knowledge discovery from a breast cancer database. In: Keravnou, E., Garbay, C., Baud, R., and Wyatt, J. (eds.) Artificial Intelligence in Medicine. AIME'97 Springer, New York, 130-133.

Meyer, M., and Booker, J. (1991). Eliciting and analyzing expert judgement. Knowledge-based systems Volume 5. Academic Press, London.

Nykänen, P., Chowdhury, S., and Wigertz, O. (1991). Evaluation of decision support systems in medicine. Computer Methods and Programs in Biomedicine 34:229-238.

O'Keefe, R., Balci, O., and Smith, E. (1987). Validating expert system performance. IEEE Expert 2:81-89.

Sanson, R.L. (1993). The development of a decision support system for animal disease emergency. Unpublished PhD Thesis, Massey University, Palmerston North, New Zealand.

Scott, A.C., Clayton, J.E., and Gibson, E.L. (1991). A practical guide to knowledge acquisition. Addison-Wesley Publishing Company, Reading.

Schimansky, K. and Seidler, M.-J. (1996). Epidemiologische Ermittlungen bei Ausbruch der KSP; ein Erfahrungsbericht. Amtstierärztlicher Dienst und Lebensmittelkontrolle 3:166-172.

Terpstra, C. (1987). Epizootiology of swine fever. Veterinary Quarterly 9:50S-60S.

Vose, D. (1996). Quantitative risk analysis – A guide to Monte Carlo simulation modelling. John Wiley & Sons, Chichester.

CHAPTER 2.8

WITHIN-FARM SPREAD OF CLASSICAL SWINE FEVER VIRUS – A BLUEPRINT FOR A STOCHASTIC SIMULATION MODEL

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1. Summary

A stochastic simulation model to investigate the transmission of classical swine fever (CSF) virus within an infected farm is described. The model is structured in processes which occur within and between management groups (pig units or houses). It uses the individual pig as the unit of interest and estimates the number of animals in the states 'susceptible', 'infected', 'infectious', 'carrier' and 'removed' for each day of the disease incident. Probabilities are assigned to the transitions between states. The probability of a pig becoming infected is made dependent on the virulence of the virus strain involved, the infection pressure in a pig unit and the housing system. The more pigs become infected in one unit, the more likely is spread to another management group on the farm. Ultimately, the probability that movements of pigs off the farm will include at least one infected pig can be estimated to identifying high-risk movements during a CSF epidemic. To finalise the input parameters and for model validation more experimental data and field data from CSF outbreaks will be needed.

2. Introduction

Recent outbreaks of classical swine fever (CSF) in Europe have demonstrated the difficulty of controlling outbreaks of contagious livestock diseases, particularly in densely populated areas. In order to implement appropriate control measures the temporal and spatial dynamics of the disease have to be understood. At the beginning of the epidemic the infectious agent is introduced to one farm (index case), within which it spreads amongst the susceptible animals. As more pigs become infected, the probability of spread to other farms increases. Thus, between-farm transmission is to some extent dependent on within-farm spread.

Simulation models can be used to investigate the quantitative aspects of the behaviour of a disease process in relation to dynamic conditions that change either deterministically or stochastically (Thrusfield, 1995). Simulation models offer a valuable tool, particularly in a case where the solution to a problem is very complex and difficult to obtain. They attempt to reproduce the behaviour of a system by including elements and their interactions, which are considered important. Typically, such simulations can only be run on a computer. This technique has therefore become more attractive with the advent of powerful and affordable personal computers.

Between-farm spread of infectious pig diseases has been modelled using different modelling approaches including simulation. Diseases studied include for example foot-and-mouth disease (Sanson, 1993), Aujeszky's disease (Houben *et al.*, 1993, Miller *et al.*, 1994), and classical swine fever. Possibilities to model within-farm spread of infectious diseases in pigs have also been described (Bouma *et al.*, 1995; Grenfell and Smith, 1990; de Jong *et al.*, 1994; Smith and Bryan, 1990). All these models are of Aujeszky's disease, and they are deterministic in the sense that there is no consideration of uncertainty with respect to the calculated outcome. This chapter describes the theoretical basis for modelling within-farm CSF transmission using Monte Carlo simulation techniques. It describes how such a model could be built and how it could be used in disease control. Current problems for the practical implementation of the model are highlighted.

3. Modelling concepts

In an outbreak situation, a within-herd CSF model would be run whenever a newly infected herd has been reported. The objective is to simulate the past spread of infection among the susceptible animals on that farm. The model estimates the number of animals that are in the different disease states at a particular point in time. The individual pig is the unit of observation, and events are simulated for each pig. Events are the transitions from one possible state to another. Possible states are susceptible, infected, infectious, carrier and removed. The output of the model is the numbers of pigs in the different states for each day after the farm becomes infected.

Susceptible animals are pigs of all breeds and ages (Dahle and Liess, 1995). When virus is introduced on to a farm, not all pigs are exposed at an equal level because a pig farm typically consists of several physically separate units. The simulation of the spread of infection on the farm has to take account of this heterogeneity of exposure, for example by simulating the disease spread according to management groups (FIGURE 42). A management group is defined as a group of pigs of approximately the same age and production status which are kept physically separate (e.g. in different buildings) from similar or different groups. The transmission from one management group to another is considered less likely than transmission within one management group.

Virus can be introduced to one management group (for example through purchase of infected animals) or to several management groups (for example if contaminated swill is fed to the animals). Transmission is then simulated within the primarily exposed management group(s) before it is propagated to secondary infected management groups. For each day, the simulation model updates the output matrix with the number of animals in the different states for each management group.

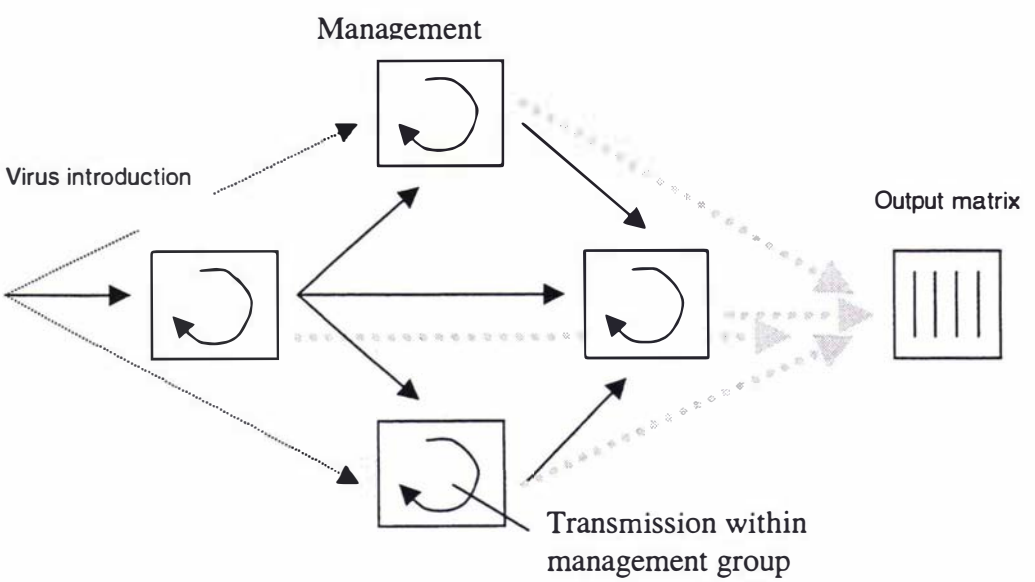


FIGURE 42. Elements of simulation process on a farm consisting of several management groups.

Because some of the input variables are expected to be unknown and all event dates during the simulation are subject to variability, the model is based on stochastic principles using Monte Carlo simulation techniques (Monney, 1997). There are thus two reasons to introduce probability distributions in a stochastic model: 1) lack of knowledge (uncertainty) about the underlying processes and 2) true variability in these biological processes (Vose, 1997). While performing experiments and collecting additional empirical information can be used to reduce the first, the second type will always remain.

The starting point for the simulation is the date of infection of the farm. If this date is not known, the model estimates it using subsidiary dates such as the date of onset of clinical signs or the date of diagnosis. From these dates one can estimate the day of infection by subtracting one or several expected incubation periods.

Other model inputs are: size of the initial infection group, the number of pigs on the farm in different management groups and the date of diagnosis. The model also uses information about the mechanism of virus introduction to the farm and the virulence of the virus.

4. Model characteristics

4.1 Start of infection

The process of infection begins with virus introduction to the farm. If the source of the infection is known, for example purchase of pigs from another infected farm, then the simulation starts on the date of virus introduction. If the date of infection is not known, but clinical signs are present, then the date of infection can be estimated by subtraction of the likely incubation period. If no clinical signs are observed or if the onset date is not known, then the date of infection is estimated by subtracting the sum of the likely number of days from clinical signs to diagnosis and the incubation period. Estimates for the different time periods depend on the virus strain involved and the efficiency of veterinary services. The time periods are highly variable. The distribution of incubation periods can best be described by the lognormal distribution (Sartwell, 1950).

If a group of purchased animals is the source of infection, it is assumed that virus is already being shed by them upon arrival. If there is a source suspected, other than live animals, such as contaminated swill feed, a first group of pigs on the farm is taken through an incubation period before additional animals become infected (FIGURE 43). Afterwards the infection is simulated within and between management groups as described in the next part of this chapter.

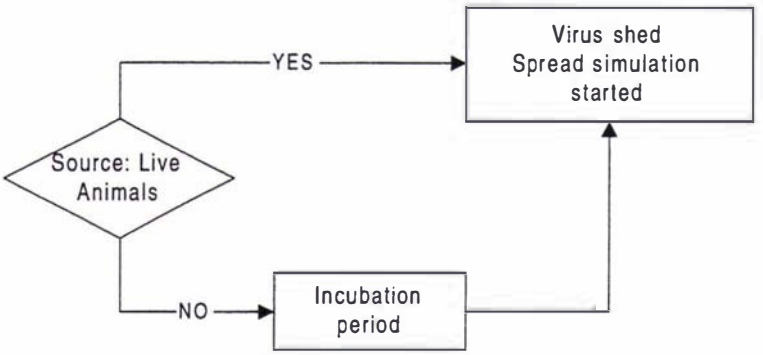


FIGURE 43. Starting point of classical swine fever infection depending on virus source

The infection begins in the management group where the virus was first introduced, such as for example the one where infected animals have been added. If it is not known which management group was first infected, the model will randomly select one group or the user will be able to select the most likely group.

4.2 Management groups

Information with respect to type and size of management groups needs to be obtained during the first farm visit to an infected premises. Each management group consists of pigs of different age categories. The following age categories are suggested:

I. Piglets = suckling piglets housed with their dams

II. Weaned pigs = pigs housed separately from their dams, <30 kg

III. Growing pigs = pigs >30 kg

IV. Breeding pigs = adult pigs used for breeding, including boars

Information about management groups is stored in a data table (TABLE 66). Information about the type of housing is also relevant, because disease spread is assumed to be faster if pigs are housed in groups as opposed to individual housing, e.g. crates or stands (Depner *et al.*, 1995).

TABLE 66. Example of a table containing information on management groups

Group	I Piglets	II Weaned pigs	III Growing pigs	IV Breeding pigs	Housing
1	0	88	0	0	Group
2	0	0	6	62	Individual
3	130	0	0	12	Individual
...					
...					
Total	130	88	6	74	

4.3 Transition probabilities

Each pig within a management group has a certain probability to change its infection state at a given time within the simulation. The following requirement has to be fulfilled:

$$\sum_{1}^{m} p_{it}(state_m) = 1$$

where p_{it} is the probability of pig i on day t to be in state m.

The possible state transitions are limited in terms of biologically possible changes of infection states (TABLE 67). It is assumed that transient infections are not possible. Therefore an infected animal cannot become susceptible again. The difference between infected and infectious is that the infected animal is still in the incubation period and therefore not yet shedding virus. Only pigs in the infectious group are relevant for infecting other pigs on the farm although both incubating and shedding pigs can spread the virus if they are sold to another farm. A removed pig is a pig that has been sold or a pig that died due to CSF or another disease. Recovery from disease is not being considered at this stage.

TABLE 67. State transition possibilities for the simulation of spread of classical swine fever virus

	Susceptible	Infected	Infectious	Carrier[a]	Removed
Susceptible	✓	✓	x	✓	✓
Infected	x	✓	✓	x	✓
Infectious	x	X	✓	x	✓
Carrier[a]	x	X	x	✓	✓
Removed	x	X	x	x	✓

[a]Prenatally infected piglets.

The probability of a transition from one state to another depends on a series of variables. For example, the transition susceptible → infected depends on the possibility of close contact with infectious animals within a particular management group. This is dependent on the housing system and the number of infectious animals present. Once a contact has occurred, the probability for successful transmission is modelled as a binomial process.

Another example is the transition infected → infectious (shedding virus). This probability depends on the incubation period, which itself is related to the virus strain (Terpstra, 1991). The probability to become infectious increases dramatically after the incubation period is over. If the incubation period is simulated at the individual animal level, the cumulative probability of becoming infectious is 1 after the maximum incubation time has elapsed.

Recent evidence also suggests an influence of breed on the course of the disease (Depner *et al.*, 1997). According to these authors Pietrain x German Landrace crossbred weaners were more likely to develop chronic disease forms with prolonged virus excretion, whereas German Landrace pigs developed an acute form of the disease. More research is required to quantify the breed effect.

The age of the animal (Carbrey *et al.*, 1966; Koenen *et al.*, 1996), immune competence, nutritional status (van Oirschot, 1992) can also influence the course of the disease as well as the quantity of virus exposure and the transmission route. All transition probabilities have to be examined using all available evidence from experiments and outbreak data. At this stage the probabilities are based on expert opinion due to insufficient data from experiments and particularly field observations. Expert opinion in such cases is best modelled using the BetaPERT distribution or a modified BetaPERT distribution as suggested by Vose (1996).

Transmission probabilities between management groups depend on the type of management group under consideration, because only animals from particular groups are likely to be transferred to other management groups during the simulation. For example, dry sows can be transferred to the farrowing unit. This obviously increases the probability of disease spread to the next management group. If biosecurity measures are in place the probability of transmission between groups is reduced. This could for example be the case if specific staff members exclusively look after certain management groups. As a general rule, virulent strains will spread faster within a herd than less virulent strains (Terpstra, 1991).

4.4 Virus strain

CSF virus strains differ with respect to their virulence and pathogenicity (van Oirschot, 1992). These characteristics influence a range of variables used by the model, e.g. the incubation period or the time from infection to onset of clinical signs (Terpstra, 1991; TABLE 68).

TABLE 68. Epidemiological differences between classical swine fever virus strains of low, moderate and high virulence

	Low - moderate virulence	High virulence
Incubation period	Longer	Shorter
Virus excretion quantity	Low	High
Excretion duration	Short	Long
Morbidity	Low	High
Mortality	Late and low (40%)	Early and high (90 %)
Carrier sow syndrome	Yes	No
Clinical signs	Weak	Distinct
Spread within herd	Slow	Fast

TABLE 69. Characteristics of classical swine fever virus isolates

Virulence	Strain	Infection route / dose	Viraemia (days p.i.[a])	Fever (days p.i.)	Antibodies (days p.i.)	Source
LOW	NSW 1960/61	Subcutaneous $10^{3\,25}$ $TCID_{50}$[b]	6	4-5	n.r.[c]	Kamolsiri-prichaiporn *et al.*, 1992a,b
LOW-MODERATE	Visbek/Han95	Intranasal 500 $TCID_{50}$	n.r.	3-14	n.r.	Depner *et al.*, 1996
	FIN 3086	Contact (26 days p.i.)	16	16	19	Plateau *et al.*, 1980
	Glentorf/315	Intranasal				Dahle and Liess, 1995
		$10^{3\,7}$ $TCID_{50}$	5.25	4.5	n.r.	
		$10^{2.7}$ $TCID_{50}$	10	8.7		
		$10^{1.7}$ $TCID_{50}$	7	6.5		
		$10^{0\,7}$ $TCID_{50}$	17.6	17.6		
		<1 $TCID_{50}$	n.r.	no fever		
		Contact	13.3	13.3	18.3	
HIGH	Alfort/187	Intranasal	n.r.		n.r.	Dahle and Liess, 1995
		$10^{3,2}$ $TCID_{50}$		3.3		
		$10^{2\,2}$ $TCID_{50}$		4		
		$10^{1.2}$ $TCID_{50}$		4.8		
		$10^{0\,2}$ $TCID_{50}$		no fever		
	Diepholz1/ Han94	Intranasal 250 $TCID_{50}$	4	7	14	Depner *et al.*, 1994
	Weybridge/86/8	Intranasal $10^{6,5}$ $TCID_{50}$	2-4	3-4	12-13	Wood *et al.*, 1988
	Brescia	Per oral $10^{6\,0}$ PFU	1-2 excretion in urine and faeces after 5-6	n.r.	n.r.	Ressang, 1973

[a] p.i. = post infection
[b] $TCID_{50}$ = 50% of total cell infective dose
[c] n.r. = not reported

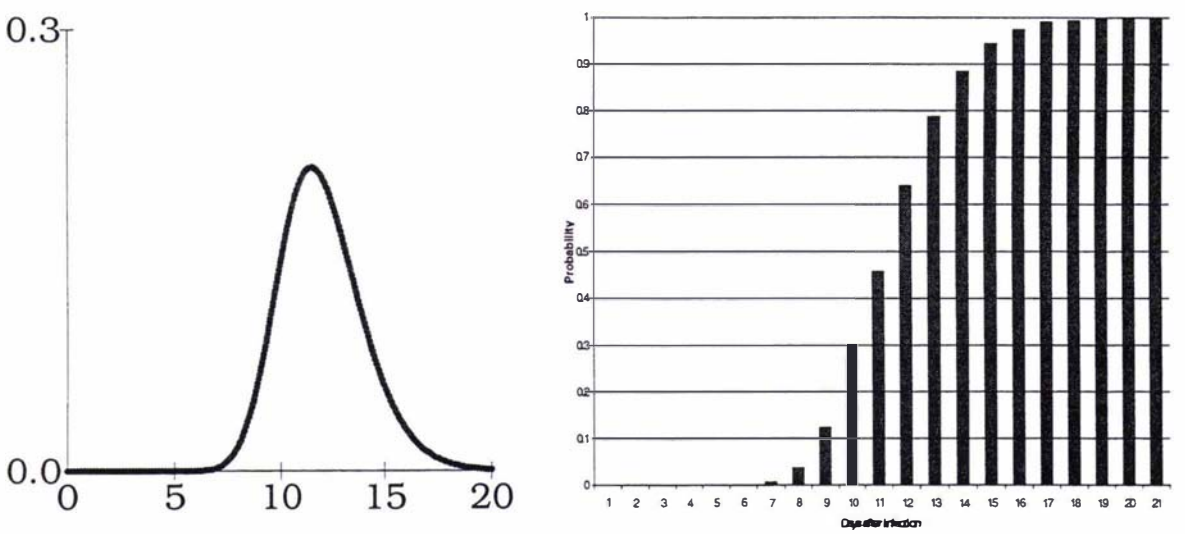
Information about the virus strain involved in an outbreak may not be available during the early stages of the epidemic. The model then uses a set of default values for virus characteris-

tics. A specific set for either low-moderate virulence or high virulence can be chosen as soon as the information becomes available.

4.4.1 Incubation period

The incubation period depends on the virus strain and the route of transmission as well as the infection dose (TABLE 69). Unfortunately, most data about incubation periods is derived from experiments and these do not necessarily represent the field situation.

The incubation period has to be simulated for every individual within an infected group. It is assumed to be a probabilistic process based on a lognormal distribution (Sartwell's model, Sartwell, 1950) assigning a value for each day following infection. The shape of the distribution is determined by the virus strain. For example, if the incubation time for a low-moderately virulent strain is approximated by a lognormal distribution with a mean of 12 days and a standard deviation of 2 days, the probability of beginning to shed virus can be determined for each day as shown in FIGURE 44. The incubation period is assumed to remain constant during the simulation. It is also assumed that a pig will continue to shed virus until it either dies or is culled.



A) **B)**

FIGURE 44. A) Distribution of incubation time for a low-moderately virulent classical swine fever strain(lognormal distribution, mean = 12, standard deviation = 2); B) Cumulative probability of terminating incubation period

4.5 Carrier sow syndrome

When pregnant sows are infected with classical swine fever virus strains of low virulence, they often do not develop clinical signs, but the virus is transmitted to the foetus and the piglets will shed the virus for up to several months without showing clinical signs (Dahle and Liess, 1992; van Oirschot and Terpstra, 1977). The critical time period for development of this syndrome is infection between 70 and 90 days of pregnancy. The carrier piglets will be born 3-6 weeks later. Thus, if the farm under consideration is a breeding farm, the model will introduce carrier piglets 3-6 weeks after the date of infection, if a low virulence virus strain is involved.

5. Model output

The model produces a matrix with the number of susceptible, infected, infectious, carrier and removed pigs for each day of the simulation by age and management group. This information is used to calculate the risk for pig movements to other farms or to the abattoir to contain at least one infected pig. Because all animals incubating or shedding the virus are relevant for virus spread both within and between herds, this figure is of particular importance.

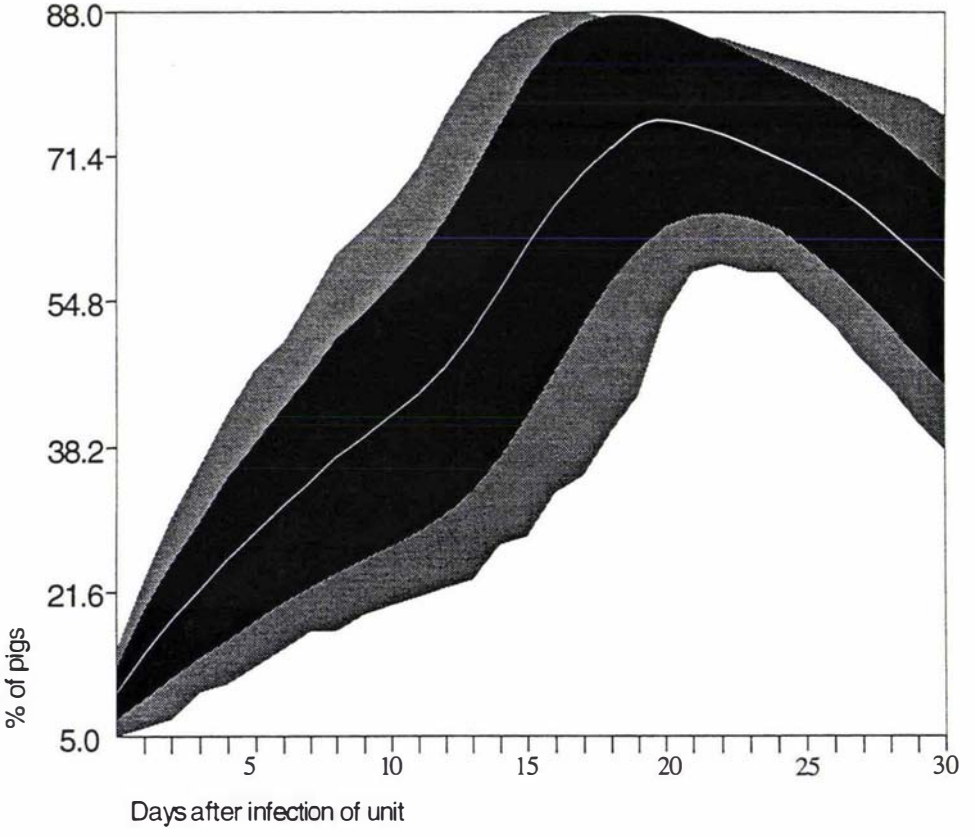


FIGURE 45. Percentage of pigs incubating or shedding virus in an infected pig unit during a 30-day simulation period of classical swine fever transmission (white line = mean, ■ = + / - 1 standard deviation, ▨ = 5% and 95% percentiles).

The model can produce the number of animals in any of these infection states for each day following infection. Due to the stochastic variability in the transmission process the number of animals will vary between upper and lower limits (FIGURE 45). It is then up to the decision maker to either choose a risk averse attitude and select the higher boundary (worst case scenario) or choose another value.

As airborne transmission represents a minor risk, the amount of virus released into the atmosphere is not calculated.

6. Validation

The validation is a crucial step in the development of every simulation model. As a model is intended to be a representation of a 'real world' process, it has to be made sure, that the simulation results are biologically plausible and realistic. Ideally, the output of the model is compared with 'real world' data from either animal experiments or field data (Martin *et al.*, 1987). Unfortunately, both types of data are difficult to obtain for exotic animal diseases. In the published literature reports of transmission dynamics are rare.

With respect to CSF, one possible source of data on the transmission of CSF has been described by Carbrey and McDaniel (1984). Their data are based on events in a pen housing 100 pigs (FIGURE 46). However, it is not clear from this report whether these data were recorded under experimental or field circumstances and what type of virus was involved.

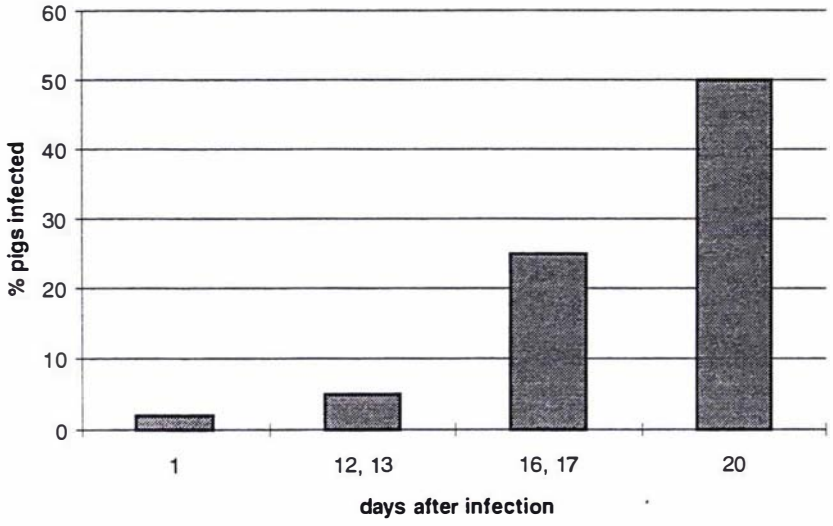


FIGURE 46. Spread of classical swine fever in a pen with 100 pigs (Carbrey and McDaniel, 1984)

A more recent study was conducted under experimental conditions by Laevens *et al.* (Laevens *et al.*, 1997). They placed 15 weaner pigs each in three adjacent pens and experimentally infected one pig in the middle pen. Then they monitored the development of antibodies in all pens. The first in-contact pig became viraemic after 12 days and in adjacent pens pigs became

viraemic on day 18, indicating a 6-day delay for between-pen transmission. The report does not present data relating to the further spread of the disease. In the same publication serology results from three farms involved in a CSF outbreak in Belgium were reported. It becomes clear that all pigs were sampled for laboratory investigation during this outbreak. Once this data becomes available it will be an ideal data set for validating the CSF disease spread model described in this paper. In the meantime however, a validation cannot be based on quantitative field data.

The evaluation of the appropriate simulation of the transmission between units within a farm also needs to be further researched. Depner *et al.* (1995) described a case where CSF was diagnosed in a mixed breeding-fattening farm with three pig units. From the level of anti-CSF virus antibodies in the different units they concluded that the infection must have occurred a relatively long time in the past. The time delay between the onset of the disease in the different units is reflected in the different prevalence of antibody-positive animals and their titres in the three units. The onset of disease in weaned pigs seemed to be delayed due to maternal antibodies. However, it could not be determined in which unit the infection process began.

7. Discussion

A large number of different approaches can be used to develop simulation models of disease dynamics in veterinary epidemiology (Hurd and Kaneene, 1993). The model described here is designed to explain the behaviour of CSF within a farm consisting of individual animals (discrete entity model based on individuals) which are clustered within management units. This is in contrast to mathematical models which often treat the entire population at risk as a single entity and therefore do not measure the processes in a system in detail ('black box' approach). In the case of the CSF model described here, there should be some understanding of the mechanisms represented in the model and their relationships. The infection status of individual pigs is simulated on a day-by-day basis (discrete-time approach). Elements of chance are included, i.e. it is a stochastic as opposed to a deterministic model, where the output does not contain uncertainty. Stochastic models have the advantage of reflecting the realistic aspects of chance and uncertainty in a model's behaviour (Hurd and Kaneene, 1993).

The simulation of CSF transmission within a farm is a rather complex process. The system includes events at the individual animal level (for example, the incubation period), events between animals (contact and subsequent disease transmission) and events between buildings or units (transmission via animals, people or fomites). Unfortunately, a lot of the input variables required for the model have not yet been quantified, and therefore had to be based on guessed estimates. This is not an uncommon event. Simulation models are well suited to reveal deficiencies in the current knowledge of a disease because they require the significant elements within a system to be named and quantified (Martin *et al.*, 1987). At this stage, it seems that before we can realistically simulate the within-herd spread of CSF, we need a better understanding of the underlying transmission processes, particularly the probability of state transitions for animals under the influence of risk factors such as age, breed and virus strain. Using techniques such as sensitivity analysis critical input variables can be identified and then used to set priorities for further data collection. This additional quantitative knowledge has to be gathered on the basis of experiments and empirical analysis of CSF outbreaks. If this is not possible, the elicitation of expert opinion can be used as an alternative method for

data collection. The latter technique has recently been used to quantify the importance of risk factors for CSF transmission between herds (Stärk *et al.*, 1997).

Once parameter estimates have been finalised, the output has to be validated, because the results can only be utilised effectively if they are based on a sound model. The objective of validation is to prove that the model adequately represents reality (Neelamkavil, 1987). However because the latter is never known, validation can only be approached but ultimately never achieved. Using a more pragmatic approach, validation attempts to prove that the model performs sufficiently accurately for the intended application (Schlesinger, 1979). A possible method for assessing the validity of the model is to compare the simulation output with historic outbreak data. Agreement between model results and real-world observations supports the hypothesis that the assumptions made on the parameters and processes in the model are correct. Emphasis should be on the degree of confidence we can have in the model rather than on its absolute validity (Neelamkavil, 1987). It is hoped that the data collected during the recent series of large CSF outbreaks in Europe will become available for this purpose in the near future.

Eventually, a validated model can be integrated into a disease management system to assist disease control managers in their decision making. As discussed earlier, the transmission of disease between farms depends to some extent on the dynamics of the disease process within a farm. For example, in order to estimate the risk of a pig movements, it would be helpful to know how likely it is that a group of pigs contains at least one infected animal. This probability can be derived directly from the model output. Therefore, a simulation model similar to the one described in this manuscript is envisaged to be integrated in a future version of EpiMAN-SF (Stärk *et al.*, 1996).

References

Bouma, A., de Jong, M.C.M., and Kimman, T.G. (1995). Transmission of pseudorabies virus within pig populations is independent of the size of the population. Preventive Veterinary Medicine 23:163-172.

Carbrey, E.A., Stewart, W.C., and Young, S.H. (1966). The changing picture of hog cholera: case studies. Journal of the American Veterinary Medical Association 149:1720-1724.

Carbrey, E.A. and McDaniel, H.A. (1984). Hog cholera. In: Foreign animal diseases, their prevention, diagnosis and control Committee on Foreign Animal Diseases of the United States Animal Health Association, 202-215.

Dahle, J. and Liess, B. (1995). Comparative study with cloned classical swine fever virus strains ALFORT and GLENTORF: clinical, pathological, virological and serological findings in weaner pigs. Wiener Tierärztliche Monatsschrift 82:232-238.

Dahle, J. and Liess, B. (1992). A review on classical swine fever infections in pigs: epizootiology, clinical disease and pathology. Comparative Immunology, Microbiology and Infectious Diseases 15:203-211.

Depner, K., Gruber, A., and Liess, B. (1994). Experimental infection of weaner pigs with a field isolate of hog cholera classical swine fever virus derived from a recent outbreak in Lower Saxony .1. Clinical, virological and serological findings. Wiener Tierärztliche Monatsschrift 81:370-373.

Depner, K.R., Hinrichs, U., Bickhardt, K., Greiserwilke, I., Pohlenz, J., Moennig, V., and Liess, B. (1997). Influence of breed-related factors on the course of classical swine fever virus infection. Veterinary Record 140:506-507.

Depner, K.R., Müller, A. and Liess, B. (1995). Epidemiologische Bedeutung des Antikörper- und Antigennachweises bei der Europäischen (Klassischen) Schweinepest – ein Fallbericht. Tierärztliche Umschau 50:571-

573.

Depner, K.R., Rodriguez, A., Pohlenz, J., and Liess, B. (1996). Persistent classical swine fever virus infection in pigs infected after weaning with a virus isolated during the 1995 epidemic in Germany: Clinical, virological, serological and pathological findings. European Journal of Veterinary Pathology 2:61-66.

Grenfell, B.T. and Smith, G. (1990). Mathematical model for the impact of a pseudorabies epizootic on the productivity of a farrow-to-finish operation. American Journal of Veterinary Research 51:156-164.

Houben, E.H.P., Dijkhuizen, A.A., de Jong, M.C.M., Kimman, T.G., van der Valk, P.C., Verheijden, J.H.M., Nieuwenhuis, H.U.R., Hunneman, W.A., and Huysman, C.N. (1993). Control measured directed at Aujeszky's disease virus: a theoretical evaluation of between-farm effects. Preventive Veterinary Medicine 15:35-52.

Hurd, H.S., and Kaneene, J.B. (1993). The application of simulation models and systems analysis in epidemiology: a review. Preventive Veterinary Medicine 15:81-99.

de Jong, M.C.M., Diekmann, O., and Heesterbeek, J.A.P. (1994). The Computation of R0 for discrete-time epidemic models with dynamic heterogeneity. Mathematical Biosciences 119:97-114.

Kamolsiriprichaiporn, S., Hooper, P.T., Morrissy, C.J., and Westbury, H.A. (1992a). A comparison of the pathogenicity of two strains of hog cholera virus. 1. Clinical and pathological studies. Australian Veterinary Journal 69:240-244.

Kamolsiriprichaiporn, S., Morrissy, C.J., and Westbury, H.A. (1992b). A comparison of the pathogenicity of two strains of hog cholera virus. 2. Virological studies. Australian Veterinary Journal 69:245-248.

Koenen, F., Vancaenegem, G., Vermeersch, J.P., Vandenheede, J., and Deluyker, H. (1996). Epidemiological characteristics of an outbreak of classical swine fever in an area of high pig density. Veterinary Record 139:367-371.

Laevens, H., Deluyker, H., van Caenegem, G., Vermeersch, J.-P., and Koenen, F. (1997). An experimental infection with a classical swine fever virus on weaner pigs. Can serological data be used to estimate the day of virus introduction in natural outbreaks? Epiémiologie et Santé Animale 31-32:12.21.1-12.21.3.

Martin, S.W., Meek, A.H., and Willeberg, P. (1987). Veterinary epidemiology – principles and methods. Iowa State Univeristy Press, Ames, Iowa.

Miller, G.Y., Forster, D.L., Tsai, J., and Bech-Nielsen, S. (1994). Predicting the number of herds infected with pseudorabies virus in the United States. American Journal of Veterinary Research 55:628-635.

Monney, C.Z. (1997). Monte Carlo simulation. Sage University Paper No. 116, Series: Quantitative Applications in the Social Sciences. Sage Publications Inc., Thousand Oaks, U.S.A.

Neelamkavil, F. (1987). Computer simulation and modelling. John Wiley & Sons, Chichester.

van Oirschot, J.T. (1992). Hog Cholera. In: A.D. Leman, B.E. Straw, W.L. Mengerling, S. D'Allaire and D.J. Taylor Diseases of Swine 7th Edition, Iowa University Press, Ames, Iowa, 274-285.

van Oirschot, J.T. and Terpstra, C. (1977). A congenital persistent swine fever infection. I. Clinical and virological observations. Veterinary Microbiology 2:121-132.

Plateau, E., Vannier, P., and Tillon, J.P. (1980). Experimental study of a mild virulence strain of hog cholera: individual variations and horizontal transmission. Zentralblatt der Veterinär-Medizin B 27:650-657.

Ressang, A.A. (1973). Studies on the pathogenesis of hog cholera. I. Demonstration of hog cholera virus subsequent to oral exposure. Zentralblatt der Veterinär-Medizin B 20:256-271.

Sanson, R.L. (1993). The development of a decision support system for animal disease emergency. Massey University, Palmerston North.

Sartwell, P.E. (1950). The distribution of incubation periods of infectious disease. American Journal of Hygiene 51:310-318.

Schlesinger, S. (1979). Terminology for model credibility. Simulation 32:103-104.

Smith, G. and Bryan, T. (1990). Population biology of pseudorabies in swine. American Journal of Veterinary

Research 51:148-155.

Stärk, K.D.C., Morris, R.S., and Sanson, R.L. (1996). EpiMAN-SF - development of a decision support system for managing a swine fever epidemic. Proceedings of the 14th IPVS Congress, Bologna, Italy, 534.

Stärk, K.D.C., Horst, H.S., Morris, R.S., and Teuffert, J. (1997). Elicitation of expert knowledge on risk factors for classical swine fever transmission in Switzerland. Proceedings 8th International Symposium on Veterinary Epidemiology and Economics, Paris. Epidémiologie et Santé Animale 31-32:12.24.1-12.24.3.

Terpstra, C. (1991). Hog cholera: an update of present knowledge. British Veterinary Journal 147:397-406.

Thrusfield, M. (1995). Veterinary epidemiology. 2nd Edition. Blackwell Science Ltd., Oxford.

Vose, D. (1996). Quantitative risk analysis - A guide to Monte Carlo simulation modelling. John Wiley & Sons, Chichester.

Vose, D. (1997). Risk analysis in relation to the importation and exportation of animal products. Revue scientifique et technique, Office International des Epizooties 16:17-29.

Wood L, Brockman S, Harkness JW, and Edwards S (1988). Classical swine fever: virulence and tissue distribution of a 1986 English isolate in pigs. Veterinary Record 122:391-394.

CHAPTER 2.9

ANALYSIS OF A CLASSICAL SWINE FEVER OUTBREAK IN LOWER SAXONY, GERMANY

1. Introduction

The epidemiological analysis of both temporal and spatial dynamics of a disease outbreak is important in order to identify risk factors for disease transmission and to investigate the behaviour of the disease under the influence of different control. These tasks are commonly assigned to decision support systems for the management of exotic disease epidemics in the veterinary domain, for example EpiMAN-FMD (Sanson, 1993). EpiMAN-FMD provides *inter alia* a set of tools to analyse the data accumulated during a foot-and-mouth disease epidemic (the 'Epidemiologist's workbench') and a model (INTERSPREAD-FMD; Sanson *et al.*, 1994) to simulate the further development of the disease and to investigated the impact of current and alternative control strategies (Jalvingh *et al.*, 1995).

A number of classical swine fever (CSF) outbreaks have occurred in Europe over the last 5 years. Some of these were large and are currently being used as a basis for the development of transmission models. During the Belgian epidemic 1993-1994 (Vantemsche, 1996) detailed epidemiological data were recorded. They are now subject to analysis (Deluyker, personal communication). Another example is the 1997 outbreak in the Netherlands. These data will also be used for further research (Dijkhuizen, personal communication). We had access to data from the German outbreak 1993-1996. This data set has been used before to investigate CSF transmission dynamics (Roberts, 1995; Kramer *et al.*, 1995; Staubach *et al.*, 1997). The objectives of the analyses described in this chapter were:

a) to explore the usefulness of field data for describing and modelling disease dynamics

b) to describe the temporal and spatial dynamics of CSF in selected areas, and

c) to make suggestions on how a decision support system such as EpiMAN-SF could help improve the epidemiologist's capacity to support sound decision-making in an outbreak situation.

2. Material and methods

2.1 Outbreak data

The CSF outbreaks analysed in this chapter occurred in two districts (Landkreise) in the state (Land) of Lower Saxony, in the North of Germany (FIGURE 47), between October 1993 and October 1995. This is an area with high pig density (FIGURE 48) with approximately 2 pig farms per km^2 and 720 pigs per km^2 at the time of the outbreak. In total, 37 outbreaks occurred, out of which 3 consisted of more than one farm belonging to the same owner. This resulted in a total of 44 infected properties (IPs, TABLE 70). This data set represents a subset of the larger CSF epidemic during which a total of 272 farms became infected in different parts of Germany between 1993 and 1996. The area was selected because spatial as well as detailed epidemiological data were available for these districts.

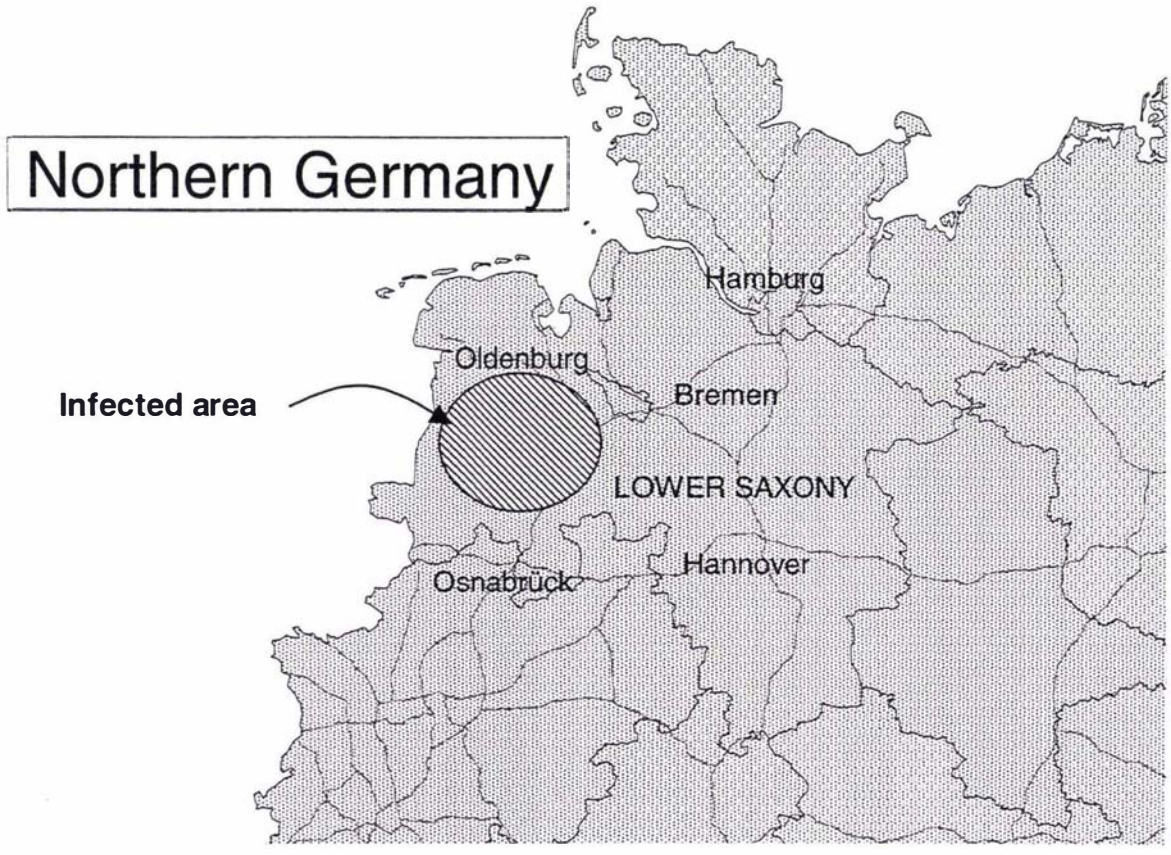


FIGURE 47. Geographic location of German classical swine fever epidemic analysed in this chapter

TABLE 70. Descriptive statistics of farms in two classical swine fever-infected districts in Germany

	District 1		District 2	
	Farms at risk[a]	Infected farms	Farms at risk	Infected farms
Number of breeding farms	33	8	611	1
Number of mixed farms	115	11	754	6
Number of finishing farms	62	7	1542	11
Total farms	210	26	2907	18
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Breeding pigs per breeding/mixed farm	122.4 (250.1)	380.3 (476.8)	51.3 (68.4)	110.5 (78.0)
Piglets per breeding/mixed farm	348.7 (862.6)	1075.2 (1763.3)	151.3 (183.5)	288.6 (240.8)
Finishing pigs per mixed/finishing farm	389.2 (326.6)	484.2 (212.5)	333.6 (1078.9)	593.8 (340.0)

[a]Farms in 1000m zones around infected properties only.

The disease was introduced in this region by a transport of infected animals from Baden-Wurttemberg, another part of Germany where CSF was occurring at the time.

FIGURE 48. Pig density in Germany based on State figures (Federal Office of Statistics, 1996).

After CSF was confirmed in this region, control measures in accordance with German and European Union legislation were introduced. These consisted of protection (3 km) and surveillance (10 km) zones with a total standstill of all animal movements within the protection zone in a first phase and restricted trade in a second phase (*Anonymous*, 1994). All animals on infected properties were killed and destroyed. Pre-emptive slaughter was applied on a case-by-case basis in an area of up to 1000 m around an IP.

The restrictions in the two districts were lifted in a stepwise manner. The last restriction was lifted in District 2 on 18 May 1995 (duration of epidemic: 569 days) and in District 1 on 15 November 1995 (duration of epidemic: 387 days).

For each outbreak the following variables were collected: Date of first clinical signs, date of suspicion, date of confirmation of infection (laboratory diagnosis), date of stamping-out, type of farm, animal numbers by age group (piglets, finishing pigs, breeding pigs), source of infection (identification of source IP), type of transmission (animals, visitor, truck, neighbour-

hood infection, unknown), morbidity and mortality by age group, laboratory results, distance to next pig farm. Information on farms in a 3 km and 10 km zone around each IP was also collected, but most of the screening results of these farms were not available for this analysis.

The local veterinary authority investigated the outbreak originally and collected the data. After the epidemic, additional data were collected with help of a questionnaire by staff of the Federal Research Centre for Virus Diseases of Animals and the Hannover School of Veterinary Medicine.

2.2 Spatial data and mapping

The geographical co-ordinates of all infected properties were known for both districts (point location of farm building). If several farm units belonging to the same owner were involved in an outbreak, only one co-ordinate was recorded. Additionally, in District 1 the location was known for all pig farms within 1000 m of an infected property. In District 2, the geo-referenced location of all pig farms in the entire district was available.

All farms, their attributes and geographical location were stored in ArcView V.3.0 (Environmental Systems Research Institute Inc., Redlands, U.S.A.). A raster scan of 1:50,000 maps (Niedersächsisches Landesverwaltungsamt, Landesvermessung, 1993-1994) was used as a background image. The district borders and main roads were manually digitised off these maps using a digitising tablet. ArcView was used to produce thematic maps of the outbreak area.

2.3 Analytical methods and simulation

Non-spatial statistical analyses were performed using the statistical software packages SPSS V.7.5 (SPSS Inc., Chicago, U.S.A.) and NCSS97 (Number Cruncher Statistical Systems, Kaysville, Utah). Survival curves were calculated using the Kaplan-Meier estimator. Differences between survival curves were tested with the log-rank test (Kleinbaum, 1997).

Descriptive graphs such as epidemic curves as described by Thrusfield (1995) and network of disease spread were generated in Microsoft Excel97 (Microsoft Corporation, Redmond, U.S.A.).

The INTERSPREAD computer simulation model (32-bit version) which has been developed by Sanson (1993) and Stern (1993, 1997) and modified by Jalvingh *et al.* (1996) was used for the simulation of the outbreak. This program simulates the development of an epidemic in a population of farms using a series of user-defined transmission probabilities (Sanson *et al.*, 1994; Jalvingh *et al.*, in press). The pig farms in District 2 with their spatial location (point location of farm building) were used as the population at risk within which CSF was simulated (2907 farms).

The simulation was started with the farm that was first infected (index case) in the area in October 1993. The date of first clinical suspicion of CSF was available (20.10.93). From this day, an estimated mean incubation period of 12 days was subtracted to estimate the day of infection (8.10.93). The infection was officially confirmed on 26.10.93, and all animals on this farm were killed and destroyed on 27.10.93. Starting with the estimated day of infection

the disease spread was simulated using different spread mechanisms. Neighbourhood spread (local spread), and movement-related spread mechanisms were considered. The disease transmission from the secondarily infected farms was also simulated. All processes were modelled stochastically by drawing random numbers from probability distributions. INTERSPREAD was run using the settings listed in TABLE 71.

Two phases are distinguished when a farm was infected: the first time period lasts from the time of infection of the farm until the incubation period has elapsed, and the second period lasts from the end of the incubation period until the farm is diagnosed with CSF. The incubation period was approximated by a lognormal distribution with a mean of 12 days (SD = 2 days) and the time from onset of clinical signs until diagnosis by a lognormal distribution with a mean of 12.8 days (SD = 10 days, maximum = 28 days). The median duration of both time periods was approximately 12 days in this simulation.

Each simulated movement is associated with one of three risk categories for disease transmission, high risk, medium risk and low risk. The high-risk category includes movements of susceptible animals (pigs), the medium-risk category covers movements of people and vehicles with pig contacts including slurry and semen for artificial insemination, and the low-risk category includes movements of people and vehicles without pig contact as well as for other animals (e.g. cattle) and products. The frequency of high-risk movements was based on data recorded during the outbreak. Data for medium-risk and low-risk movements were not available, and therefore, empirical data from the Netherlands and Switzerland (CHAPTER 2.5) were used to estimate these parameters as well as the distribution of movements over distance classes. The probabilities of infection by different contacts are based on expert opinion. The probability of transmission before the incubation period had elapsed was assumed to be much lower than afterwards (TABLE 71). Currently, INTERSPREAD is not designed to handle two levels of infectivity. Therefore, a weighted average value of the probabilities was used in the model. As both periods have the same median length, the mean of the two transmission probabilities was used.

TABLE 71. Input parameters related to between-farm spread as used by INTERSPREAD to simulate the dynamics of classical swine fever

Daily probabilities of infection of pig farms within certain distance ranges of an infected farm (local spread)[a]					
Distance	0-0.1 km	0.1-0.25 km	0.25-0.5 km	0.5-1 km	
Probability	0.04	0.015	0.01	0.003	
Spread related to movements (movement spread)					
Risk category	High	Medium	Low		
Number per day	0.050	0.070	0.200		
Probability of infection[b]	0.60	0.45	0.01		
Tracing delay (days)	1	1	2		
Proportion of movements remembered by farmer (%)	100	80	50		
Distribution of movements off farms over distance classes	0-3 km	3-10 km	10-20 km	20-30 km	>30 km
Probability of occurrence	0.635	0.195	0.095	0.028	0.047

[a]Local spread was simulated from -2 to 28 days after the day of onset of clinical signs.
[b]Probabilities during incubation period for high, medium and low-risk contacts: 0.3, 0.1, 0, probabilities after the incubation period for high, medium and low-risk contacts: 0.9, 0.8, 0.02.

INTERSPREAD offers the user the choice among a range of control strategies. The following basic set of interventions reflecting the requirements set by German legislation (*Anonymous*, 1994) were used in this simulation: stamping-out of IPs, protection zone of 3 km around an IP, surveillance zone of 10 km around an IP and surveillance. Within each zone, two phases were distinguished: phase 1 with complete standstill of animal movements, and phase 2 with restricted trade. During the outbreak, each farm in the protection zone was visited (level of surveillance = 100%). The level of surveillance in the surveillance zone was set to 50%. In addition, all farms that had experienced a high-risk or medium-risk movement contact were put under surveillance. The effect of being under surveillance for a farm is that movements off the farm are restricted and if the farm is infected, the time until diagnosis is reduced. In the basic scenario movement control was performed as described in TABLE 72.

TABLE 72. Control measures applied in all scenarios

	Protection zone	Surveillance zone	Farms under surveillance[a]
Radius	3 km	3km - 10 km	-
Level of surveillance (%)	100	50[b]	100
Movement control Phase 1			
Start	day of diagnosis	Day of diagnosis	After trace is completed
Duration	21 days	7 days	40 days
(low/medium/high)	Proportions of movements no longer allowed per risk category of movement		
- within same zone	0.9/0/0	0.9/0/0	1/0.2/0
- out of zone	0.9/0/0	0.9/0/0	1/0.2/0
- into zone	0.9/0/0	0.9/0/0	1/0.2/0
Movement control Phase 2			
Start	22 days after diagnosis	8 day after diagnosis	
Duration	18 days	17 days	
(low/medium/high)	Proportions of movements no longer allowed per risk category of movement		
- within same zone	1/0/0.5	1/0/0.5	
- out of zone	1/0/0	1/0/0	
- into zone	1/0/1	1/0/1	

[a]Proportion of farms set under surveillance after risky contacts (contact risk level = high/medium/low): 1/1/0. If such a farm lies within a restricted zone, the control measures defined for the zone apply.
[b]Farms that had experienced a high or medium-risk contact were also put under surveillance for 40 days.

In addition, the simulation was run with and without pre-emptive slaughter of neighbouring properties within up to 1 km of an IP or pre-emptive slaughter based on risk of infection of a farm. In the latter scenario, all farms that had experienced a contact originating from an infected farm were pre-emptively emptied. For a detailed description of the control scenarios refer to TABLE 73. As vaccination is currently not allowed in the European Union, it was not known how exactly this strategy would be applied if it was considered. It was assumed that similarly to a foot-and-mouth disease outbreak, a vaccination buffer would be created around the infected area. Additional assumptions made when running INTERSPREAD are listed in TABLE 74.

TABLE 73. Combination of control strategies

	Basic control [a]	Risk-based pre-emptive slaughter	Distance-based pre-emptive slaughter	Vaccination
Scenario 1	✓	✘	✘	✘
Scenario 2a	✓	✓[b]	✘	✘
Scenario 2b	✓	✓[c]	✘	✘
Scenario 2c	✓	✓[d]	✘	✘
Scenario 3a	✓	✘	✓[e]	✘
Scenario 3b	✓	✘	✓[f]	✘
Scenario 3c	✓	✘	✓[g]	✘
Scenario 4	✓	✘	✘	✓[h]
Scenario 5	✓	✘	✓[f]	✓[h]

[a]Patrol zone 0-3 km around IP, surveillance zone 3-10 km around IP, surveillance within restricted zones and of farms with very-high-risk and high-risk contacts.
[b]Pre-emptive slaughter of farms with very-high-risk contract.
[c]Pre-emptive slaughter of farms with very-high-risk or high-risk contract.
[d]Pre-emptive slaughter of farms with very-high-risk, high-risk or medium-risk contract.
[e]Pre-emptive slaughter of all farms 0-250 m around an IP.
[f]Pre-emptive slaughter of all farms 0-500 m around an IP.
[g]Pre-emptive slaughter of all farms 0-1000 m around an IP.
[h]Vaccination buffer 3-10 km around an IP.

TABLE 74. Further assumptions for classical swine fever simulation with INTERSPREAD

Issue	Assumption	
Time needed for slaughtering farm	≤5,000 animals	1 day
	>5,000 and ≤ 10,000	2 days
	> 10,000 animals	3 days
Max. number of farms that may be slaughtered on one day	5	
Time needed for tracing a movement	Very-high risk	1 day
	High risk	1 day
	Medium risk	2 days
Time needed to find a farm by back-tracing	1 day	
% of local spread remaining after farm is under surveillance	80	
Number of days to become immune after vaccination	14	
Efficacy of vaccine	100 %	
Resources available for vaccination	Unlimited	
Time needed for vaccination	6 days	
Resources available for pre-emptive slaughter	Unlimited	
Time needed for pre-emptive slaughter	0-250 m	2 days
	0-500 m	2 days
	0-1000 m	2 days

The simulation was run for each scenario until the relative error of the mean number of IPs between iterations was <0.5. The duration of the runs was flexible. It either lasted until 30

days after the time when no more farms were infected or for a maximum of 360 days from the day of diagnosis or 378 days from the day of first infection.

For each scenario descriptive statistics were calculated for the number of IPs that had occurred and for the duration of the epidemic (days). The Aspin-Welch-test for differences between the means were applied (NCSS97, Number Cruncher Statistical Systems, Kaysville, Utah). The number of IPs was transformed by taking the square root in order to approximate the characteristics of a normal distribution. Aspects of the dynamics of the epidemics under the different control scenarios were compared using survival curves. Survival curves display the probability of experiencing a time-related event for individuals in a study cohort. In this case the time-related event was the end of the epidemic and the curve was calculated based on the results of all iterations performed with the same control scenario. 'Survival' in this case was defined as the duration of the epidemic in days. A shorter survival time means that on average, the outbreak is eliminated more quickly.

3. Results

3.1 Maps of outbreak area

FIGURE 49 shows a map produced with ArcView. On this map the location and the status of individual farms is displayed for District 2. While the locations of cases in District 2 appear to be rather dispersed, the ones in District 1 are mainly located in the north of the district (data not shown). Because of the small number of cases and incomplete location data for non-infected farms in District 1, no formal test for spatial clustering was applied.

In FIGURE 50 the same sort of information is provided with a scanned road map as background image. This is an example of a map as it could be used by field personnel or to illustrate the status of the epidemic to other interested parties. Additional information such as boundaries of restricted zones could be added if available.

3.2 Epidemic curve

In a first step to describe the temporal dynamics of the epidemic, an epidemic curve was produced (FIGURE 51). In the same graph both the date of confirmation of infection of a farm and the date of first clinical signs were plotted. As the date of first clinical signs was not known for all IPs, the distribution of this value is probably biased.

The graph shows that the epidemic in this region consisted of two waves of infection, the first of which was initiated by an outbreak due to purchase of infected animals from outside the region. Secondary outbreaks related to the index case were not diagnosed until February 1994. Whether the second wave of outbreaks starting in October 1994 is actually related to the first wave or whether it is due to a second introduction from outside, cannot be determined, because the source of the first IPs in the second wave could not be identified.

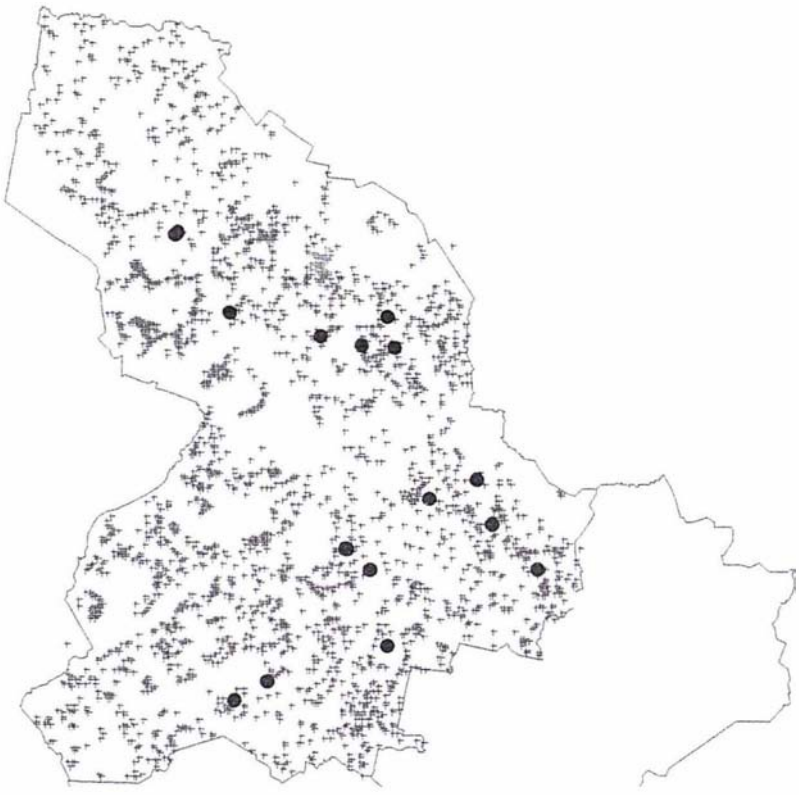


FIGURE 49. Map of pig farm locations in District 2: + = uninfected, ● = infected

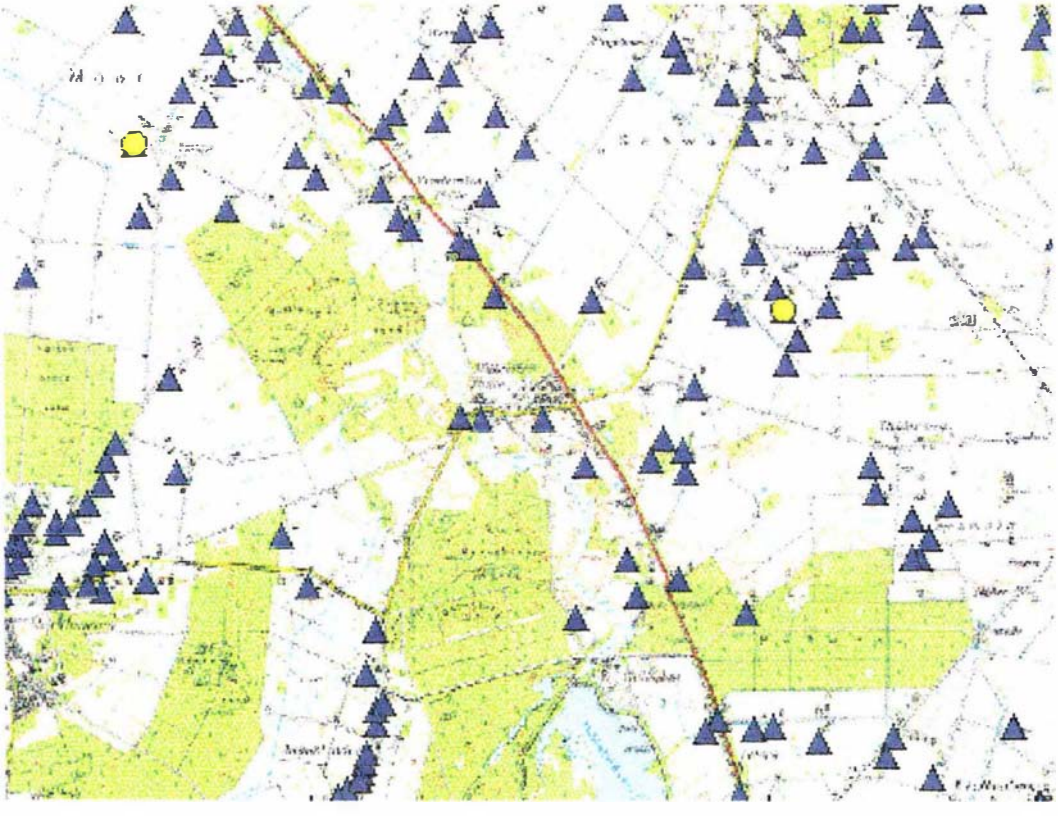


FIGURE 50. Example of a thematic map: ▲ = uninfected, ● = infected

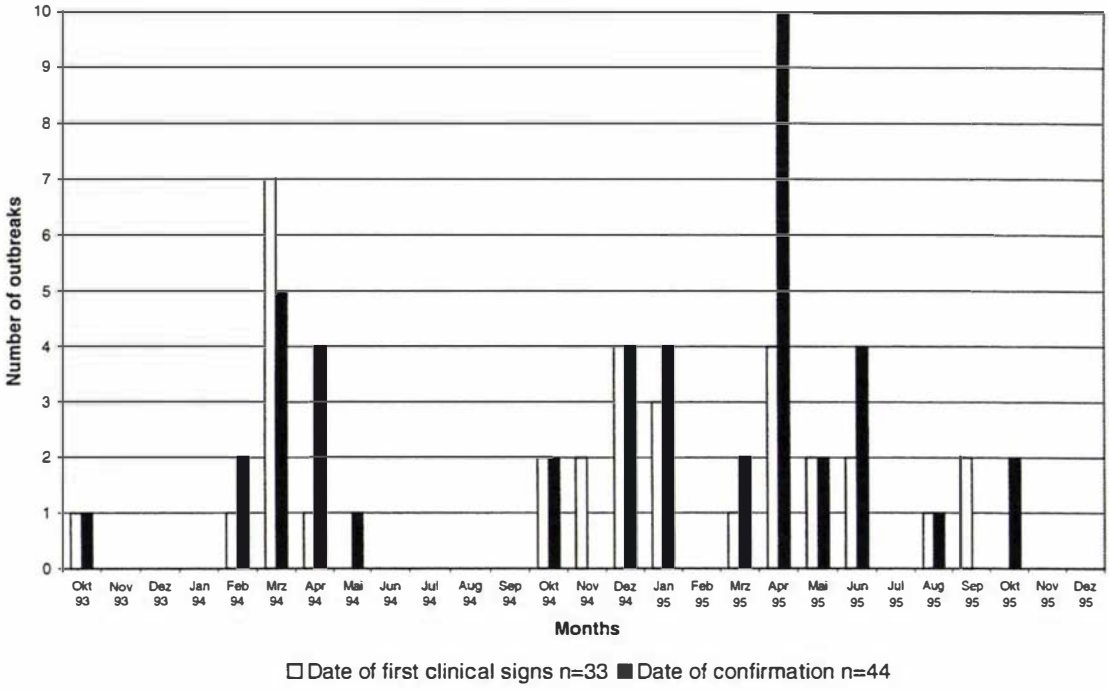


FIGURE 51. Epidemic curve of classical swine fever outbreaks two districts in Lower Saxony between October 1993 and October 1995

For the farms with a known date of first clinical signs (n=33), the time interval between onset of disease and confirmation of disease averaged 12.8 days (SD = 10.0). And the average time between first clinical signs and stamping out was 12.5 (SD = 9.4).

3.3 Network of spread

In order to describe the relationships between infected farms, epidemiologists often use graphs where related IPs are linked. The links can be symbolised using different types of arrows depending on the type of transmission that has been identified. If the relationships of the infected farms are plotted over time, this network of spread is helpful for visualising the transmission dynamics of the disease. FIGURE 52 displays the network of spread for the farms used in this analysis.

This graph shows that the source of infection was known or suspected for 22 (50%) of all outbreaks, 32% of which were diagnosed as within-property transmission, i.e. the disease spread among farms owned by the same person or family.

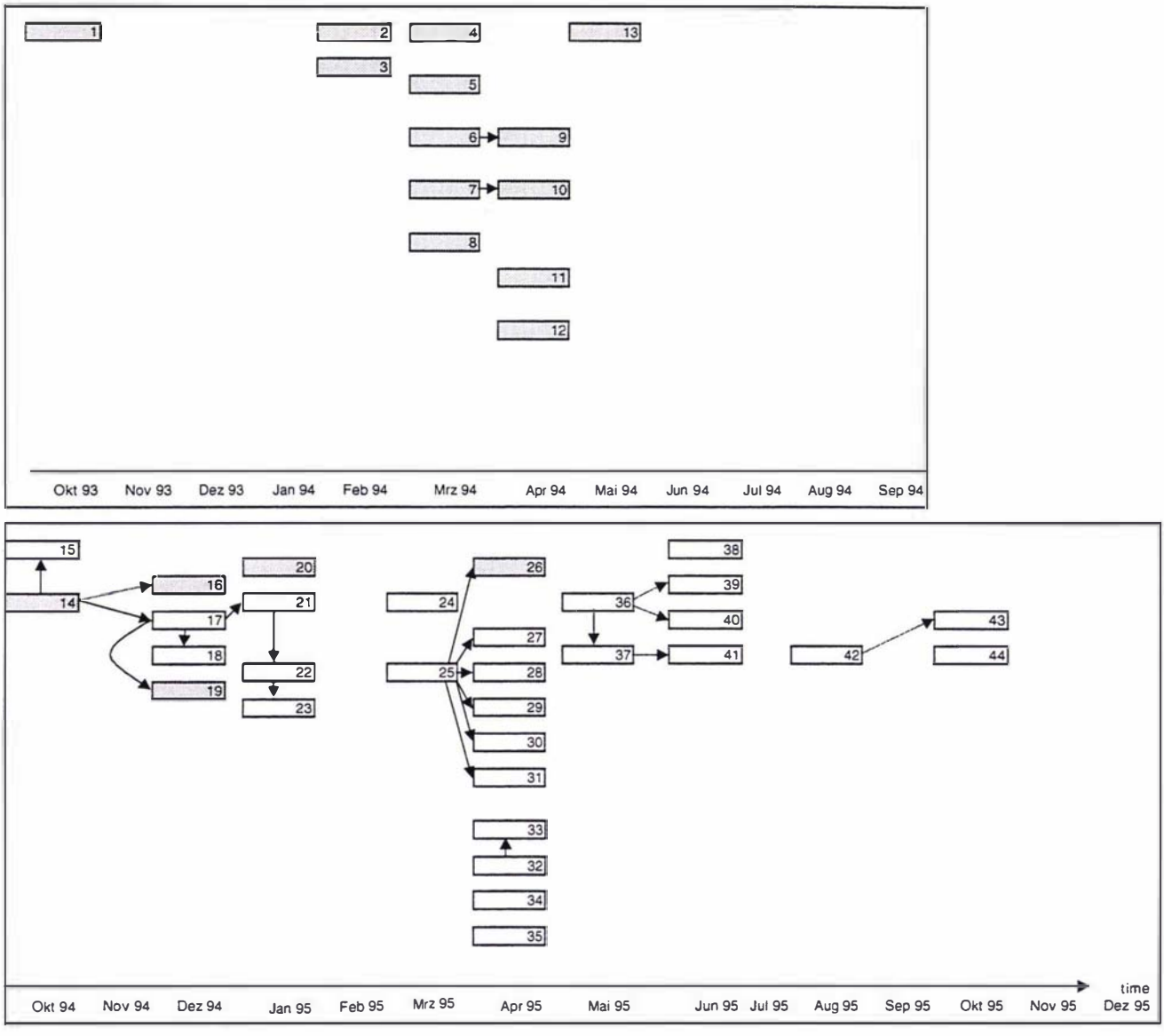


FIGURE 52. Network of spread of classical swine fever in two districts in Lower Saxony, Germany, between October 1993 and October 1995 (44 outbreaks)

Confirmed transmission
Suspected transmission
Farm in District 2
Farm in District 1

3.4 Survival curve and dissemination rate

The probability of farms becoming infected with CSF in relation to time can be presented using survival curves. Because one must know the total number of farms at risk to calculate the survival function, this could only be done for District 2.

Because the population at risk in District 2 was very large (more than 2900 pig farms) and the number of IPs was relatively small (18 cases) the survival probability decreases only slowly over time (FIGURE 53). However, the two phases with an increased number of outbreaks

(two peaks in epidemic curve, FIGURE 51) are still visible as steeper declines in the survival probability.

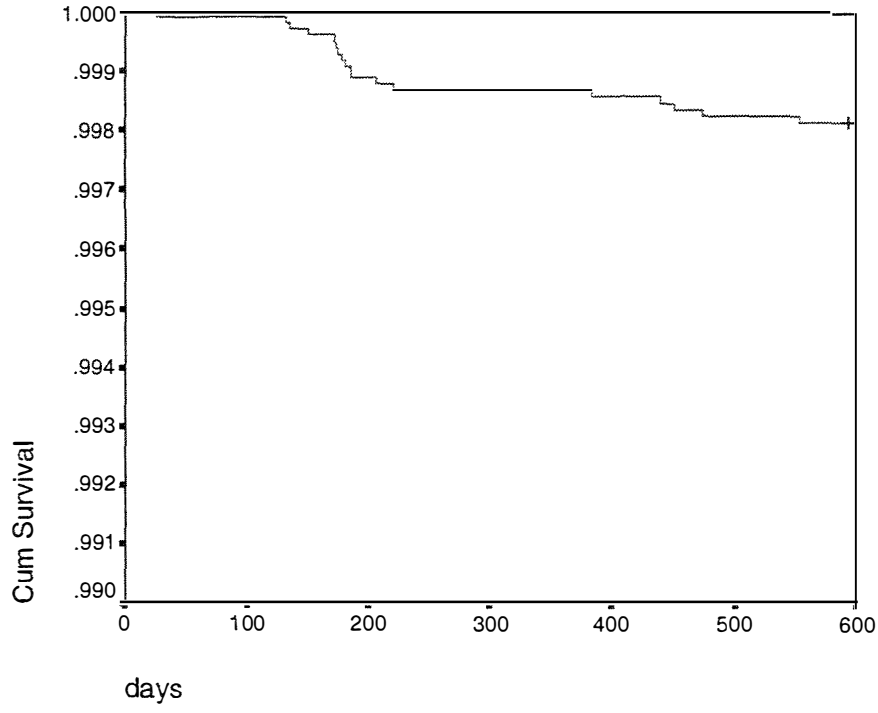


FIGURE 53. Survival function for farms in District 2 between 01/Oct/93 and 18/May/95

3.5 Proportion of IP caused by episode types

The relative importance of different modes of transmission is important for identifying high-risk properties and for the evaluation of control strategies. In this outbreak, infection was partly due to contaminated trucks and personal contacts between farms (TABLE 75). Neighbourhood infection was also identified as a means of transmission in a number of cases. Although an intensive epidemiological investigation was performed for all farms, the mode of transmission of infection could not be identified in over 50% of all cases. In 7 cases, contaminated vehicles were suspected, but the source could not be confirmed. Four farms were in very close neighbourhood of infected properties and could also have been infected by neighbourhood spread. Slurry was suspected as a means of transmission in one case.

TABLE 75. Distribution of sources of classical swine fever infection for 37 outbreaks in Germany[a]

Source	Confirmed (number of farms)	%	Suspected (number of farms)	% including confirmed and suspected
Pig transports	7	15.9		15.9
Person contact	2	4.5		4.5
Contaminated vehicle	0	0.0	7	15.9
Same management	7	15.9		15.9
Neighbourhood infection	4	9.1	4	18.2
Slurry	0	0.0	1	2.3
Unknown	24	54.5		27.3

[a]Based on data available by October 97.

3.6 Simulation modelling

When the epidemic was simulated without any control methods in place, a mean number of 1424.2 farms became infected and the epidemic lasted on average for 249.7 days (TABLE 76). With respect to the transmission mechanisms, 44.6 % of the farms were infected through local spread, the remainder through movement spread (TABLE 77). All control scenarios significantly reduced both the number of infected farms as well as the duration of the uncontrolled epidemic (data not shown).

As TABLE 77 demonstrates the basic control strategy mainly reduced the number of outbreaks due to movement spread. Local spread then accounted for 80 % of all infections. From all control measures, area-based pre-emptive slaughter seemed to be most suitable for preventing local spread by reducing its importance to the level where it accounted for 54% of all IPs.

The chosen control methods (including pre-emptive slaughter) were then compared with the basic scenario. The epidemic curves calculated on the basis of the mean weekly numbers of IPs are shown in FIGURE 54 A and B. When applying area-wide pre-emptive slaughter, the mean number of IPs was reduced from 28.6 with the basic control to 14.9 – 9.7 (TABLE 76). However, because 23 -31 farms had to be pre-emptively slaughtered, the total number of culled farms was still high, even higher than with the basic control strategy (TABLE 77). This effect was much less dramatic when applying risk-based pre-emptive slaughter. Slaughtering farms on a risk basis, however, reduced the mean number of IPs by a maximum of 30 %. The reduction of the mean number of IPs was statistically significant at the 5%-level comparing the basic control and all scenarios with area-based pre-emptive slaughter (p=0.001-0.009, Aspin-Welch test).

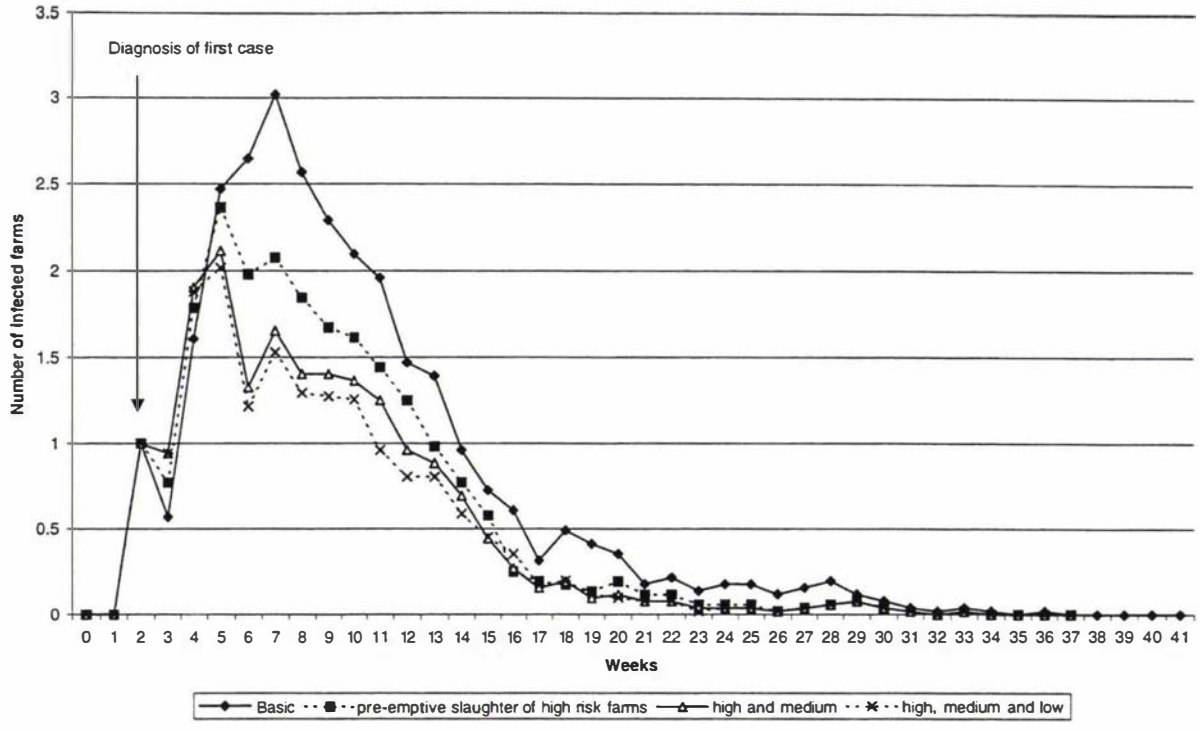
A scenario including vaccination was significantly more effective than the basic scenario only if combined with pre-emptive slaughter (TABLE 76).

TABLE 76. Descriptive statistics of INTERSPREAD simulations

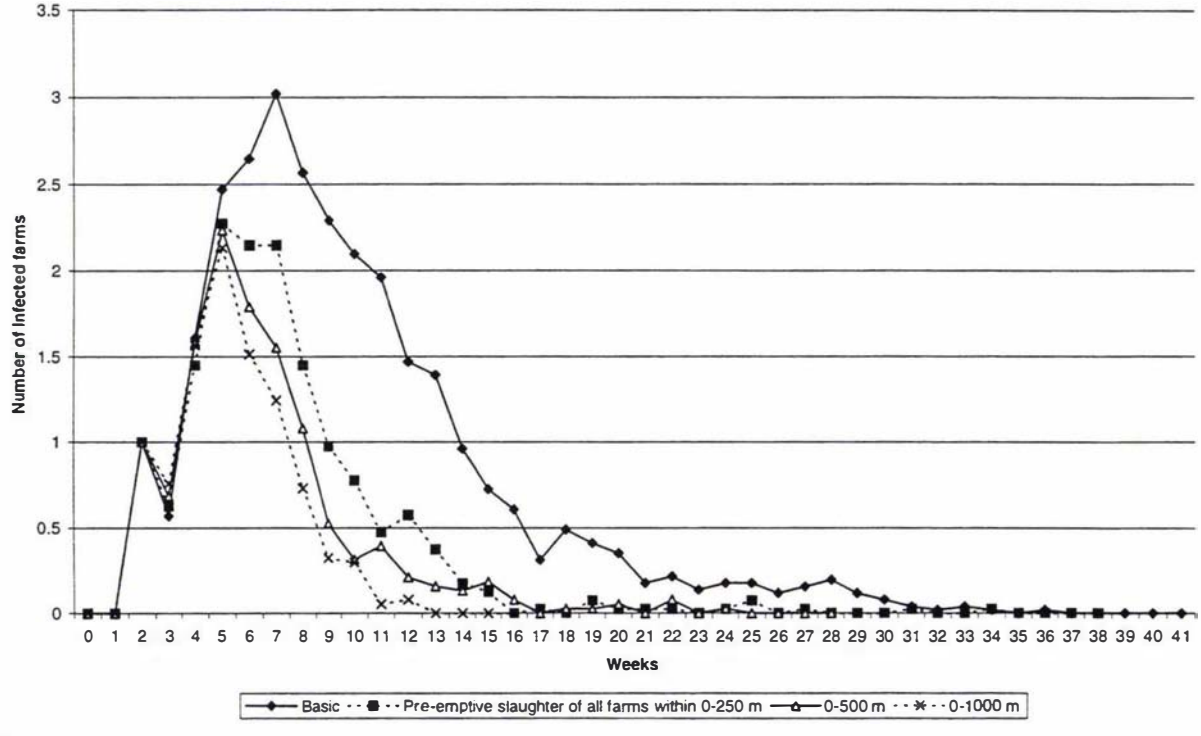
Control scenario	#Runs	IP				Duration (days)			
		Mean	Lower 95% C.I.	Upper 95% C.I.	SD	Mean	Lower 95% C.I.	Upper 95% C.I.	SD
No control	15	1424.2	846.8	2001.6	1042.7	249.7	169.1	330.3	145.6
Movement Control and Surveillance (= Basic)	51	28.6	17.4	39.9	39.9	109.4	93.2	125.7	57.8
Pre-emptive slaughter of very-high risk herds	52	21.8	13.1	30.4	31.0	101.0	88.0	114.1	46.8
Pre-emptive slaughter of very-high and high-risk herds	52	18.7	11.4	26.0	26.1	97.6	84.9	110.3	45.5
Pre-emptive slaughter of very-high, high, and medium-risk herds	51	17.5	10.6	24.4	24.6	96.7	83.9	109.5	45.5
Pre-emptive slaughter 0-250 m around IP	40	14.9	9.1	20.7	18.1	92.3	79.2	105.3	40.7
Pre-emptive slaughter 0-500 m around IP	38	12.1	7.3	16.9	14.6	87.9	75.9	100.0	36.6
Pre-emptive slaughter 0-1000 m around IP	37	9.7	5.8	13.6	11.6	79.0	71.8	86.2	21.6
Vaccination 3-10 km	43	18.8	11.3	26.3	24.3	98.1	84.6	111.5	43.7
Vaccination 3-10 km and pre-emptive slaughter of very-high, high and medium-risk herds	36	9.6	5.8	13.3	11.2	78.9	71.4	86.4	22.3

TABLE 77. Number of farms affected by different infection and control mechanisms

Control	Mean number of farms						% local spread
	Local spread	Movement spread	Pre-emptively culled (% of emptied farms)		Vaccinated	Emptied in total	
No control	634.4	788.9	0		0	1423.3	44.6
Movement Control and Surveillance (= Basic)	22.1	5.5	0		0	27.6	80.0
Pre-emptive slaughter of very-high risk herds	16.4	4.7	1.8	(7.9)	0	22.9	77.7
Pre-emptive slaughter of very-high and high-risk herds	13.7	4.8	4.2	(18.5)	0	22.7	74.1
Pre-emptive slaughter of very-high, high, and medium-risk herds	12.8	4.4	12.7	(42.5)	0	29.9	74.4
Pre-emptive slaughter 0-250 m around IP	10.5	4.6	22.7	(60.1)	0	37.8	69.5
Pre-emptive slaughter 0-500 m around IP	8.0	4.6	30.8	(71.0)	0	43.4	63.5
Pre-emptive slaughter 0-1000 m around IP	5.6	4.6	31.3	(75.4)	0	41.5	54.9
Vaccination 3-5 km	13.1	4.6	0		170.2	17.7	74.0
Vaccination 3-5 km and pre-emptive slaughter of very-high, high and medium-risk herds	5.1	4.4	17.8	(65.2)	174.7	27.3	53.7

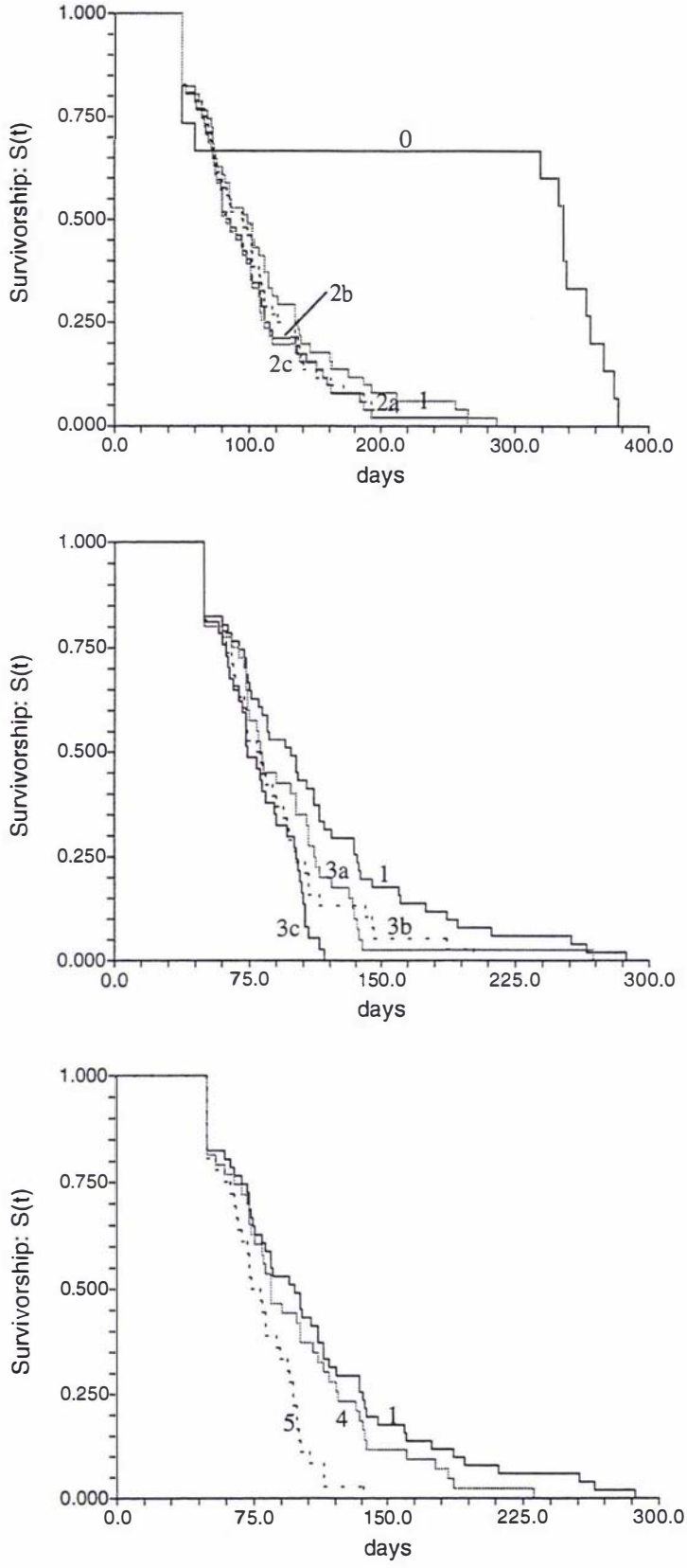


A)



B)

FIGURE 54. Typical epidemic curves of mean weekly numbers of classical swine fever outbreaks simulated with INTERSPREAD and applying different control strategies (n = number of iterations)



0 = uncontrolled
1 = basic control (EU legislation)
2a = pre-emptive slaughter of high-risk herds
2b = pre-emptive slaughter of high-risk herds and medium-risk herds
2c = pre-emptive slaughter of high-risk herds, medium-risk and low-risk herds
3a = pre-emptive slaughter in 250 m radius
3b = pre-emptive slaughter in 500 m radius
3c = pre-emptive slaughter in 1000 m radius
4 = vaccination in 3-10 km area
5 = 2c and 4 combined

FIGURE 55. Survival curves for duration of classical swine fever epidemics

In order to compare the effect of the control strategies on the duration of the epidemic, survival analysis was used. The time from initial infection to extinction of the epidemic was used as the time-related event in the survival curves shown in FIGURE 55. The median survival time for scenarios 1, 2a, 2b, 2c, 3a, 3b, and 3c was 98, 94, 83, 83, 81, 81 and 74 days, respectively. Using the log-rank test it was shown, that the survival curves for the duration of the epidemic were significantly differente at the 5%-level comparing the basic scenario and the scenario including area-based pre-emptive slaughter of farms up to 500 m (χ^2= 4.12, 1 d.f, p=0.042) and 1000 m (χ^2=9.57, 1 d.f., p=0.002) around an IP. When vaccination was used, the duration was only significantly reduced if pre-emptive slaughter was applied as well (χ^2=9.35, 1 d.f., p=0.002).

4. Discussion

4.1 Suitability of field data

During a CSF outbreak data are needed for analysing the dynamics of the epidemic in order to assess the effectiveness of control measures. The disease control headquarter needs to know whether the epidemic is still growing at an increasing rate, slowing down its rate of growth or starting to decrease as a result of the current control strategy. This can be determined by using a set of performance measures such as the incidence of new cases, the dissemination rate (number of new cases divided by earlier cases), the time to detection of new outbreaks or the pattern of infection sources. With help of the Epidemiologist's Workbench as included in EpiMAN, such performance measures can be calculated repeatedly during an epidemic ('real time'). One of the objectives of this chapter was to evaluate the suitability of field data that was not collected with a system similar to EpiMAN-SF but using the recording systems currently in place at the district level in Germany.

Most districts were using paper-based recording systems at the time of the 1993-1995 CSF epidemic. Data were collected by field teams and hard copies of their reports were filed in the district capital. The data used for international reporting were forwarded to a national centre for further processing. More detailed data however were only stored at the district level. Some more detailed data were collected by questionnaire after the outbreak had been successfully controlled.

Several difficulties were observed with this system:

1. Data are only available as hard copies or in separate databases at the district-level. If data entry or merging of databases is required, 'real time' analysis becomes more difficult. Also data ownership is at the district level and it may therefore be difficult to obtain data for analysis at higher levels within the administrative structure. As districts are relatively small administrative units, epidemics will almost always involve several districts and possibly federal states. The 1993-1995 CSF outbreak for example involved 47 districts in 9 states (Länder). In Lower Saxony 16 districts were affected (Teuffert *et al.*, 1997).
2. The level of detail of data recording may be different in each district and is generally insufficient for detailed epidemiological analyses. If it is unclear how specific values in the data base fields are defined or coded, it is necessary to keep in frequent contact with the

district level to clarify the questions. This is time-consuming and some questions may still remain unanswered. More detailed information may be available on hard copies at the district level, but data entry will be required and again, real time analysis is not possible.

3. Because the people responsible for data collection and entering may not know what type of analysis is needed, they may not collect the most suitable information and a lot of time and effort may be invested in vain. If the people recording the data do not get feedback such as reports with the results of the epidemiological analysis and if they do not appreciate the importance of the data, data quality may also suffer.
4. Data are mainly recorded for infected farms, but only little information is collected for the other farms affected by the outbreak. For example field teams visit all farms in the protection and in the surveillance zone and samples are collected for laboratory analysis. This key information is currently not available in electronic form and therefore not accessible for analysis at the time of the outbreak.
5. Spatial data may not be available at the time of the outbreak or it is stored in a different database, and merging the data may be technically difficult. Data ownership may also be complicating the access to spatial data.

The conclusion is that field data as it is currently collected in many countries is not suitable for quantitative epidemiological analysis during a CSF epidemic, mainly because it is not electronically accessible at a regional level. Also, the routinely recorded data are unlikely to be sufficiently detailed, and they are too much focussed on the IP as opposed to all farms at risk. Jalvingh *et al.* (in press) made similar observations when using Dutch CSF data for a simulation and concluded that outbreak data often do not meet the needs of a simulation model.

An additional problem is that spatial data may not be available for every farm. This point is likely to be similarly problematic in most countries which do not have a national farm data base with information on farm location. The development of a national database requires considerable time and resources as was demonstrated in the cases of New Zealand (Sanson and Pearson, 1997) and the Netherlands (Nielen *et al.*, 1996).

Despite the limitations of the current German data recording system in terms of immediate and region-wide analysis during a disease emergency, the data are valuable for retrospective analyses similar to the ones reported in this chapter.

4.2 Outbreak dynamics

Data from two districts in Lower Saxony were used to investigate the dynamics of the 1993-1995 CSF epidemic. However, the events in the two districts were probably strongly influenced by outbreaks occurring in neighbouring areas which were affected by the disease at the same time. It is therefore difficult to conclusively interpret the results in isolation from related events. The results should rather be seen as an illustration of what could be performed during an outbreak when using an electronic data recording system in combination with epidemiological analysis.

The epidemic curve of the two districts we analysed had a bimodal shape. Whether the second peak is due to a new introduction of the virus from a contiguous area is not known. However,

given the long duration of the epidemic, it is likely to have happened. This is supported by the fact that such long epidemics never occurred when CSF was simulated with INTERSPREAD. The mean duration for an uncontrolled epidemic was 250 days as opposed to 569 days for District 2 and 387 days for District 1. When the data for District 2 is split into two phases for the two peaks of the epidemic this results in two time periods with a duration of 210 and 359 days, respectively. Both figures are outside the upper 95% confidence limit for any controlled epidemic. Given the fact, that CSF occurred in neighbouring districts simultaneously, the long duration may indicate repeated introductions of virus into the area.

Although the network of spread is incomplete and should be connected with data from surrounding areas, it shows that the transmission between farm units that belong to the same owner can occur via direct or indirect contacts. Most farms were infected by movements of animals, people or trucks. Including cases where the source is only suspected, the odds to become infected by a movement as opposed to local spread were about 2:1. In the simulations however, more infections were due to local spread than to movements. It is however difficult to interpret the significance of this finding as for at least 27% of the IPs the source of infection remained unknown, and it cannot be assumed that neighbourhood and movement infections are equally represented in this group of farms.

4.3 Simulation and selection of control strategy

4.3.1 Assumptions and model parameters

As Jalvingh *et al.* (in press) pointed out, assigning parameters for CSF spread in a simulation model is difficult due to the lack of adequate field data. The simulation parameters used in this exercise were based on field observations and a sensitivity analysis previously performed with INTERSPREAD (see APPENDIX H) as well as expert opinion. The sensitivity analysis provided estimates of the relative sensitivity of the model output with respect to the input parameters. Both the duration of the epidemic and the number of IPs were mainly influenced by the time from first clinical signs to diagnosis. This time period had a mean of 13.63 days (SD 10.47, n=16) in District 2 and 11.65 (SD = 8.75, n=23) in District 1. The default value of INTERSPREAD assumes a lognormal distribution with a mean of 12.8 (SD = 10), which is a reasonable approximation to the values in this real epidemic.

The incubation period of the virus strain involved in the German outbreak was investigated under experimental conditions and reported to be approximately 6-7 days indicating a relatively high virulence (Depner *et al.*, 1994,1997). The default values of INTERSPREAD assume a less virulent strain with a longer incubation period represented using Sartwell's model (Sartwell, 1950; Glickman *et al.*, 1987) by a lognormal distribution with a mean of 12 days and a SD of 2. Given the laboratory results mentioned above, a distribution with a mean of 7 days would have been more realistic. However, as INTERSPREAD simulates disease transmission between farms and not animals, the incubation period is technically not the correct interval to use. What needs to be simulated is the transition of a farm from a period of low infectivity (only few animals shedding virus) to a period of high infectivity (many animals infected). The low-infectivity period is longer than the incubation period because several disease cycles may be necessary to build up a critical number of infectious animals. This number

is dependent on the virus strain, the age group of animals affected and the physical separation of pig houses on a farm. Currently, no empirical data is available to estimate the duration of the low-infectivity period because the day of infection is not known for most farms in a real outbreak. However, a simulation model for within-farm spread as described in CHAPTER 2.8 could be used to explore the dynamics of the disease within a farm. A similar approach was used by Jalvingh *et al.* (in press) when they adapted INTERSPREAD to simulate the dynamics of infectious bovine rhinotracheitis (IBR). In the IBR model, a deterministic state-transition model was used to calculate the number of infectious animals on a farm for each week. This figure then determined the risk of transmission of IBR to other farms.

An alternative to simulation is the use of expert opinion. German CSF experts suggest that two disease cycles would be a reasonable duration for the low-infectivity period on a farm. Simulations using a time interval with a lognormal distribution with a mean of 24 and a SD of 10 and a maximum of 36 days (three disease cycles) were subsequently performed to explore the effect of the change. The mean number of IPs and the mean duration under the basic control scenario were 45 farms and 203 days, respectively. Our sensitivity analysis had already demonstrated (APPENDIX H) that mean duration of the epidemic is strongly influenced by the low-risk time period (formerly called 'incubation period'). Therefore, this combination of parameters allows to obtain longer epidemics. More research is needed however, to determine the input variables more accurately.

The number of movements between pig farms was only available for high-risk movements in this analysis. It was assumed that the frequency of the other movement categories was likely to be similar to those recorded for Switzerland and the Netherlands (see CHAPTER 2.5). Given the fact that the number of movements was comparable in two countries with very different production systems such as the Netherlands and Switzerland, this assumption seems to be justifiable. However, field data should be collected in order to better quantify this variable under German conditions.

It was also not possible to quantify two other important input variables: the probability of infection by a contact (movement) and the probability of local spread. INTERSPREAD currently only allows the use of one transmission probability for the entire duration of the infection on one farm. However, from the above described suggestion to use two intervals with different infectivity levels follows directly that movements during the two intervals should be associated with different risks to transmit the disease. In this simulation an average value was used, but it is planned that INTERSPREAD will be able to handle two periods of infectivity in the future. Another suggestion to improve the model behaviour is the use of value distributions for the transmission probabilities instead of one mean value. This would allow for the adequate representation of the uncertainty related to these values. Experiments could then be conducted to quantify expert knowledge of these variables.

The transmission probabilities used for local spread are currently based on field data (Staubach *et al.*, 1997; Benard, personal communication) and expert opinion and represent 'best guesses' given the incomplete knowledge about this factor and more research is necessary to quantify this variable. The high proportion of IPs due to local spread may be an indication that the current values may be too high. On the other hand, Roberts (1995) reported that in approximately 50% of all infections could be due to local spread in an area with a high pig density. The probability of local spread should also take into account the change of infectivity of the farm over time. This mechanism is envisaged to be included in a new version of IN-

TERSPREAD that will be specifiaclly designed to simulate swine fever epidemics (INTER-SPREAD-SF). However, while the probability of transmission by a movement can be expected to be reasonably comparable between outbreaks in different regions, the probability of local spread may depend on factors related to a particular area, for example the farm density or the occurrence of wildlife. It therefore may have to be adjusted each time when analysing a particular outbreak.

In summary, given our incomplete knowledge about CSF dynamics, the parameter values used by INTERSPREAD in this simulation appear to be reasonably realistic for a crude analysis at the beginning of an epidemic.

4.3.2 Comparison of control strategies

Using the basic control strategy, the mean number of outbreaks is expected to fall between 17.4 and 39.9 in 95% of the time. In the actual outbreak, pre-emptive slaughter was used on a case-by-case basis. If pre-emptive slaughter was applied, it was performed within a radius of up to 1000 m around each IP. The simulation predicts a mean number of outbreaks between 5.8 and 13.6 for this scenario. However, when the longer low-infectivity period is used instead of the incubation period, as explained above, the number of outbreaks predicted for this scenario is between 9.0 and 21.1 (mean = 15.0). The real number of outbreaks in District 2 (18) falls in this range.

When looking at the duration of the epidemic, the results of the simulation and the real observations are different. The observed epidemic lasted much longer than the model predicted. Even in an uncontrolled situation, INTERSPREAD predicts that outbreaks of more than 346 days are unlikely. Given any of the control strategies in place, the epidemic would hardly have lasted longer than 100 days. Using the period of low-infectivity instead of the incubation period the upper 95% confidence interval under scenario 3c is at 152 days (mean=128.7 days). In reality, however, the epidemic lasted much longer. Possible explanations for this discrepancy could be a too short low-risk period or, as mentioned earlier, a repeated introduction of virus into the area.

The comparison of the different control strategies revealed a strong effect of area-based pre-emptive slaughter. The necessity to slaughter farms before signs of disease are detected is crucial for gaining time and thus to get the epidemic under control. While the area-based strategy may be logistically easier to apply because it does not require effective tracing, it results in very large numbers of emptied farms. In this simulation between 60.1-75.4 % of all culled herds were slaughtered because of area-based control measures and only the remaining percentage was due to diagnosed infection. When risk-based slaughter was applied, a maximum of 42.5 % of farms were culled due to pre-emptive slaughter, the rest due to infection. The lowest number of emptied farms was achieved with a risk-based pre-emptive scenario where only very-high-risk and high-risk farms were culled and when vaccination only was applied.

Although differences between the effects of control strategies were tested for statistical significance in this paper, the latter may not have real meaning for the decision-maker because statistical significance does not imply biological or economical significance. Significance testing was useful for exploratory data analysis but on its own is not considered as a recommended method for selecting control strategies during an epidemic.

In order to be able to choose between different control strategies, a monetary value for the direct costs incurred by each IP needs to be assigned. In Germany, Küttler (1996, cited by Teuffert *et al.*, 1997) calculated an average cost for compensating slaughtered animals and for laboratory analyses of 2.069 million DM for each IP (including costs for pre-emptively slaughtered farms). Meuwissen *et al.* (1997) demonstrated that the cost of eradication is only a small amount of the total costs incurred. Therefore additional costs due to production losses on affected farms and in areas with movement control have to be included as well as expenses for organising the eradication campaign. Indirect costs due to market support and international trade disruptions also need to be considered. The latter are probably more dependent on the duration of the epidemic than the actual number of outbreaks. Both these aspects need to be included in an economic model similar to the one developed for INTERSPREAD for foot-and-mouth disease (Jalvingh *et al.*, 1996, 1997) or as outlined by Horst (1997) for CSF.

Without the economic dimension, a selection among control strategies can only be made on the assumption that the shortest outbreak with the minimum amount of emptied farms would be most preferable. Assuming equal weight for both parameters and using a sum of ranks, the scenario with risk-based slaughter of high and medium-risk farms appeared to be preferable. If vaccination was allowed, this strategy particularly in combination with risk-based pre-emptive slaughter could also be considered. The second choice would be a scenario with area-based pre-emptive slaughter. Although these strategies required a very high number of farms to be emptied they were successful in controlling the epidemic quickly probably because of their efficiency in preventing local spread.

The disadvantage of this method of evaluating control strategies is that the variability of results is not taken into account. In order to provide useful decision support during a real outbreak, the model needs to provide the probability of achieving certain results given a particular control measure. It is then up to the decision-maker to evaluate the results adopting a risk-averse or a risk-taking attitude. Again, the inclusion of economic figures is indispensable for realistic decision support. Once these elements are added to INTERSPREAD-SF, this model can become one of the most powerful decision tools for online support of managers in a CSF emergency.

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References

Anonymous (1994). Verordnung zum Schutz gegen die Schweinepest und die Afrikanische Schweinepest. Bundesgesetzblatt Teil I, 3163-3174.

Depner, K., Gruber, A., and Liess, B. (1994). Experimental infection of weaner pigs with a field isolate of hog cholera classical swine fever virus derived from a recent outbreak in Lower Saxony. 1. Clinical, virological

and serological findings. Wiener Tierärztliche Monatsschrift 81:370-373.

Depner, K.R., Hinrichs, U., Bickhardt, K., Greiserwilke, I., Pohlenz, J., Moennig, V., and Liess, B. (1997). Influence of breed-related factors on the course of classical swine fever virus infection. Veterinary Record 140:506-507.

Glickman, L.T., Lee, L.A., and Cohen, N.D. (1987). Logarithmic normal distribution of incubation times for canine diseases (Sartwell's model). American Journal of Veterinary Research 48:842-847.

Jalvingh, A.W., Nielen, M., Dijkhuizen, A.A., and Morris, R.S. (1995). A computerized decision support system for contagious animal disease control. Pig News and Information 16:9N-12N.

Jalvingh, A.W., Nielen, M., Dijkhuizen, A.A. and Huirne, R.B.M. (1996). Development of economic models for integration into EpiMAN (EU). In: EpiMAN(EU): A computer system for the management of epidemiological data and prediction of risk and economic consequences during outbreaks of foot-and-mouth disease. Unpublished final report for the European Union (Contract AIR3-CT92-0652), Wageningen Agributiral Univeristy, The Netherlands.

Jalvingh, A.W., Nielen, M., Meuwissen, M.P.M., Dijkhuizen, A.A., and Morris, R.S. (1997). Economic evaluation of foot-and-mouth disease control strategies using spatial and stochastic simulation. Epidémiologie et Santé Animale 31-32:10.22.1-10.22.3.

Jalvingh, A.W., Vonk Noordegraaf, A., Nielen, M., Maurice, H., and Dijkhuizen, A.A. (in press). Epidemiological and economic evaluation of disease control strategies and spatial simulation: General framework and two applications. Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine.

Horst, H.S. (1997) Risk and economic consequences of contagious animal disease introduction. PhD thesis, Wageningen Agricultural University, Wageningen, the Netherlands.

Kleinbaum, D.G. (1997). Survival analysis: a self-learning text. Springer Verlag, New York.

Kramer, M., Ahl, R., Teuffert, J., Kroschewski, K., Schlüter, H., and Otte, J. (1995). Classical swine fever in Germany - some epidemiological aspects. Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine, Reading 110-118.

Meuwissen, M.P.M., Horst, H.S., Huirne, R.B.M., and Dijkhuizen, A.A. (1997). Insurance against losses from contagious animal disease. Epidémiologie et Santé Animale 31-32:10.13.1-10.13.3.

Nielen, M., Jalvingh, A.W., Dijkhuizen, A.A., and Lattuada, R. (1996). Requirements for a geographical information system to be used during a foot-and-mouth disease outbreak. Proceedings of the Annual Meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Glasgow, 178-184.

Roberts, M. (1995). Evaluation of optimal size of restriction zones in disease control with particular reference to classical swine fever. Proceedings of the Annual Meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Reading 119-130.

Sanson, R.L. (1993). The development of a decision support system for an animal disease emergency. PhD Thesis, Massey University, Palmerston North, New Zealand.

Sanson, R.L., Stern, M.W., and Morris, R.S. (1994). INTERSPREAD – a spatial stochastic simulation model of epidemic foot-and-mouth disease. The Kenya Veterinarian 18:493-495.

Sanson, R.L., and Pearson, A. (1997). Agribase – a national spatial farm database. Epidémiologie et Santé Animale 31-32:12.16.1-12.16.2

Sartwell, P.E. (1950). The distribution of incubation periods of infectious disease. American Journal of Hygiene 51:310-318.

Staubach, C., Teuffert, J., and Thulke, H.-H. (1997). Risk analysis and local spread mechanisms of classical swine fever. Epidémiologie et Santé Animale 13-32:06.12.1-06.12.3.

Stern, M.W. (1993) INTERSPREAD FMD epidemic simulation tool, user requirements. Unpublished document, Massey University, Palmerston North.

Stern, M.W. (1997). INTERSPREAD-KERNEL User guide. Unpublished document, Massey University, Palmerston North.

Teuffert, J., Schlüter, H, and Kramer, M. (1997). Europäische Schweinepest – Übersicht zur internationalen (Europa) und nationalen Schweinepestsituation – ermittelte Einschleppungsursachen und Verschleppungsrisiken. Deutsches Tierärzteblatt 45:1078-1080.

Thrusfield, M. (1995). Veterinary epidemiology. 2nd Edition. Blackwell Science Ltd., Oxford, 114-120.

Vantemsche, V. (1996). Classical swine fever 1993-1994 Belgium. The Pig Journal 37:43-53.

GENERAL DISCUSSION

1. What is a system?

The title of this thesis is: Systems for the prevention and control of infectious diseases in pigs. The term 'systems' therefore appears to play quite a key role and it seems useful to define the expression for this general discussion. In the Merriam-Webster dictionary a system is defined as (http:\\www.m-w.com/netdict.htm):

"*a regularly interacting or interdependent group of items forming a unified whole*"

or also as

"*a group of devices or artificial objects or an organisation forming a network especially for distributing something or serving a common purpose*".

Indeed, the term 'system' is used in many different meanings in this thesis, probably mostly in the sense of the second, more technical definition. For example, animal identification systems, information systems, expert systems are all technical terms describing interconnected items that attempt to provide some kind of service.

However, there is also a different aspect of 'systems' used in this thesis according to the first definition given above. In CHAPTER 1.1, a network of risk factors (a system) was described. The complexity of the interactions of these risk factors was postulated to be the main problem in understanding and controlling respiratory diseases in pigs.

It thus appears that this thesis deals with two types of systems, biological systems and technical systems. Biological systems are inherently complex and technical systems can help us understand and manage them better.

2. Biological systems: investigating the web of causation

The concept of multiple causation has developed in epidemiology since the 1960s (Krieger, 1994). Schwabe (1982) describes the difficult situation of preventive veterinary medicine in the presence of multiple, possibly interacting disease determinants. He writes that the key to the problem is the identification of those factors that are susceptible to economical interventions by means of an epidemiological diagnosis which encompasses the clinical and the laboratory diagnosis.

Kovác (1978) estimated that 80-85 % of all losses in animal production are due to multifactorial diseases. Although the detailed causes of most disease complexes were not fully understood at that time, Kovács expected the control of such diseases to be difficult. The pathogenesis of multifactorial diseases in pigs was described as being related to immunosuppressive effects of inadequate climate, feeding, husbandry, management and veterinary treatment (Bollwahn, 1989). Although our knowledge of multifactorial diseases may be more complete today, the same problem areas are still being investigated. As the review of the literature on the epidemiology of respiratory diseases in pigs showed, the traditional tools used in observational research, even when applied appropriately, may not be sufficient to investigate multifactorial diseases. This was observed in the abattoir survey (CHAPTER 1.2) conducted in New Zealand. Although advanced analytical tools were applied, the results were

limited in terms of additional information provided. However, the effect of numerous environmental risk factors were quantified in a comprehensive multivariate model, and earlier results from more basic studies were confirmed. It was shown that the inclusion of a random effect in the model to account for the clustered structure of the data significantly improved the model. This indicates that despite the large number of risk factors considered some important variables such as the herd effect were still inadequately measured.

Technical difficulties appear to be common if working with complex data sets and numerous variables (Dohoo *et al.*, 1996; Martin, 1997) as is often the case when studying multifactorial diseases. New tools may therefore be needed to handle such problems. A selection of alternative analytical tools was evaluated in CHAPTER 1.5. Although the power of these tools in terms of correct classification of problem farms was not superior to traditional analysis, they provided additional insight into the structure of the data set and the relationships among the variables. This is important information for the development of causal webs.

An important component of the transmission of enzootic pneumonia, however, was substantiated. It was shown that the causal agent *Mycoplasma hyopneumoniae* can be isolated from the air in rooms housing clinically ill pigs. This finding is significant for within-farm transmission and probably for between-farm transmission as well. If airborne mycoplasma can survive while travelling over short distances, airborne transmission is likely to occur in regions with high farm densities. This hypothesis was developed in Switzerland many years ago and is now confirmed with hard data for the first time.

Airborne transmission appears to be an important way of transmission for a series of infectious diseases in pigs (CHAPTER 1.3). An experiment to isolate airborne classical swine fever (CSF) virus provided negative results. However, as this may be due to technical limitations, the occurrence of airborne transmission cannot be excluded. Given the patterns of the disease in recent outbreaks in Europe the hypothesis of airborne CSF appears still sustainable. Further research both using experimental and epidemiological studies is required to further improve the understanding of the dynamics of this disease.

Another possibly important element of the causal network of infectious diseases in pigs may be the human-pig interaction. As Seabrook (1984) pointed out, this aspect has been the subject of only limited research, although there seems to be evidence of correlation between human behaviour and production performance. Bigras-Poulin *et al.* (1984/85a,b) investigated the association between socio-psychological variables and performance and health of dairy cows. The authors measured significant associations and concluded that attitudes act as effect modifiers in the dairy farm system. Personality assessments conducted by Seabrook (1987) and Seabrook and Mount (1993) demonstrated psychological differences between stockpersons had higher and lower achievement levels. One possible contributor to this difference was quantified by Hemsworth and Barnett (1991) who measured a significant influence of unpleasant handling on the growth performance and on corticosteroid levels of young pigs. Similarly, Ravel *et al.* (1996) demonstrated an influence of the personality of the stockperson on preweaning performance on swine farms. Hemsworth *et al.* (1994) also demonstrated that it was possible to improve attitude and behaviour of stockpersons and thus to achieve a positive effect on the number of pigs born per sow per year. Although such associations may be technically difficult to measure, these variables help complete our knowledge of multifactorial diseases.

At this stage, however, strategies for the control of respiratory disease problems on pig farms have to be implemented despite our incomplete understanding of the factors that are involved. We may adopt the pragmatic approach suggested by MacMahon *et al.* (1960, cited by Krieger, 1994) that "*to effect preventive measures, it is not necessary to understand causal mechanisms in their entirety*". Control measures can and must be taken based on the information that is available (Robertson, 1998). Petersen (1984) suggests that the key to the epidemiological diagnosis of multifactorial diseases is to make use of all information available on a farm. She describes a computer-based information system supporting the early detection and prevention of multifactorial diseases. This idea, to use information and integrate it in computer-based systems to support decision making is one of the central concepts in this thesis. The next part of the discussion explains the different types of information systems and their potential in controlling infectious diseases in pigs.

3. Information systems

As mentioned in the introduction of this thesis, an information system "*is a collection of people, procedures and equipment designed, built, operated and maintained to collect, record, process, store, retrieve and display information*" (Teichroew, 1993). Health information systems specifically deal with information related to patients. In the field of veterinary medicine, health information is required as a basis for decision making by farmers, veterinarians and animal health policy makers alike. The justification for the development of information system is based on the potential benefit that will result for decision-makers (Lewis, 1994).

Computer-based systems are becoming more widely used as the amount of data to be processed increases. Such systems are typically organised around a central database which is accessed through a user interface using a query language (Teichroew, 1993). This is the case with both systems described in this thesis, RestiMATE and EpiMAN-SF. Both of them,, however, include more technically advanced elements than database management systems. EpiMAN-SF for example also contains simulation model and expert system components.

3.1 Decision-support systems

It can be argued that all information systems are decision-support systems (DSS) because they deal with information, and information represents data that has been processed for the purpose to aid some kind of decision (Curtis, 1989; Nunamsker *et al.*, 1992). In the context of the following discussion, decisions are defined as organisational responses to a problem (Ivancevitch and Matteson, 1996). Traditionally decision-making was associated with the work of 'managers'. Today, its importance importance at all levels of an organisation is recognised because it appears that top managers often ratify decisions suggested by their staff (Ginzberg and Stohr, 1982).

Decision-support systems (DSS) are a sub-field of information systems theory, the development of which began in the early 70s (Konsynski *et al.*, 1992). Such systems are designed to support users in decision tasks that require judgement and therefore cannot be made according to a strict routine. The emphasis of DSS is clearly on supporting decision-making and not on actually making the decision. From this follows directly that such systems do only intend to

extend the capabilities of the decision-maker and not to replace them. All processes remain user-initiated and user-controlled.

The decisions required to control an exotic disease are so-called unprogrammed decisions. Although there are some established control procedures, some aspects are handled on a case-by-case basis, because two outbreaks never occur in exactly the same way and because these decisions are extremely important (Ivancevitch and Matteson, 1996). Such decisions require judgement, intuition, and creativity. This type of decision is the main concern of DSS. They are also referred to as unstructured decisions, as they cannot be fully automated (Konsynski *et al.*, 1992).

Similarly, when assessing possible interventions against respiratory diseases, each farm has to be treated individually. An advice that may be apropriate for one farm may not work on another. This is therefore also described as an unstructured problem.

An issue that deserves particular attention is decision making under uncertainty. As explained in earlier paragraphs, our knowledge of diseases is incomplete and therefore all predictions of the effect of an intervention are subject to uncertainty. While theoretical models are available to structure the decision making process (e.g. Simon's model; Simon, 1960), these may be quite unrealistic (Bazerman, 1994), because in reality decisions are influenced by values, ethical decisions (what is morally acceptable to society) and the law. Human decision making is generally biased (Bazerman, 1994) as reactions towards uncertainty (risk-seeking, risk aversion) are not consistent within one individual let alone among different individuals or between cultures. This has also been observed when evaluating the expert system of EpiMAN-SF (CHAPTER 2.7). Technical solutions on how to deal with uncertainty in DSS are explained below.

All information systems require knowledge, but knowledge-based systems (KBS) need a special type namely facts, heuristics and rules. Similar to DSS, KBS are concerned with ill-structured problems. However, KBS are not only assisting but can be used to replace human decision making in a limited domain (Konsynski *et al.*, 1992). There is an overlap between DSS and KBS as they may contain concepts of each other. KBS also provide modelling tools to integrate with DSS. Many authors do not make a distinction between expert systems (ES) and KBS, but others use a more open definition incorporating all techniques (including fuzzy logic, learning techniques, machine learning) that can be used for problem solving in a KBS environment (Partridge and Hussain, 1995). ES are then considered a sub-field of KBS (FIGURE 56).

When designing an ES knowledge has to be collated and structured either using empirical techniques or using human experts. Both options have been considered in this thesis. RestiMATE uses rules based on the analysis of a field data set as well as rules elicited from a respiratory disease expert. How they compare in terms of practicality and accuracy cannot be answered at this stage due to incomplete field testing. When building the ES for EpiMAN-SF expert opinion was considered to rate CSF transmission between farms (CHAPTER 2.3). Although some people have reservations with respect to this (or any similar) technique because of the inherent subjectivity of human opinion, the results of this experiment were comparable with field data. Recent examples (Horst, 1997) have illustrated that techniques for eliciting expert knowledge are valuable tools, particularly when dealing with exotic diseases where no experimental data may be obtained.

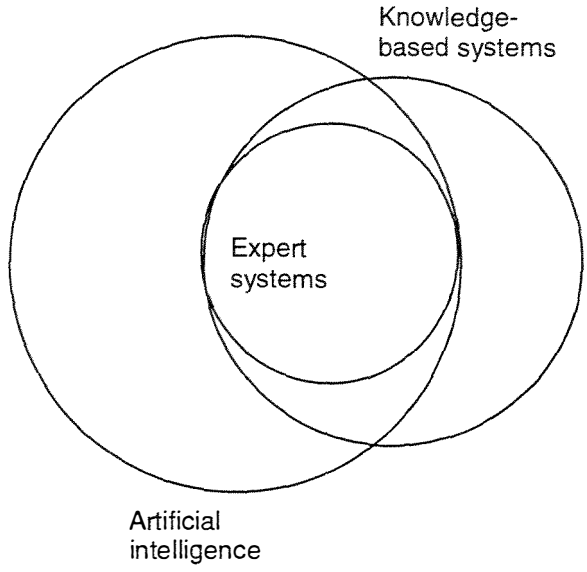

FIGURE 56. Relationship between artificial intelligence, expert systems and knowledge-based systems (modified after Partridge and Hussain, 1995).

3.2 Simulation models as elements of decision-support systems

Simulation models are tools of great potential in decision support because they allow to perform 'what-if' scenarios. Using this technique, a manager is capable of exploring the consequences of the decision options he/she is currently considering. The effects of the decisions can be quantified and compared. The stochastic simulation model INTERSPREAD-SF (CHAPTER 2.9) is designed to perform exactly this task during an outbreak of classical swine fever. The person responsible of making control decisions in the emergency headquarters can use INTERSPREAD-SF to assess the consequences of alternative control strategies including stamping-out or pre-emptive-slaughter policies, or possibly vaccination. Not only will the model allow the user to obtain quantitative information as a basis for making a decision, but it will also provide solid data for justifying and communicating the final choice.

3.3 How to deal with uncertainty

Simulation models can also be used to make predictions under uncertainty. For example, if a farm is infected with swine fever, the virus spreads through the herd in a fashion that is difficult to predict because of biological variability and lack of knowledge. A stochastic simulation model such as the one outlined in CHAPTER 2.8 can deal with this situation by including elements of chance. This is in contrast with deterministic models, where the output does not contain uncertainty. Stochastic simulation models are preferable to deterministic models because uncertainty is handled in a realistic fashion (Hurd and Kaneene, 1993). This approach is analogous to the concepts used in quantitative risk analysis (Vose, 1996).

Another technical solution to the problem of uncertainty in KBS is the use of fuzzy logic (Partridge and Hussain, 1995). Fuzzy logic is used in artificial intelligence, control engineering and 'smart' products. It is based on the concept of absence of sharply defined classification criteria rather than on the presence of random variables (Zadeh, 1965). If a decision rule can exactly classify all possible cases (no ambiguity about class membership), the result is called a *crisp* set. In contrast, the borders between fuzzy sets overlap. Consequently, a case can have partial membership in several classes. Fuzzy sets contain elements with varying degrees of membership using graded statements rather than ones that are strictly true or false (Zadeh, 1984, cited by Partridge and Hussain, 1995). However, the grade of membership is very subjective in nature. Fuzziness thus captures the vagueness about an event as opposed to probability that expresses the frequency of an event. In the case of an exotic disease outbreak, this vagueness would cause legal problems. For example, some farms with identical contact patterns with an infected farms would be treated as 'very-high-risk' and some as 'high-risk'. This situation is legally not acceptable. It was therefore decided that the use of fuzzy logic techniques was not an option for EpiMAN-SF.

Other methods of dealing with uncertainty in KBS are certainty and probability factors (Partridge and Hussain, 1995). The latter option was used in RestiMATE (CHAPTER 1.6). In RestiMATE, the classification of a farm in terms of respiratory disease problems is reported as "*Farm A is in the high-risk group with a probability of 0.9*". The advantage of this approach is that it is easy to implement. The disadvantage, however, is that data has to be available in order to compute the probabilities. Additionally, a good understanding of probabilities is expected from the user.

3.4 Validation of knowledge-based systems and simulation models

Verification and validation are part of the evaluation process of KBS and SM. While verifications attempts to answer the question: "*Did we build the system right?*", validation is concerned with the question: "*Did we build the right system?*" (Partridge and Hussain, 1995). The objective of verification and validation is also to enhance user acceptance and confidence (Green and Keyes, 1991) and it provides feedback for improving the system (Liebowitz, 1991).

The basis of KBS validation should be specifications defined at the beginning of the design process (Chandrasekaran, 1991; Green and Keyes, 1991). However, specifications are often non-existent or imprecise. For the ES modules of both RestiMATE and EpiMAN-SF no specifications are defined yet. If validation was to consist of a formal statistical acceptance test (quantitative validation, O'Leary, 1991) the specifications would have to be defined first. The problem with formal testing is that there may not be a single best but several acceptable answers, or there may be no agreement on what is an acceptable answer (Green and Keyes, 1991).

Another issue is what to validate against, test cases with confirmed result or human experts (Turing test). O'Leary (1991) suggests that if a system is designed to perform as an expert it should be tested against an expert. The selection of experts however needs to be carefully performed. In Turing tests, the performance of the ES and human experts are evaluated by someone not knowing the subject performer's identity (blind evaluation; O'Keefe *et al.*, 1991).

Blinding is necessary in order to prevent bias (Chandrasekaran, 1991). The Turing test is one potential technique for evaluating the ES component of EpiMAN-SF. Test cases have already been submitted to both the ES and human experts (CHAPTER 2.7). In a next step the results have to be evaluated by another human expert who was not involved in the first round. After each case has been classified in terms of how well it was handled, formal statistical tests can be applied to measure whether the performance of the ES was acceptable.

Another option to evaluate ES is field testing. Typically, field testing is used early in the development with ES prototypes. The ES is distributed to a group of selected users who apply the ES to perform the task it is designed for (O'Keefe et al, 1987). The users report back the performance of the system and any other suggestions they may have. Field testing allows not only to test the knowledge base but also to detect other weaknesses such as an inadequate user interface. However, field testing is only possible with non-critical applications, such as RestiMATE, where the users can assess whether the result delivered by the expert system is correct or not. For RestiMATE, field testing can start as soon as a reliable prototype is available. Staff at Massey University and selected users elsewhere will use RestiMATE during their routine clinical work. The users should be representative of real-world users and work under real-world conditions (Cochran and Hutchins 1991). RestiMATE needs to be confronted with cases that are representative of the range of problems that may be encountered by the system (Chandrasekaran, 1991; O'Leary, 1991). This coverage of the domain is more important than the number of cases. If statistical testing is used for the final evaluation, however, the number needs to be sufficiently high in order to be able to draw meaningful conclusions.

Sensitivity analysis is another powerful quantitative validation technique. Introducing slight changes in the knowledge base or the weights (O'Leary, 1991) can assess the stability of a KBS. However, sensitivity analysis is more frequently used for the validation of simulation models (Kleijnen, 1992; Ackerman, 1994). Sensitivity analysis allows identification of influential input parameters but it can also help reveal conceptual errors (Kleijnen *et al.*, 1992). Sensitivity analysis typically requires the use of experimental design principles (Kleijnen, 1992; APPENDIX H). Alternatively, latin hypercube sampling techniques could be used, which is considered more efficient (Iman and Helton, 1988).

Simulation models can also be evaluated through comparison with observational data (Ackerman, 1994). This method was applied when evaluating INTERSPREAD-SF based on data from a German CSF outbreak. The results showed that it was possible to replicate the outbreak in terms of the number of infected farms (CHAPTER 2.9). Some model parameters, however, need further evaluation with larger data sets. Results of the latter analyses will become available in the near future (Jalvingh *et al.*, in press).

3.5 Use and success of information systems

Lippeveld *et al.* (1997) acknowledged that information systems in the medical field still struggle to become fully accepted. This may be due to a lack of direct use of the data by the person who does the recording or because the information is not up-to date and therefore often useless. Managers also apparently prefer to rely on their intuition rather than on information.

However, in the case of an exotic disease outbreak the advantages of using an integrated decision support system such as EpiMAN-SF as compared with both traditional paper-based sys-

tems and with partially computer-based systems are numerous (Morris *et al.*, 1993). The most significant points are probably the permanent access to updated information on the epidemic and the ability to instantly analyse it. This allows users at all levels to make their decisions based on all available information. The use of a central database also ensures that everyone works with the same 'version' of the data, which avoids contradicting statements and subsequent confusion. The data can also be used to produce updated reports that are tailored to the demands of different parties. This facilitates communication during the epidemic. For example producer groups or the media can be provided with illustrated reports on the current situation and control measures. Good communication helps increase understanding and compliance of both farmers and the public, which will eventually allow the epidemic to be brought under control faster.

The expert system element helps set priorities consistently when the amount of data would otherwise be overwhelming. A structured presentation of information will provide the user with a global view of the epidemic, and permit the right choices to be made amongst competing tasks under time pressure and with resource restrictions. If inexperienced personnel need to be recruited (which is likely in a large outbreak), the expert system will guarantee uniform processing of the data.

In recent CSF outbreaks in Europe great care was taken to document the epidemic. For this reason detailed information in terms of epidemiological investigations and laboratory analyses was collected. However, as the analysis of these data has a comparatively low priority during the control phase of the epidemic, the data often are not consistently or not at all entered. Consequently, the data are of limited value for decision making and a lot of the data collection effort is in vain. EpiMAN-SF makes sure that data are entered systematically and promptly so that they can be used as quickly as possible. The fact that all episodes are recorded and not just the ones resulting in infection allows the calculation of true infection rates and probabilities.

The decision support provided by EpiMAN-SF includes various epidemiological tools for online analysis of the epidemic. INTERSPREAD-SF allows the comparison of control strategies and their economic effects. The economic module of INTERSPREAD is an urgently needed addition to the model, because the direct and indirect costs of disease control in emergency situations are high and disease control programmes cannot be evaluated without economic assessments (Buijtels *et al.*, 1996).

The success of an information system does not only depend on methodological factors but the political, socio-cultural and administrative context have also to be taken into account (Lippeveld *et al.*, 1997). The developments in the European Union over the last decade have resulted in an open market and new trade principles (Thierman, 1997). In 1988, Bendixen predicted an increased health risk as a consequence of these changes. The frequent outbreaks of exotic diseases in the recent past seem to confirm this prognosis. The then described needed structural changes in terms of maximal size of holdings, maximum density, separation of holdings and transport relationships now seem to be imminent. Under these new conditions, member countries are under great pressure to rapidly control diseases and to provide accurate and up-to-date data to prevent trade restrictions. The use of information systems could provide veterinary cervices with the necessary tools (Donaldson and Kihm, 1996). It is therefore not surprising that a number of countries are currently working to adapt EpiMAN to their specific needs. EpiMAN-SF is likely to be a welcome addition to their disease control armoury.

4. Systems thinking

Considering the discussion of multifactorial diseases and the use of information for decision support above it appears that we do need methods to study complex things as a whole rather than in isolation. This concept has been adopted by scientists and philosophers as well as business professionals. The concept of 'systems thinking' was developed. This idea is based on the notion that a system is a set of inter-related components organised together to form an entity that as a whole has emerging properties that belong to no single component or subset of the components of which it is formed (Lewis, 1994). Aristotle founded this concept by saying: "*The whole is more than the sum of its parts*". This is in contrast to the reductionist approach of Descartes who assumed that each problem could be divided into small problems and that by solving the small problems, the primary problem would be solved as well. However, this method neglects interactions, and the increasingly complex problems of the modern world are difficult to tackle with this mindset (Ballé, 1994). Systems thinking is a pragmatic alternative that focuses on relationships rather than on parts, and on patterns rather than on events. It also introduces circular causality. Through feedback links the system can cause its own dynamic behaviour. The systems approach provides a thinking framework that allows to remain analytical but also to become synthetic, dynamic and holistic.

Systems thinking has been widely applied in the fields of cybernetics, biology (Sattler, 1986), computer science (Lewis, 1994) and business science (Ballé, 1994). Demands for more integrated approaches have also been made for the fields of medicine (including veterinary medicine) and particularly epidemiology. The recent discussion in the field of epidemiology was ignited through an article by Taubes (1995) in which the author criticised the methods used by epidemiology and the lack of benefit to public health. Subsequently, a need for a paradigm shift was postulated (Koopman ,1996; Pearce, 1996) to move away from risk factor epidemiology to a systems approach, where epidemiology would analyse natural systems that generate patterns of disease in populations to produce results supporting decision making in public health policy. Currently a change similar to the transition from biology to ecology was observed in epidemiology by Susser and Susser (1996a,b). Such trends can also be observed in the veterinary field with the introduction of the concept of agroecosystem health (Conway, 1987; Nielsen, 1992; Faye *et al.*, 1997)

The systems discussed in this thesis apply the concepts of systems thinking by pursuing structural and functional integration of elements to solve animal health problems. New methodologies, particularly additional tools for computer modelling, the development of probabilistic systems and to study disease patterns will be needed. The successful use of these methods will require interdisciplinary collaboration between veterinary epidemiologists, computer scientists, economists and agronomists.

References

Ackerman, E. (1994). Simulation of micropopulations in epidemiology: Tutorial 3.Simulation model evaluation methods. International Journal of Bio-Medical Computing 37:195-204.

Ballé, M. (1994). Managing with systems thinking. McGraw-Hill Book Company, London.

Bazerman, M.H. (1994). Judgement in managerial decision making. 3rd Edition. John Wiley and Sons, New York.

Bendixen, H.J. (1988). Health hazards and intensified animal productions. Acta Veterinaria Scandinavica Suppl. 84:29-41.

Bigras-Poulin, M., Meek, A.H., and Martin, S.W. (1984/85a). Attitudes, management practices and herd performance - A study of Ontario dairy farm managers. I. Descriptive aspects. Preventive Veterinary Medicine 3:227-240.

Bigras-Poulin, M., Meek, A.H., Blackburn, D.J., and Martin, S.W. (1984/85b). Attitudes, management practices and herd performance - A study of Ontario dairy farm managers. II. Associations. Preventive Veterinary Medicine 3:241-250.

Bollwahn, W. (1989). Infektiöse Faktorenkrankheiten beim Schwein - Pathogenese und Bekämpfung. Berliner und Münchener Tierärztliche Wochenschrift 102:410-412.

Buijtels, J.A.A.M., Huirne, R.B.M., Dijkhuizen, A.A., Renkema, J.A., and Noordhuizen, J.P.T.M. (1996). Basic framework for the economic evaluation of animal health control programmes. Revue scientifique et technique de l'Office International des Epizooties 15:775-795.

Chandrasekaran, B. (1991). On evaluating artificial intelligence systems for medical diagnosis. In: Gupta, U.G. (ed.) Validating and verifying knowledge-based systems IEEE Computer Society Press, Los Alamitos, 260-265.

Cochran, E.L. and Hutchins, B.L. (1991). Testing, verifying and releasing an expert system: the case history of Mentor. In: Gupta, U.G. (ed.) Validating and verifying knowledge-based systems IEEE Computer Society Press, Los Alamitos, 304-308.

Conway, G.R. (1987). The properties of agroecosystems. Agricultural Systems 24:95-117.

Curtis, G. (1989). Business information systems: analysis, design and practice. Addison-Wesley, Wokingham.

Dohoo, I.R., Ducrot, C., Fourichon, C., Donald, A., and Hurnik, D. (1996). An overview of techniques for dealing with large numbers of independent variables in epidemiologic studies. Preventive Veterinary Medicine 29:221-239.

Donaldson, A.I. and Kihm, U. (1996). Research and technological developments required for more rapid control and eradication of foot and mouth disease. Revue scientifique et technique de l'Office International des Epizooties 15:863-873.

Faye, B., Waltner-Toews, D., and McDermott, J. (1997). From "ecopatholgy" to "agrosystem health". Epidémiologie et Santé Animale 31-32:XVII-XXI.

Ginzberg, M.J., and Stohr, E.A. (1982). Decision support systems: issues and perspectives. In: Ginzberg, M.J., Reitman, W., and Stohr, E.A. (eds.) Decision support systems, North-Holland Publishers, Amsterdam, 9-31.

Green, C.J.R. and Keyes, M.M. (1991). Verification and validation of expert systems. In: Gupta, U.G. (ed.) Validating and verifying knowledge-based systems IEEE Computer Society Press, Los Alamitos, 20-25.

Hemsworth, P.H. and Barnett, J.L. (1991). The effects of aversively handling pigs, either individually or in groups, on their behaviour, growth and corticosteroids. Applied Animal Behaviour Science 30:61-72.

Hemsworth, P.H., Coleman, G.J., and Barnett, J.L. (1994). Improving the attitude and behaviour of stockpersons towards pigs and the consequences on the behaviour and reproductive performance of commercial pigs. Applied Animal Behaviour Science 39:349-362.

Horst, H.S. (1997). Risk and economic consequences of contagious animal disease introduction. PhD thesis, Wageningen Agricultural University, Wageningen, The Netherlands.

Hurd, H.S. and Kaneene, J.B. (1993). The application of simulation models and systems analysis in epidemiology: a review. Preventive Veterinary Medicine 15:81-99.

Iman, R.L. and Helton, J.C. (1988). An investigation of uncertainty and sensitivity analysis techniques for computer models. Risk Analysis 8:71-90.

Ivancevich, J.M., and Matteson, M.T. (1996). Organisational behaviour and management. 4th Edition. Irwin, Chicago.

Jalvingh, A. Epidemiological and economic evaluation of disease control strategies using stochastic and spatial

simulation: general framework and two applications. Proceedings of the Annual Conference of the Society for Veterinary Epidemiology and Preventive Medicine 1998, Ennis, County Clare, Ireland, in press.

Kleijnen, J.P.C. (1992) Verification and validation of models Department of Information Systems and Auditing, Tilburg University, Tilburg, The Netherlands.

Kleijnen, J.P.C., van Ham, G., and Rotmans, J. (1992). Techniques for Sensitivity Analysis of Simulation Models: A Case Study of the CO_2 Greenhouse Effect. Simulation 58:410-417.

Koopman, J.S. (1996). Comment - emerging objectives and methods in epidemiology. American Journal of Public Health 86:630-632.

Konsynski, B.R., Stohr, E.A., and McGee, J.V. (1992). Review and critique of decision-support systems. In: Stohr, E.A., and Konsynski, B.R. (eds.) Information systems and decision processes. IEEE Computer Society Press, Los Alamitos, 7-26.

Kovács, F. (1978). Role of animal hygiene in the prevention of multifactorial diseases. Berliner und Münchner Tierärztliche Wochenschrift 91:64-68.

Krieger, N. (1994). Epidemiology and the web of causation: has anyone seen the spider? Social Science and Medicine 39:887-903.

Liebowitz, J. (1991). Useful approach for evaluating expert systems. In: Gupta, U.G. (ed.) Validating and verifying knowledge-based systems IEEE Computer Society Press, Los Alamitos, 249-259.

Lippeveld, T., Sauerborn, R., and Sapirie, S. (1997). Health information systems - making them work. World Health Forum 18:176-184.

Lewis, P. (1994). Information-systems development. Pitman Publishing, London.

Martin, W. (1997). A structured approach for analysing survey data and making useful causal inferences. Epidémiologie et Santé Animale 31-32:13.01.1-13.01.3

Morris, R.S., Sanson, R.L., McKenzie, J.S., and Marsh, W.E. (1993). Decision support systems in animal health programmes. Proceedings of the Annual Meeting of the Society of Veterinary Epidemiology and Preventive Medicine, Exeter, 188-199.

Nielsen, N.O. (1992). Ecosystem health and veterinary medicine. Canadian Veterinary Journal 33:23-26.

Nunamaker, J.F., Applegate, L.M., Culnan, M.J., Fedorowicz, J., George, J.F., Gutek, B., Henderson, J.C., King, J.L., Konsynski, B.R., Star, S.L., Swanson, E.B., Valacich, J.S., and Zmud, R.W. (1992). Organisational decision support systems. In: Stohr, E.A., and Konsynski, B.R. (eds.) Information systems and decision processes. IEEE Computer Society Press, Los Alamitos, 137-166.

O'Leary, D.E. (1991). Validation of expert systems-with applications to auditing and accounting expert systems. In: Gupta, U.G. (ed.) Validating and verifying knowledge-based systems IEEE Computer Society Press, Los Alamitos, 57-75.

Partridge, D., and Hussain, K.M. (1995). Knowledge-based information systems. McGraw-Hill Book Company, London.O'Keefe, R., Balci, O., and Smith, E. (1987). Validating expert system performance. IEEE Expert 2:81-89.

Pearce, N. (1996). Traditional epidemiology, modern epidemiology and public health. American Journal of Public Health 86:678-683.

Petersen, B. (1984). Prerequisites for introducing an integrated, production-related performance and health monitoring system for piglet- producing units. Der Praktische Tierarzt 65:1003-1006, 1009-1012.

Ravel, A., D'Allaire, S., and Bigras-Poulin, M. (1996). Influence of management, housing and personality of the stockperson on preweaning performances on independent and integrated swine farms in Québec. Preventive Veterinary Medicine 29:37-57.

Robertson, L.S. (1998). Causal webs, preventive brooms, and housekeepers. Social Science & Medicine 46:53-58.

Sattler, R. (1986). Biophilosophy – Analytic and holistic perspectives. Springer-Verlag, Berlin.

Schwabe, C. (1982). The current epidemiological revolution in veterinary medicine. Part I. Preventive Veterinary Medicine 1:5-15.

Seabrook, M.F. (1984). The psychological interaction between the stockman and his animals and its influence on performance of pigs and dairy cows. The Veterinary Record 115:84-87.

Seabrook, M.F. (1987). The role of the stockman in livestock productivity and management. Research epistemology : the holistic approach. The role of the stockman in livestock productivity and management EUR 10982 EN Commission of the European Communities, Luxembourg, 39-50.

Seabrook, M.F. and Mount, N.C. (1993). Good stockmanship - good for animals, good for profit. Journal of the Royal Agricultural Society of England 154:104-115.

Simon, H.A. (1960). The new science of management decisions. Prentice Hall, Englewood Cliffs.

Susser, M. and Susser, E. (1996a). Choosing a future for epidemiology. 1. Eras and paradigms. American Journal of Public Health 86:668-673.

Susser, M. and Susser, E. (1996b). Choosing a future for epidemiology. 2. From black box to Chinese boxes and eco-epidemiology. American Journal of Public Health 86:674-677.

Taubes, G. (1995). Epidemiology faces its limits. Science 269:164.

Thierman, A.B. (1997). The relationship between the World Trade Organisation and the Office International des Epizooties. Revue scientifique et technique. Office internationale des Epizooties 16:13-16.

Vose, D. (1996). Quantitative risk analysis - A guide to Monte Carlo simulation modelling. John Wiley & Sons, Chichester.

Zadeh, L.A. (1965). Fuzzy sets. Information and Control 8:338-353.

List of related publications

Stärk K.D.C., and Morris R.S. (1996): Risk factors for respiratory diseases in New Zealand pig farms. *2nd Pan-pacific Veterinary Conference, Christchurch, New Zealand,* 37-40.

Stärk K.D.C., Morris R.S., and Sanson R.L. (1996): EpiMAN-SF - development of a decision support system for managing a swine fever epidemic. *Proceedings of the 14th IPVS Congress, Bologna, Italy,* 534.

Stärk K.D.C., Pfeiffer D.U., and Morris R.S. (1996): Predicting respiratory disease levels from farm risk factors by computer program. *Proceedings of the 14th IPVS Congress, Bologna, Italy,* 535.

Stärk K.D.C., Horst H.S., Morris, R.S., and Teuffert, J. (1997). Elicitation of expert knowledge on risk factors for classical swine fever transmission in Switzerland. *Proceedings of the 8th International Symposium on Veterinary Epidemiology and Economics, Paris. Epidémiologie et Santé Animale* 31-32, 12.24.1-12.24.3.

Stärk K.D.C., Pfeiffer D.U., and Morris R.S. (1997): The use of field data for building an expert system for respiratory diseases in pigs . *Proceedings of the 8th International Symposium on Veterinary Epidemiology and Economics, Paris. Epidémiologie et Santé Animale* 31-32, 12.C.28.

Horst, H.S., Stärk, K.D.C., Huirne, R.B.M., and Dijkhuizen, A.A. (1997). Use of expert knowledge in risk analysis. *Proceedings of the 10th Congress of the Society for Risk Analysis in Europe, Stockholm,* 44-48.

Stärk, K.D.C., Nicolet, J., and Frey, J. (1998). Detecetion of *Mycoplasma hyopneumoniae* by air sampling with a nested PCR assay. *Applied and Environmental Microbiology* 64, 543-548

Stärk K.D.C., Pfeiffer D.U., and Morris R.S. Risk factors for respiratory diseases in New Zealand pig herds. *New Zealand Veterinary Journal* (in press).

Stärk K.D.C., Morris R.S., and Pfeiffer, D.U. Comparison of electronic and visual identification systems in pigs. *Livestock Production Science* (in press).

Stärk, K.D.C., Frey, J., Nicolet, J., Thür, B., and Morris, R.S. Assessment of aerosol transmission in the epidemiology of infectious diseases in swine using air sampling and polymerase chain reaction assays. *Proceedings of the 15th IPVS Congress, Birmingham* (in press).

Stärk, K.D.C., Nielen, M., and Morris, R. Setting priorities for investigating movement traces during a classical swine fever epidemic. *Proceedings of the 15th IPVS Congress, Birmingham* (in press).

Stärk, K.D.C., and Pfeiffer D.U. Comparison of alternative classification methods in animal health data. *Preventive Veterinary Medicine* (submitted).

Stärk, K.D.C., Pfeiffer, D.U., and Morris, R.S. Within-farm spread of classical swine fever virus – a blueprint for a stochastic simulation model. *Veterinary Quarterly* (submitted).

Stärk, K.D.C., Morris, R.S., Stern, M.W., and Benard, H.J. EpiMAN-SF – A decision-support system for managing swine fever epidemics. *Revue scientifique et technique Office International des Epizooties* (submitted).

APPENDIX

APPENDIX A. Questionnaire used for data collection on environmental risk factors for respiratory diseases on New Zealand pig farms

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Palmerston North

Ph 06 350 50 20
Fax 06 350 56 16

ENVIRONMENTAL RISK FACTORS AND PNEUMONIA IN PIGS

QUESTIONNAIRE

1. Identification

Name: _____

Farm name: _____

Address: _____

Ph. / Fax: _____

Best time to ring: _____

Abattoir name, place _____

How often are pigs transported to the abattoir? *(please tick):* weekly ☐ ;fortnightly ☐

Average number of pigs shipped _____. Usual day of slaughter *(please tick)* :

Monday ☐ ; Tuesday ☐ ; Wednesday ☐ ; Thursday ☐ ; Friday ☐ ; variable. ☐

1.1. Farm type: *(please tick one)*

Breeding and fattening ☐

Breeding only (sell weaners ☐

Fattening only (buy weaners) ☐

1.2. Herd size: *(today's inventory)*

Number of sows:

Number of growers (4 weeks and older)

1.3. Neighbourhood

Distance to next pig farm from my pig facilities (km) _____

2. Current health status of the herd

2.1. Are you using any of the following preventive measures? *(please tick)*

Vaccination against *Mycoplasma hyopneumoniae* (enzootic pneumonia)	☐yes ☐no	Vaccination against *Actinobacillus pleuropneumoniae* (pleuropneumonia)	☐yes ☐no

2.2. Have you observed any of the following conditions during the last two weeks? *(please tick)*

Coughing in piglets 4-11 weeks old (weaners)	☐ yes ☐ no	Diarrhoea in any age group	☐ yes ☐ no
Coughing in pigs 12 or more weeks old (growers)	☐yes ☐no		

2.3. Have you been using antibiotic medicated feed or other antibiotic treatments during the last two weeks for any health disorder or as a prevention? *(please tick)*

in-feed medication of pigs on the weaner decks	☐yes ☐no	individual treatments of weaner pigs by injection	☐yes ☐no
in-feed medication of pigs in the grower/finisher stage	☐yes ☐no	individual treatments of growers/finishers by injection	☐yes ☐no

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3. Management and Hygiene

3.1. Purchase policy *(tick one)*

I don't buy any stock	☐
I currently buy all stock from one supplier only	☐
I buy breeding stock from multiple suppliers, but not weaners	☐
I sometimes buy weaners from a market	☐

3.2. I consider the hygiene status of my farm to be *(please tick one)*

Good	☐
Adequate	☐
Could be better	☐

3.3. I consider environmental factors (temperature, humidity, air quality etc.) to be important for swine health *(please tick one)*

Yes, very important	☐	No, not very important	☐
Yes, some importance	☐	No, doesn't make any difference	☐

3.4. I consider the current environment for the pigs on my farm to be: *(please tick one)*

Good	☐	Could be better	☐
Ädequate	☐		

4. Housing

If your management system does not fit these descriptions in any point please tick this box ☐ and explain on back of sheet.

4.1. Weaner deck *(please tick)*

Ventilation is supported by fans	☐yes ☐no
The ventilation is automatically controlled (thermostat)	☐yes ☐no
The threshold temperature in the nursery is set to	°C
Pigs are mostly fed wet feed, e.g. wet-dry feeding	☐yes ☐no
Pigs have unlimited access to drinking water (ad lib)	☐yes ☐no
There is a liquid manure system and slatted floors	☐yes ☐no
Effluent is removed	☐daily ☐less ☐more
Bedding (such as straw, wood shavings) is being used	☐yes ☐no
All pen separations are solid (no possibility for nose to nose contact)	☐yes ☐no
Pigs less than 12 weeks old are sharing room with pigs 5 or more weeks older than them	☐yes ☐no
Average size of pen (m^2)	_____
Average number of pigs per pen	_____
Maximal number of pigs per pen	_____
Average number of pigs per closed weaner deck room	_____
Maximal number of pigs per closed weaner deck room	_____
Pigs are moved through the weaner decks following an all-in/all-out system, i.e. sections will be completely emptied, cleaned and disinfected between batches	☐yes ☐no

4.2. Grower/Finisher area

Ventilation is supported by fans	☐yes ☐no
The ventilation is automatically controlled (thermostat)	☐yes ☐no
The threshold temperature in the grower area is set to	°C
Pigs are mostly fed wet feed, e.g. wet-dry feeding	☐yes ☐no
Pigs have unlimited access to drinking water (ad lib)	☐yes ☐no
There is a liquid manure system and slatted floors	☐yes ☐no
Effluent is removed	☐daily ☐less ☐more
Bedding (straw, wood shavings) is being used	☐yes ☐no
All pen separations are solid (no possibility for nose to nose contact)	☐yes ☐no
Pigs more than 12 weeks are sharing room with pigs 5 or more weeks younger than them	☐yes ☐no
Average size of pen (m^2)	_____
Average number of pigs per pen	_____
Maximal number of pigs per pen	_____
Average number of pigs per closed grower/ finisher room	_____
Maximal number of pigs per closed grower/ finisher room	_____
Pigs are moved through the grower/finisher sections following an all-in/all-out system, i.e. sections will be completely emptied, cleaned and disinfected between batches	☐yes ☐no

5. Accomodation type and number of movements/stages of pigs during a production cycle on your farm.

During a production cycle different stages (e.g. mating, dry period, farrowing) can be distinguished. When a pig goes from one stage to the next, it is often shifted to a different room.

A room is a single air space separated from other rooms so that air does not circulate between them. Therefore, a movement from one pen to another within the same room does not fit our definition. A shift is a room-to-room movement.

We are interested to find out how many of such shifts are normally occurring on your farm during a production cycle.

5.1. Please fill in the following table, giving details on type of accomodation, number of rooms available and number of shifts per production stage.

If your management system does not fit these descriptions in any point please tick this box ☐ and explain on back of sheet.

	Accomodation type(s)*	**Total no. of such rooms available**	**no. of shifts for an individual animal during one production cycle**
Sows:			
- Farrowing	_____	_____	sow in lactation: _____
- Mating/dry sow	_____	_____	dry sow _____
Growers:			
- Weaners	_____	_____	weaning until 10 weeks of age _____
- Growers	_____	_____	from 10 weeks to slaughter: _____

* for example: crate, tethered, group housing, decks, fully slatted, sawdust, indoors, outdoors...

Written Consent

I herby give my formal consent that slaughterhouse check data for pneumonia from pigs coming from my farm may be collected twice by investigators affiliated with this project.

Date: Signature:

This is a mutual agreement that data will be treated with confidentiality and that it will exclusively be used for this project. Data will not be forwarded to third parties except in summary tables not allowing for tracing back to individual farms.

June, 1995 K. Stark, Massey University

APPENDIX B. Table of indicator variables used by RestiMATE

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
ABDOSE	AB currently used at therapeutic level	EXP	-	Step 4	=	0.0	=	1.0			2.00		
ABUSE	AB are currently used	EXP, LIT	General	Step 4	=	0.0	=	1.0	1.3	0.6	3.00		The use of antibiotic treatment is an indicator of a respiratory diseases problem. Investigate other risk factors and review medication!
CC1	Cough count in 4-7 week old pigs (couhgs/100 pigs/1minute)	EXP	-	Step 2, Step 3	<=	2.0	>	2.0			2.00		
CC2	Cough count in 8-14 week old pigs (cougs/100 pigs/1minute)	EXP	-	Step 2, Step 3	<=	6.0	>	6.0			2.00		
CC3	Cough count in pigs > 14 weeks old (cougs/100 pigs/1minute)	EXP	-	Step 2, Step 3	<=	10.0	>	10.0			2.00		
CPROP1	Proportion of coughing pigs in 4-7 week old pigs	EXP, LIT	Weaner	step 2, step 3	<=	2.0	>	2.0	1.0	1.7	3.00		Clinical signs of respiratory diseases in young age group. This is probably not EP. Discuss the problem with your vet.
CPROP2	Proportion of coughing pigs in 8-14 week old pigs	EXP	-	Step 2, Step 3	<=	5.0	>	5.0			3.00		
CPROP3	Proportion of coughing pigs >14 weeks old	EXP	-	Step 2, Step 3	<=	10.0	>	10.0			3.00		

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
GAB	In-feed AB in growers	EXP, LIT	Grower	-	=	0.0	=	1.0	1.3	0.6			The use of antibiotic treatment is an indicator of a respiratory disease problem. Investigate other risk factors and review medication!
GAIR	Air quality	EXP	-	Air flow	<=	2.0	>	2.0			3.24		
GBED	Bedding used in grower rooms	LIT	Grower	-	=	0.0	=	1.0	1.0	1.0			The use of bedding can cause hygiene problems. Check the quality and management of bedding material!
GCD	Cleaning and disinfecting between batches	EXP	-	Environment	=	1.0	=	0.0			3.00		
GCOND	Evidence of condensation	EXP	-	Air flow	=	0.0	=	1.0			3.24		
GCOUGH	Coughing in grower pigs	LIT	Grower	-	=	0.0	=	1.0	1.8	1.0			Clinical signs of respiratory diseases on your farm. Investigate management risk factors! Discuss short-term medication with your vet!
GDIA	Signs of diarrhoea in growers	EXP, LIT	General	Step 1	=	0.0	=	1.0	1.0	1.3	2.00		Pigs that suffer from other diseases such as diarrhoea are more susceptible to respiratory diseases. Treat the problem!
GDUST	Amount of dust	EXP	-	Environment	<	1.0	>	1.0			2.00		
GEMPTY	Rooms completely empty between batches	EXP	-	Environment	=	1.0	=	0.0			3.00		
GFED	Wet or wet/dry feeding system	EXP, LIT	Grower	Environment	=	1.0	=	0.0	1.2	1.0	2.00		
GFLOOR	Slatted floor	EXP	-	Environment	=	0.0	=	1.0			1.00		
GFLOW	Continuous production flow	EXP, LIT	Grower	Environment	=	0.0	=	1.0	1.3	1.0	3.00		Running an all-in/all-out system is a very effective means of interrupting the infection cycle. Consider introducing this practice!

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
GMAN	Liquid manure system in grower rooms	EXP, LIT	Grower	Environment	=	0.0	=	1.0	1.0	2.0	1.00		
GOUT	Type of air outlet	EXP	-	Air flow	<	3.0	>	3.0			3.24		
GOUT-COV	Air outlet is covered	EXP	-	Air flow	=	1.0	=	0.0			3.24		
GOUTLOC	Location of outlet in ceiling	EXP	-	Air flow	=	1.0	=	0.0			3.24		
GOUT-SIZE	Proportion air outlet capacity by inlet capacity	EXP	-	Air flow	>=	1.0	<	1.0			3.24		
GPPP	Number of pigs per pen in grower rooms	LIT	Grower	-	<	12.0	>	15.0	1.0	1.3		More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. You are getting close to this level. Try reducing the numbers.	More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. Try reducing the numbers.
GPPR	Number of growing pigs per airspace	EXP, LIT	Grower	Environment	<=	200.0	>	300.0	1.0	1.4	3.00	More than 300-500 pigs per room are increasing the risk of maintaining infectious diseases. You are getting close to this level. Try reducing the numbers.	More than 300-500 pigs per room are increasing the risk of maintaining infectious diseases. You are getting close to this level. Try reducing the numbers.
GREM	Frequency of manure removal	LIT	Grower	-	=	2.0	<	2.0	1.4	3.2		Remember that manure needs to be removed from under the pens regularly!	You need to remove manure from under the pens more regularly!
GROW	Number of growing pigs	LIT	General	-	<	500.0	>	1000.0	1.0	1.7		Larger farms are at higher risk of suffering from airborne infectious diseases. No recommendation.	Large farms are at higher risk of suffering from airborne infectious diseases. No recommendation.
GROWTH1	Growth rate in 4-7 week old pigs	EXP	-	Step 1	>=	350.0	<	350.0			3.00		
GROWTH2	Growth rate in 8-14 week old pigs	EXP	-	Step 1	>=	500.0	<	500.0			3.00		

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
GROWTH 3	Growth rate in pigs >14 weeks old	EXP	-	Step 1	>=	650.0	<	650.0			3.00		
GSEP	Solid pen separations	EXP, LIT	Grower	Environment	=	1.0	=	0.0	1.3	0.8	3.00		Solid pen separations help prevent disease transmission. Check whether they could be introduced on your farm.
GSHA	Different age groups in one airspace	EXP	-	Environment	=	0.0	=	1.0			3.00		
GSPACE	Proportion of space occupied by pigs (%)	EXP	-	Environment	<=	60.0	>	60.0	1.0	1.0	2.00		Over-crowding can have an adverse effect on pig health in general. You do not have a problem now, yet you should plan to reduce the numbers in the future.
GVENT	Type of ventilation	EXP, LIT	Grower	Air flow	>	3.0	<	3.0	1.0	1.2	3.24		A good ventilation system is crucial for the wellbeing of the pigs. Check your system for appropriate design!
GWIDTH	Width of building (m)	EXP	-	Air flow	<=	12.0	>	12.0			3.24		
HYG	Hygiene status	LIT	General	-	>	1.0	<	2.0	1.0	1.7		High hygiene levels are essential for the prevention of respiratory diseases. Check your procedures!	High hygiene levels are essential for the prevention of respiratory diseases. You need to improve your procedures!
ISLAND	Island	LIT	General	-	=	1.0	=	0.0	1.0	2.8			Pleuropneumonia is more prevalent on the South Island due to the widespread occurrence of Actinobacillus. No recommendation.
MOR-RESP1	Mortality due to respiratory diseases in 4-7 week old pigs	EXP	-	Step 3	<=	1.0	>	1.0			3.00		
MOR-RESP2	Mortality due to respiratory diseases in 8-14 week old pigs	EXP	-	Step 3	<=	2.0	>	2.0			3.00		

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
MOR-RESP3	Mortality due to respiratory diseases in pigs >14 weeks old	EXP	-	Step 3	<=	0.5	>	0.5			3.00		
MORT1	Mortality in 4-7 week old pigs	EXP	-	Step 1, Step 2	<=	5.0	>	5.0			3.00		
MORT2	Mortality in 8-14 week old pigs	EXP	-	Step 1, Step 2	<=	3.0	>	3.0			3.00		
MORT3	Mortality in pigs >14 weeks old	EXP	-	Step 1, Step 2	<=	1.0	>	1.0			3.00		
NEIB	Distance to nearest piggery	LIT	General	-	>	2.0	<	1.0	1.2	1.2		The risk of airborne infection with respiratory diseases is increased if you have a neighbour who lives closer than 2 km. No recommendations.	The risk of airborne infection with respiratory diseases is increased if there are pig farms closer than 2 km. No recommendations.
PURCH	Purchase practice	EXP, LIT	General	Notes	<	3.0	>	3.0	1.3	4.8	3.00	Purchasing pigs from several farms is a risky procedure. Try reducing the number of sources!	Purchasing pigs from several farms and markets are risky procedures. Try reducing the number of sources and avoid buying from markets!
SEASON	Current season	EXP	-	Step 4	=	0.0	=	1.0			2.00		
SIGN1	Signs of severe pneumonia	EXP	-	Step 2	=	0.0	=	1.0			3.00		
SIGN2	Signs of APP	EXP	-	Step 2	=	0.0	=	1.0			3.00		
SIZE	Number of pigs sold per year	EXP	-	Notes	<=	4000.0	>	4000.0			2.00		
SOWS	Number of sows	LIT	General	-	<	500.0	>	750.0	1.0	1.8		Larger farms are at higher risk of suffering from airborne infectious diseases. No recommendation.	Large farms are at higher risk of suffering from airborne infectious diseases. No recommendation.
SQUO	Environment status	LIT	General	-	=	1.0	=	3.0	1.4	1.5		The current environment on your farm is generally not ideal for the respiratory health of your pigs. Explore other management factors and consider interventions.	The current environment on your farm is currently hampering the respiratory health of your pigs. Explore other management factors and plan interventions.

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
STATUS	Free of EP or APP	EXP	-	Notes	=	0.0	=	1.0			3.00		
TREAT	Proportion of pigs that are indvidually treated for disease (not injury)	EXP	-	Step 1	<=	2.0	>	2.0			2.00		
TYPE	Mixed farm or only fattening farm	LIT	General	-	=	0.0	=	1.0	1.0	1.0			Growier-only farms are at higher risk of having respiratory disease problems because they need to purchase animals from outside. Check the health status of your suppliers.
VACC	Currently vaccintaing against EP and/or APP	EXP, LIT	General	Step 4	=	0.0	=	1.0	1.3	0.3	3.00		Farms which vaccinate against enzootic pneumonia are mostly those which have respiratory disease problems. Investigate other factors and plan interventions!
WAIAO	All-in/all-out system in weaner rooms	LIT	Weaner	-	=	1.0	=	0.0	1.6	1.2			Running an all-in/all-out system is a very effective means of interrupting the infection circle. Consider introducing this practice!
WBED	Bedding used in weaner rooms	LIT	Weaner	-	=	0.0	=	1.0	1.0	1.6			
WFED	Wet-dry feeding in weaners	LIT	Weaner	-	=	1.0	=	0.0	1.5	1.3			Wet/dry feeding helps reduce dust levels in pig rooms and improve air quality. Consider alternative feeding practices!
WFLUC	Temperature fluctuation in weaning rooms	EXP	-	Weaner	<=	0.5	>	0.5			2.00		
WMAN	Liquid manure system in weaner rooms	LIT	Weaner	-	=	0.0	=	1.0	2.4	1.0			Liquid manure systems may have an adverse effect on air quality if not properly operated. Check your operations!
WPPP	Number of pigs per pen in weaner rooms	EXP, LIT	Weaner	-	<	12.0	>	15.0	1.2	1.6		More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. You are getting close to this level. Try reducing the numbers.	More than 15 pigs per pen are increasing the risk of transmitting infectious diseases. Try reducing the numbers.

ID	FullName	Origin	Level Tree	Level Expert	Operator 1	Target	Operator 2	Action	Weight EP	Weight PLPN	Weight EXP	Advice Warning	Advice Action
WPPR	Number of pigs in weaner room	LIT	Weaner	-	<	100.0	>	200.0	1.0	1.0		More than 200 pigs per room are increasing the risk of maintaining infectious diseases. You are getting close to this level. Try reducing the numbers.	More than 200 pigs per room are increasing the risk of maintaining infectious diseases. Try reducing the numbers.
WREM	Frequency of manure removal in weaner rooms	LIT	Weaner	-	=	2.0	<	2.0	1.4	2.0		Remember that manure needs to be removed from under the pens regularly!	You need to remove manure from under the pens more regularly!
WSEP	Solid pen separation in weaner rooms	EXP, LIT	Weaner	-	=	1.0	=	0.0	1.0	1.0			Solid pen separations help prevent disease transmission. Check whether they could be introduced on your farm.
WSHA	Weaners sharing rooms with >12 week-old pigs	LIT	Weaner	-	=	0.0	=	1.0	1.5	1.4			Young pigs mustn't share a room with older pigs or they will be infected with respiratory diseases.
WTEMP	Temperature for 4-7 week old pigs	EXP, LIT	Weaner	Weaner	>	24.0	<	22.0	1.0	1.0	2.00	The ideal temperature for weaner pigs is >24 degrees Celsius. It is too cold in your buildings.	The ideal temperature for weaner pigs is >24 degrees Celsius. It is too cold in your buildings.

APPENDIX C. Report modules used by the expert-based method in RestiMATE

Module ID	Sub-heading	Text	Comments
Module 1		In order to improve the situation on this farm, the airflow in the grower and finisher buildings should be improved.	To be inserted in advice sheets (see below)
Module 2		The general environment and hygiene in the grower/finisher buildings appear to be a problem area. The stocking density should be checked. It is recommended that less than 200 pigs should share an airspace and less than 15 pigs should be housed in the same pen. Manure management is crucial to avoid an adverse environment. Hygiene may be improved by all-in/all-out pig flow. Measures to avoid contact between pigs in different pens and of different age groups are likely to reduce the infection pressure.	To be inserted in advice sheets (see below)
Module 3		Note: Results from this analysis are for a typical herd producing <4000 pigs per year and not buying in stock. The results have to be interpreted with caution because this farm has a larger size or a different purchase practice. Both factors are known to have a negative influence on respiratory diseases in pigs.	To be inserted in advice sheets (see below)
Module 4		Note: It was indicated that this farm may be free of enzootic pneumonia or APP or both. The results of this analysis have to be interpreted with caution as some of the major agents causing respiratory problems may be absent on this farm.	To be inserted in advice sheets (see below)
Module 5		Note: Signs of severe pneumonia and/or APP have been observed on this farm. It is recommended that the status of this farm be determined more specifically using slaughter checks or other diagnostic techniques. This issue should be discussed with the veterinary advisor.	To be inserted in advice sheets (see below)
Advice sheet A		No signs of respiratory disease were detected on this farm and the general health status is high. No interventions are needed at this stage.	
Advice sheet B		No signs of respiratory disease were detected on this farm. However, other health problems seem to be present. Possible causes and interventions should be discussed with the veterinary advisor.	
Advice sheet C		Signs of respiratory disease have been detected in the 4-7 week-old pigs on this farm. The cause is probably not enzootic pneumonia. Look at ways to improve the environment in the nurseries. Temperature fluctuations have to be minimised and the temperature possibly needs to be increased. Check for draughts and general hygiene.	
Advice sheet D		<insert text module 3 and/or 4 and/or 5 here> Mild signs of respiratory disease were detected on this farm. <insert text module 1 and/or 2 here>	

	Short-term intervention	Check possibilities to improve the airflow or reduce stocking density by changes in the management.	
	Medium-term intervention	Evaluate low-cost changes to buildings in order to improve airflow and general environment of the pigs.	
	Long-term intervention	Not applicable	
Advice sheet E		<insert text module 3 and/or 4 and/or 5 here> This farm appears to have a moderate problem with respiratory diseases. <text module 1 and/or 2>	
	Short-term intervention	The possibility of starting a low-dosage antibiotic treatment should be considered. If medication is already in use, product and dosage need to be reviewed. Check for possibilities to improve the airflow or reduce stocking density by changes in the management. Try to use all-in/all-out pig flow and cleaning and disinfecting between batches.	
	Medium-term intervention	Consider starting a vaccination programme. Evaluate low-cost changes to buildings in order to improve air flow and general environment of the pigs.	
	Long-term intervention	Plan permanent changes to farm buildings with respect to ventilation and sub-division of rooms.	
Advice sheet F		<insert text module 3 and/or 4 and/or 5 here> This farm appears to have a severe problem with respiratory diseases. <insert text module 1 and/or 2 here>	
	Short-term intervention	The start of an antibiotic treatment at therapeutic level should be discussed with the veterinary advisor. If medication is already in use, product and dosage need to be reviewed. Consider options for immediately improvement of airflow or reduce stocking density by changes in the management. Try to use all-in/all-out pig flow and cleaning and disinfecting between batches.	
	Medium-term intervention	Consider starting a vaccination programme. Evaluate low-cost changes to buildings in order to improve air flow and general environment of the pigs.	
	Long-term intervention	Plan permanent changes to farm buildings with respect to ventilation and sub-division of rooms. Discuss the possibility to eradicate respiratory diseases from this farm with the veterinary advisor.	

APPENDIX D. General farm questionnaire

Name		*Leave*
Surname		*empty*
Address		
City/Village		
Phone		
Occupation		
Jobs outside the farm		
Age		
Number of persons living on farm	..... adults ..... children	
Number of employees not living on farm		
Livestock inventory	*Number of animals*	
Breeding pigs		
Growing-finishing pigs		
Piglets		
Dairy cows		
Heifers and/or beef cattle		
Calves		
Sheep, goats		
Poultry		
Cats		
Dogs		
other		
Farm size (ha)		
Distance to next farm (in m)		
Distance to next pig farm (in m)		
Husbandry systems for pigs	☐ open-front housing ☐ outdoor pens ☐ pasture	
Health status (as certified by Swiss pig health service)		

Contacts (house)	*Number per week*	
Mail delivery		
Nurse		
Deliveries		
Family and friends		
Other		
Contacts (farm)	*Number per year*	
Feed compound delivery		
Liquid feed delivery		
Other deliveries		
Hoof trimmer		
Milking machine technician		
Farm advisor		
Animal dealer, animal transports		
Contract workers		
Mechanic		
Veterinarian		
AI technician		
Slurry pick-up		
Other		
Do you use slurry from another pig farm	☐ yes ☐ no	
From how many farms?		
Farm type	☐ breeding ☐ finishing ☐ mixed	
Breeding farm		
Do you use AI?	☐ yes ☐ no	
Do you use boars from other farms	☐ yes ☐ no	
Do you lend your boar to other farmers?	☐ yes ☐ no	
From how many farms do you buy boars?		

From how many farms do you buy gilts?		
How often do you buy gilts per year?		
How many gilts per year do you buy?		
Do you buy gilts from a dealer?		
To whom do you sell culled sows?		
How often do you sell culled sows?		
What identification system do you use for breeding stock?	☐ Ear tags ☐ Other..... ☐ Tattoo	
Multipliers		
Do you finish all your piglets?	☐ yes ☐ no	
If no, do you sell to a dealer?		
To how many farms are your piglets distributed?		
How often do you sell piglets?		
How many piglets do you sell per year?		
How do you identify your piglets?	☐ Ear tags ☐ Other ..... ☐ Tattoo ☐ not identified.	
Finishing farm		
Do you practice the all-in/all-out system?	☐ yes ☐ no	
Do you feed one of the following products?	☐ Whey ☐ Kitchen scrap ☐ By-products from food industry ☐ Overproduction from food industry	
Do you purchase piglets for finishing?	☐ yes ☐ no	
If yes: do you buy from a dealer?		
From how many farms do you purchase?		
How do you identify you pigs?	☐ Ear tags ☐ Other..... ☐ Tattoo ☐ Not identified	
How do you dispose of dead pigs?		

To whom do you sell slaughter pigs?		
Do you sell slaughter pigs to a dealer?		
Who transports the pigs to the abattoir?		
How often do you sell slaughter pigs per month?		
How many slaughter pigs do you sell per year?		
In which abattoir are your pigs normally slaughtered?		
Do you slaughter pigs on-farm?	☐ yes ☐ no	

Thank you!

APPENDIX E. Forms for recording farm and family contacts

DATE/TIME	NUMBER OF PEOPLE	Did any of these people have contact with pigs on your farm? (YES/NO)	REASON FOR VISIT	DESCRIPTION OF VEHICLE (FOR EXAMPLE: TRUCK, CAR, TRACTOR ...)	DELIVERY (everything that remains on the farm, for example feed, mail, animals etc.)	ORIGIN ■ Town/village ■ Farm with pigs ■ Farm without pigs ■ other Give name of town and estimated distance in km	CONTACT WITH PIGS AT ORIGIN? (YES/NO)	DESTINATION ■ Town/village ■ Farm with pigs ■ Farm without pigs ■ Abattoir, market ■ other Name of town (km)	CONTACT WITH PIGS AT DESTINA-TION? (YES/NO)
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO
		☐ YES ☐ NO					☐ YES ☐ NO		☐ YES ☐ NO

DATE/TIME	NAME	REASON FOR VISIT	DESCRITPTION OF VEHICLE	GOODS DELIVERED FROM FARM	GOODS DELIVERED TO FARM	DESTINATION ■ Town/village ■ Farm with pigs ■ Farm without pigs ■ Abattoir ■ other Name of place (km)	CONTACT WITH PIGS AT DESTINA-TION? (YES/NO)
							☐ YES ☐ NO
							☐ YES ☐ NO
							☐ YES ☐ NO
							☐ YES ☐ NO
							☐ YES ☐ NO
							☐ YES ☐ NO

APPENDIX F. Questionnaire used by experts for classification of contacts in a classical swine fever outbreak situation

Name (optional) .....

Questionnaire ID **B/01**

Scenario 1:

(Today's date: 1 May)

Imagine that today, classical swine fever has been diagnosed on pig **Farm X**. This farm is a mixed breeding-fattening farm (200 sows) with poultry as an additional small-scale production branch (100 hens).

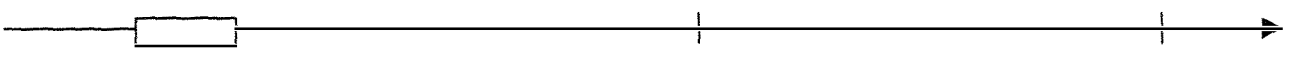
From the history of the outbreak on Farm X (clinical signs, dynamics) it looks like the virus is not highly virulent. The first clinical signs (pigs going off the feed) were observed on April 14. It has been calculated that the likely time of infection in this farm was between March 30 and April 4.

This is the first outbreak in this country since 10 years (index case). The source of the outbreak is not known. Therefore, all contacts to this farm are recorded. Attached is the list of contacts that the farmer could remember. For each contact the origin and destination are listed as well as the persons, vehicles and animals or goods involved in the contact. It is also recorded whether there was a contact with the pigs on Farm X. Note: It is assumed that feeding of heat-treated swill is allowed in this country. Swill is treated on-farm.

Each contact is considered both in terms of being a possible source of the infection (tracing back) and in terms of spreading the infection further to other non-infected farms (tracing forward). The following risk categories are to be assigned to the contacts:

No risk	0
Very low risk	1
Low risk	2
Medium risk	3
High risk	4
Very high risk	5

TIME LINE:



Likely infection date	onset of clinical signs	today
30/03-04/04	14/04	01/05

	Date of contact	Origin of contact	Contact with pigs on farm X	Description of contact	Destination of contact	**Likely source of infection (tracing back): Your score (0-5)**	**Likely spread of infection (tracing forward): Your score (0-5)**	**Comments**
1.	Daily	Various farms	No	Mail delivery: 1 person + car	Various farms			
2.	Daily	-	Yes/No	Children going to school: 2 persons + bicycles	Village			
3.	Daily	-	Yes	Son working as manager in tree nursery: 1 person + car	Town			
4.	Daily	-	No	Delivering eggs from farm to shop: 1 person, car	Town			
5.	3 times per week	-	Yes	Grocery shopping and other errands (bank, post, hair dresser etc): 1-3 persons, car, groceries	Town			
6.	2 times per week	Various farms	No	Rendering truck picking up dead animals	Various farms			
7.	March 28	Pig farm	Yes	Neighbour visiting: 1 person, walking	Pig farm			
8.	March 30	Pig Farm	Yes	Truck picking up pigs for slaughter (pigs from other farms already loaded): 1 per-	Abattoir			

	Date of contact	Origin of contact	Contact with pigs on farm X	Description of contact	Destination of contact	**Likely source of infection (tracing back): Your score (0-5)**	**Likely spread of infection (tracing forward): Your score (0-5)**	**Comments**
				son, truck, pigs				
9.	March 30	AI centre	No	Delivery of porcine semen for artificial insemination of sows: 1 person, car, semen	Pig farm			
10.	March 30	Cattle farm	No	Farmer picking up pig slurry: 1 person, slurry truck, slurry (approx 20 loads)	Pasture			
11.	April 1	Cattle farm	No	Veterinarian delivering drugs: 1 person + car	Not known			
12.	April 2	Town	No	Family visit: 3 people + car	Town			
13.	April 3	Pig farm	No	Neighbour visiting: 1 person, walking	Pig farm			
14.	April 3	Village	No	Delivery of restaurant scrubs for pig feed: 1 person, swill, truck	Not known			
15.	April 4	Village	No	Delivery of pig feed: 1 person + truck	Village			
16.	April 4	Beef farm	No	Pick-up of chicks: 1 person, truck, 15 chicks	Beef farm			
17.	April 5	Village	Yes	Pig producer association president inspecting farm: 1 person + car	Pig farm			

	Date of contact	Origin of contact	Contact with pigs on farm X	Description of contact	Destination of contact	**Likely source of infection (tracing back): Your score (0-5)**	**Likely spread of infection (tracing forward): Your score (0-5)**	**Comments**
18.	April 8	Village	No	Delivery of restaurant scrubs for pig feed: 1 person, swill, truck	Village			
19.	April 10	Butchery	Yes	Local butcher helping to slaughter pig on-farm	Butchery			
20.	April 11	Pig farm	Yes	Truck picking up pigs for slaughter (pigs from other farms already loaded): 1 person, truck, pigs	Pig farm			
21.	April 11	Pig farm	Yes	Truck delivering breeding gilts from top breeding farm (high-health farm): 1 person, truck, pigs	Pig farm			
22.	April 13	Pig farm	No	Delivery of porcine semen for artificial insemination of sows: 1 person, car, semen	AI centre			
23.	April 13	Pig farm	No	Neighbour purchasing eggs: 3 persons, eggs, no vehicles	Pig farm			
24.	April 15	Cattle farm	No	Farmer picking up pig slurry: 1 person, slurry truck, slurry	Pasture			
25.	April 16	Pig farm	No	Friend buying fresh home-made sausages (meat from 1 pig slaughtered on-farm 1 week ago): 2 persons, car, sau-	Not known			

	Date of contact	Origin of contact	Contact with pigs on farm X	Description of contact	Destination of contact	**Likely source of infection (tracing back): Your score (0-5)**	**Likely spread of infection (tracing forward): Your score (0-5)**	**Comments**
				sage				
26.	April 16	Village	No	Delivery of restaurant scrubs for pig feed: 1 person, truck, swill	Pig farm			
27.	April 18	Poultry farm	No	Truck delivering laying hens: 2 persons, truck, hens	Poultry farm			
28.	April 20	Pig farm	Yes	Neighbour picking up piglets for fattening on his farm: 1 person, trailer, car, pigs	Pig farm			
29.	April 25	Village	No	Delivery of pig feed: 1 person + truck	Not known			
30.	April 30	Pig farm	Yes	Veterinarian for herd-health visit: 1 person, car (the vet observed clinical signs in animals and reported the suspected outbreak)	Pig farm			
31.	April 30	Village	Yes	Truck picking up pigs for slaughter (empty): 1 person, truck	Pig farm			

APPENDIX G. Risk classification of farms in a classical swine fever (CSF) outbreak situation

Name (optional) .....

Questionnaire ID **B/01**

Scenario 2:

(Today's date: 1 June)

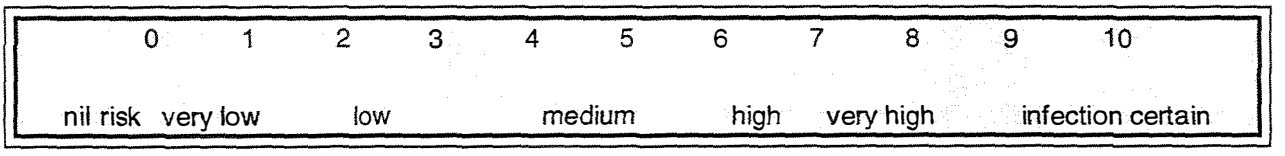
In the meantime, several more farms have been infected with classical swine fever in this outbreak. One protection zone and a surrounding surveillance zone have been declared and control measures are being executed according to directive 80/217 EEC (stand-still of movements, outbreak investigation by farm visits, no pre-emptive slaughter).

A large number of farms do now have to be visited by the field teams. You are the officer responsible to assign field teams to farms. As a rule, the farms with the highest risk to be infected have to be visited first. You need to plan the visits for the 21 farms described on the attached sheets over the next 2-3 days (an earlier completion is preferable).

Find attached a list of farms that need to be visited. For each farm, the reason why it needs to be visited is listed. If the reason is a contact to an infected farm, the risk categories of the contact(s) are also given. To illustrate the risk categories, here some typical examples:

Very high risk contact	Purchase of pigs from an infected farm
High risk contact	Person from pig farm visiting farm (pig contact)
Medium risk contact	Pickup of pig slurry from infected farm as fertiliser
Low risk contact	Purchase of straw (or similar products) from an infected farm
Very-low risk contact	Sister-in law from town visiting without pig contact

Step 1: Classify each farm according to its risk to be infected with a score between 0-10, where 0 = nil risk to be infected and 10 = infection certain:



Step 2: Rank the farms according to the sequence how they should be visited. Rank 1 = farm should be visited first, Rank 20 = farm should be visited last. You may assign the same rank to several farms, for example:

Farm 1	Rank 1
Farm 2	Rank 2
Farm 3	Rank 2
Farm 4	Rank 4
Farm 5	Rank 5

	Farm ID	Reason for visit	Contact details (all contacts are between *this* farm and an *infected* property)	**Risk to be infected: Your score (0-10)**	**Sequence of visiting: Your rank (1-21)**	Comments
32.	A	Clinical signs reported by farmer (telephone) and located in surveillance zone	No contacts			
33.	B	Located in protection zone and neighbour farm of an infected property	10 very-low risk contacts			
34.	C	Contact farm outside protection zone	15 very-low risk contacts			
35.	D	Located in protection zone	1 medium risk contact			
36.	E	Contact farm outside protection zone	1 very-high risk contact			
37.	F	Clinical signs reported by farmer (telephone) and located in protection zone	No contacts			
38.	G	Located in protection zone	No contacts			
39.	H	Contact farm outside protection zone	3 low risk contacts			
40.	I	Located in protection zone and	4 low risk contacts			

	Farm ID	Reason for visit	Contact details (all contacts are between *this* farm and an *infected* property)	**Risk to be infected: Your score (0-10)**	**Sequence of visiting: Your rank (1-21)**	Comments
		neighbour farm of an infected property				
41.	J	Located in protection zone	7 very-low risk contacts			
42.	K	Located in protection zone and neighbour farm of an infected property	No contacts			
43.	L	Located in protection zone	1 high risk contact			
44.	M	Located in protection zone and swill feeding	No contacts			
45.	N	Contact farm outside protection zone	1 medium risk contact			
46.	O	Located in protection zone and swill feeding	1 medium risk contact			
47.	P	Clinical signs reported by veterinarian by telephone and neighbour of an infected property and located in protection zone	No contacts			

XXX

	Farm ID	Reason for visit	Contact details (all contacts are between *this* farm and an *infected* property)	**Risk to be infected: Your score (0-10)**	**Sequence of visiting: Your rank (1-21)**	Comments
48.	Q	Located in protection zone	1 very-high risk contact			
49.	R	Located in protection zone	3 low risk contacts			
50.	S	Located in protection zone	1 very-low risk contact			
51.	T	Located in protection zone and neighbour farm of an infected property	1 high risk contact			
52.	U	In protection zone and neighbour farm of an infected property and swill feeding farm	No contacts			

APPENDIX H. Sensitivity analysis of INTERSPREAD-SF

Objective and introduction

The objective of a sensitivity analysis is to explore the sensitivity of a system to parameter variations (Frank, 1978). It allows the identification of influential input variables in a simulation model. Frank (1978) defines parameter sensitivity as the effect of parameter changes on the behaviour and outputs of a model. Parameter sensitivity is important because it helps identify factors that need to be most accurately determined and possibly require further research. More specifically, we wanted to identify the most influential input variables in INTERSPREAD. The main output of this model is the forecast of the average number of infected properties and the duration of a classical swine fever epidemic. These are influenced mainly by the disease-spread mechanisms and the control measures in place. Provided that the latter is kept constant, the influence of the remaining factors can be explored. Statistical methods are available to measure the effect of a change in one factor on the output (Kleijnen, 1979). The use of experimental designs (Hunter and Naylor, 1970) supports a structured approach to sensitivity analysis. Particularly fractional factorial designs seem to be widely used in sensitivity analysis (Kleijnen, 1979, 1987). The results from the experiments can be used to construct a 'meta-model' using regression analysis (Kleijnen, 1987). The term meta-model is adequate because the empirical relationships between input and output parameters of the underlying simulation model are described (Kleijnen, 1979). The coefficients of the meta-model reflect the importance of a factor (Kleijnen *et al.*, 1992).

Material and methods

A simulation experiment based on a 3^{k-p} fractional factorial design (Cochran and Cox, 1992; Montgomery, 1997) was performed using INTERSPREAD (32-bit version, Stern, 1997). Only local spread and movement spread mechanisms were considered. The 5 input variables 'time to clinical signs' (Factor 1, CLIN), 'time to diagnosis' (Factor 2, DIAG), 'local spread' (Factor 3, LOCAL), 'number of movements' (Factor 4, MOVE) and 'probability of infection by movement' (Factor 5, INF) were used on three levels each (low, medium, high). The medium level was defined first and the low and high level were calculated symmetrically by adding or subtracting 25% of the medium value. The values used for the factors under study are listed below.

1. Factor 1: Time to onset of clinical signs (CLIN)

Level 1: LogNormal (8,1.5)[1]

Level 2: LogNormal (12,2)

---

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

Level 3: LogNormal (16,2.5) truncated 0-28

TABLE 1. Probability distribution of time from infection to earliest clinical signs (days) in an outbreak of classical swine fever involving a virus strain of low-moderate virulence

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Level 1	0.000	0.000	0.000	0.006	0.064	0.207	0.263	0.220	0.114	0.070	0.046	0.007	0.002	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Level 2	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.030	0.087	0.147	0.187	0.183	0.147	0.097	0.060	0.030	0.017	0.003	0.003	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Level 3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.006	0.026	0.057	0.119	0.160	0.156	0.133	0.117	0.096	0.051	0.030	0.027	0.010	0.004	0.003	0.001	0.000	0.000

2. Factor 2: Time to diagnosis (DIAGN)

Level 1: LogNormal (8,7.5) truncated 0-28

Level 2: LogNormal (12.8,10) truncated 0-28

Level 3: LogNormal (17.6,12.5) truncated 0-28

TABLE 2. Probability distribution time of earliest clinical signs to time of diagnosis (days) in an outbreak of classical swine fever involving a virus strain of low-moderate virulence

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Level 1	0.076	0.111	0.123	0.115	0.094	0.076	0.065	0.063	0.054	0.048	0.032	0.027	0.020	0.017	0.015	0.010	0.009	0.007	0.007	0.006	0.005	0.005	0.005	0.003	0.003	0.002	0.002	0.000
Level 2	0.012	0.031	0.064	0.066	0.076	0.084	0.077	0.068	0.063	0.055	0.054	0.047	0.044	0.039	0.026	0.025	0.024	0.023	0.017	0.016	0.017	0.015	0.015	0.013	0.010	0.009	0.006	0.004
Level 3	0.001	0.005	0.013	0.027	0.042	0.046	0.062	0.062	0.064	0.058	0.059	0.054	0.053	0.053	0.050	0.045	0.040	0.040	0.036	0.036	0.026	0.024	0.021	0.021	0.020	0.016	0.016	0.010

3. Factor 3: Local spread (LOCAL)

Level 1: Level 2-25%

Level 2: based on Staubach *et al.* (1997) and Benard (personal communication)

Level 3: Level 2 + 25%

TABLE 3. Input parameters related to local spread

Daily probabilities of infection of pig farms within certain distance ranges of infected farm (local spread)				
	0-0.1 km	0.1-0.25 km	0.25-0.5 km	0.5-1 km
Level 1	0.03	0.01125	0.0075	0.00225
Level 2	0.04	0.015	0.01	0.003
Level 3	0.05	0.01875	0.0125	0.00375

4. Factor 4: Number of movements off a farm (MOVE)

Level 1: Level 2 – 25%

Level 2: empirical from Dutch and Swiss survey (CHAPTER 2.5)[2]

Level 3: Level 2 + 25%

TABLE 4. Input parameters related to contact spread: number of movements to other pig farms

Movements off farm per day			
Risk category	Very-high	High	Medium
Level 1	0.019	0.053	0.150
Level 2	0.025	0.070	0.200
Level 3	0.031	0.088	0.250

5. Factor 5: Probability of infection by movements (INF)

Level 1: Level 2-25%

Level 2: Reduced estimates used in EpiMAN expert system (see CHAPTER 2.7)

Level 3: Level 2+25%

TABLE 5. Probability of infection by movements

Risk category	Very-high	High	Medium
Level 1	0.71	0.56	0.38
Level 2	0.95	0.75	0.50
Level 3	1.00	0.93	0.63

[2] In the original calculations, no distinction was made between contacts to farms with pigs and other contacts. These figures have been re-calculated to discount all other contacts. Currently, if a farm is infected with CSF and there is a contact to another pig farm, this is always at least a medium risk. So, medium is now put instead of low, high instead of medium and very-high instead of high.

6. Control strategy

A basic control strategy including 2 radial restricted zones with a radius of 3000 m and 10000 m was used. Within the radial zones, movement control was applied. Very-high risk farms were pre-emptively slaughtered. Ten simulation iterations over a maximum of 381 days were run for each of the factor combinations. The average number of infected properties (IP) and the average duration of the epidemic in days were used for further analysis.

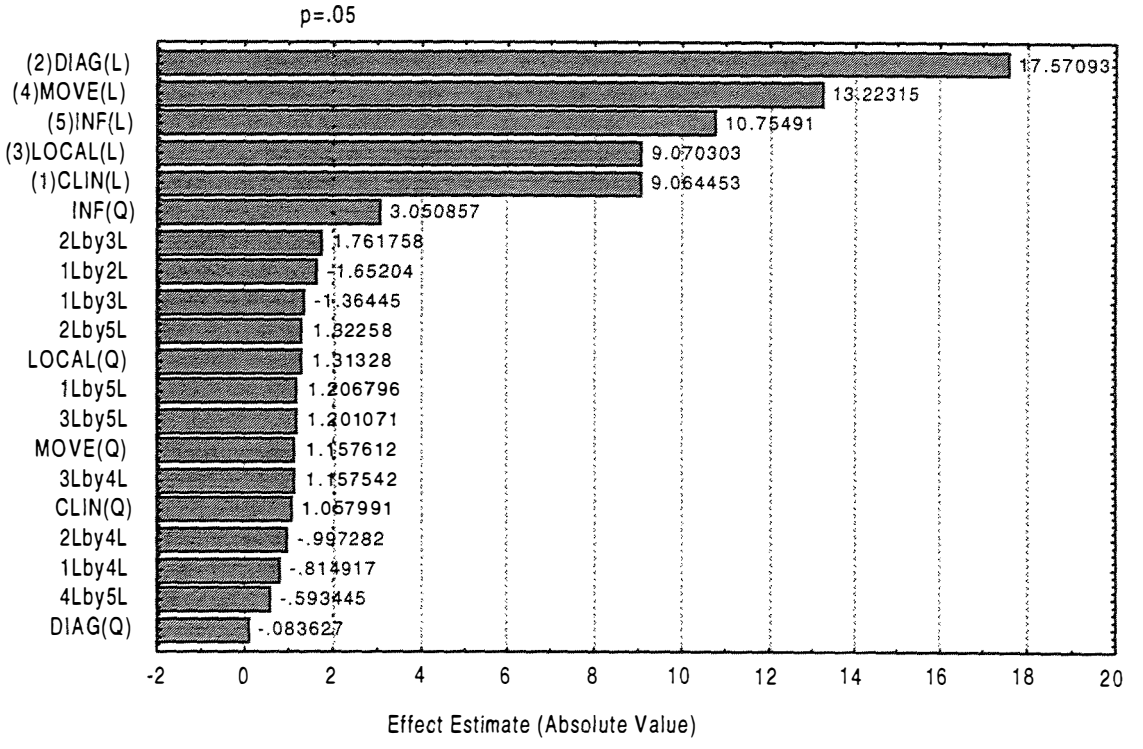
7. Experimental design and analysis

A 3^{5-1} fractional factorial design with 81 factor combinations was created using STATISTICA for Windows v.5.1 (StatSoft Inc., Tulsa). The results were analysed using the same software package. Linear and quadratic main effects as well as two-way linear interaction terms were assessed using analysis of variance (ANOVA). Regression coefficients of the significant effects were calculated. The average number of IPs was $\log_{10}$-transformed in the final analysis.

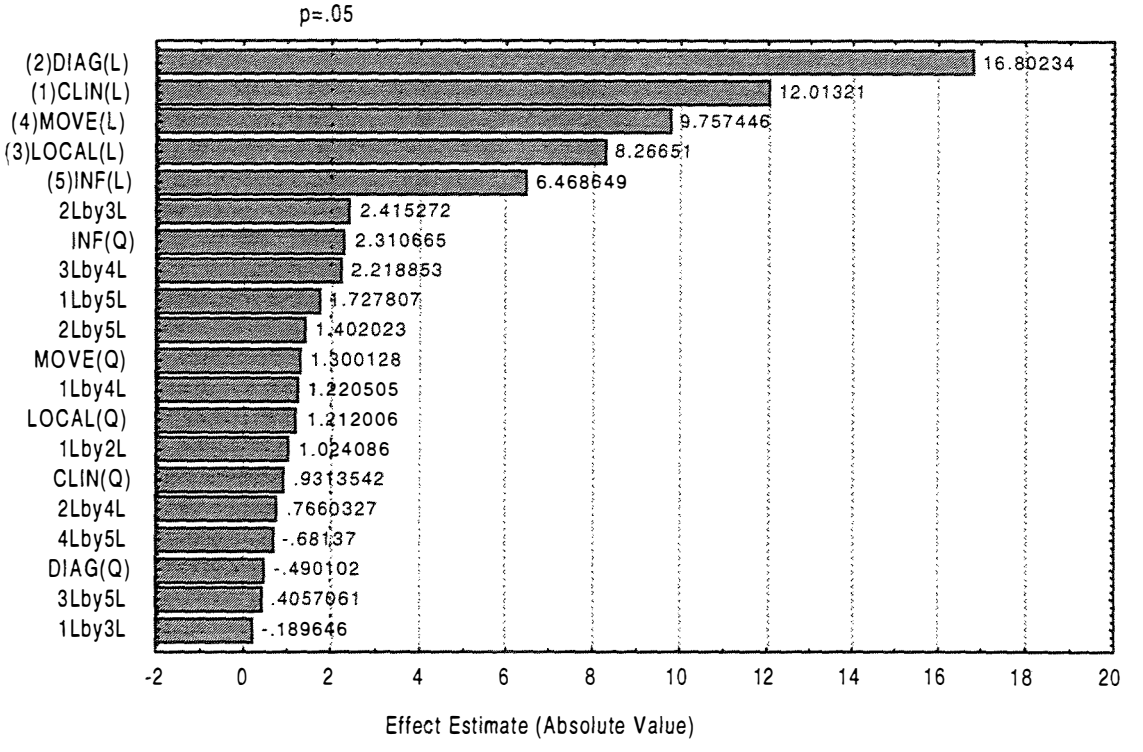
Results

With the exception of INF, only linear main effects were significantly associated ($p<0.05$) with the log of the average number of IPs (FIGURE 1A). A simple linear-main-effects model was considered sufficient to provide the results of the sensitivity analysis. Out of the main effects DIAG had the largest influence on the outcome, followed by MOVE, INF, LOCAL, and CLIN (TABLE 6). This model had an adjusted R^2 of 0.89.

Similarly, mainly linear main effects were significantly associated ($p<0.05$) with the average number of days that the epidemic lasted (FIGURE 1B). Again, a linear-main-effects model was further analysed. DIAG was the most influential variable with CLIN, MOVE, LOCAL and INF following in this sequence (TABLE 7). The model had an adjusted R^2 of 0.87.



A)



B)

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

TABLE 6. Regression coefficients for model with dependent variable = $\log_{10}$(average number of infected properties). R^2 adjusted=0.89, n=81, DF=75.

Factor	Coefficient	Std. Error	T	P	95% C.I.
Intercept	1.642	0.021	75.69	0.000	1.598-1.685
DIAG	0.428	0.027	16.11	0.000	0.375-0.481
MOVE	0.322	0.027	12.13	0.000	0.269-0.375
INF	0.262	0.027	9.86	0.000	0.209-0.314
CLIN	0.221	0.027	8.31	0.000	0.168-0.273
LOCAL	0.221	0.027	8.31	0.000	0.168-0.273

TABLE 7. Regression coefficients for model with dependent variable = average duration of epidemic in days. R^2 adjusted=0.87, n=81, DF=75.

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

8. Discussion

Performing sensitivity analysis with meta-models requires a profound knowledge of the underlying simulation model and the real world (Kleijnen *et al.*, 1992) as the potentially influential factors to be studied have to be selected by the investigator. In the experiments described here, all factors directly related to the probability of local and movement-related disease spread (MOVE, INF, LOCAL) were included plus two additional factors with an indirect influence. The time to first clinical signs (CLIN) is a characteristic of CSF virus strains. As the detection of classical swine fever largely depends on the occurrence of clinical signs, this factor is significant. The time until diagnosis (DIAG) depends on the disease awareness of farmers and the efficiency of veterinary services. The sooner the diagnosis occurs, the faster control measures are put into place and further disease spread is prevented. Unfortunately, both these latter factors are hard to obtain from field data. The results of this experiment show, that it is particularly important to have a good estimation of DIAG, because this is the most influential factor with respect to both output variables. When performing a sensitivity analysis with INTERSPREAD on foot-and-mouth disease, Jalvingh *et al.* (1996) also explored the influence of farm density and the size of the area within which the simulation took place. These were considered fixed in this experiment as they are not disease-dependent but can be controlled by the analyst. The farm density was 2.05 farms/km^2 and the area was 1417

km^2. The high R^2 of the regression models demonstrate that the significant parameters for the model variability have been included.

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

Hunter and Naylor (1970) have pointed out the importance of using a sufficiently large number of runs when performing simulation experiments. The effect of the number of runs on the results provided by INTERSPREAD was first assessed by Sanson (1993). He recommended using at least 5 iterations to obtain a reasonably stable output in terms of the average number of IPs. Jalvingh *et al.* (1996) however postulated a sample size of at least 50 runs. This is correct if one is interested in the entire course of the epidemic and not only in the average number of infected farms. Although the shapes of the epidemic curves are highly variable if only 5 runs are used (FIGURE 2), the average number of IPs stabilises quickly and is not significantly different from the figure obtained from high-run simulations. In the example shown in FIGURE 2 the total number of IPs simulated with 5, 10, 25 and 50 runs was 47.6, 27.4, 21.6 and 21.6, respectively. Additionally, in this experiment we were less interested in the actual output values than in the influence of the relative importance of the co-variates. Therefore, the 10 runs used in this experiment to calculate the average number of IPs was considered sufficient.

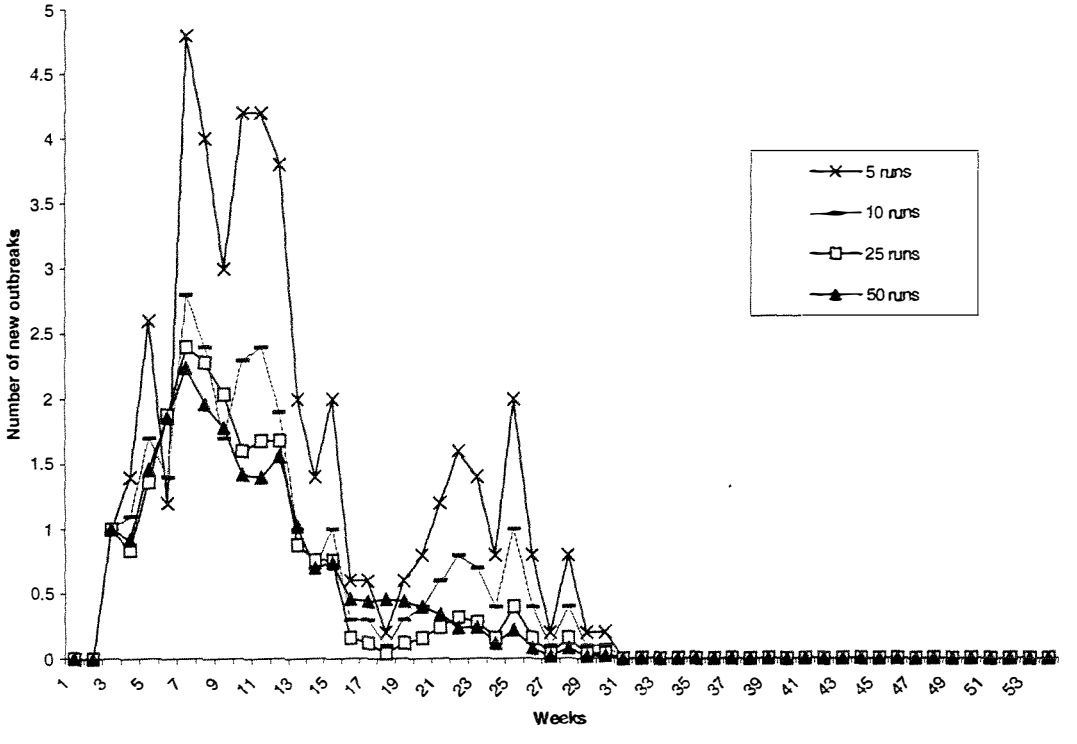


FIGURE 2. Comparison of epidemic curves simulated by INTERSPREAD using different numbers of replications (runs)

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

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

All regression coefficients in our models have a positive sign as they logically should have. This is reassuring because coefficients with 'wrong' signs are indicators of computer errors or conceptual errors (Kleijnen, 1992). The magnitude of the coefficient is directly proportional to the importance of the factor. Collecting more information on important variables is crucial for accurate results. In the CSF case, DIAG was most influential. Both the number of farms that got infected in an epidemic as well as the duration of the epidemic were greatly influenced by this factor. However, this factor is difficult to estimate as the onset of clinical signs as well as the time of infection are not known for most infected farms in real outbreaks. Consequently, the probability of diagnosis is difficult to estimate for any given day. In such a case, principles of risk analysis could be applied (Kleijnen, 1992). The factor under consideration is then replaced by a probability distribution of input values which may be based on expert opinion. INTERSPREAD already implements this approach. The figures used for DIAG and CLIN represent results of sampling from lognormal distributions (medium factor levels LOGNORMAL(12.8,10) and LOGNORMAL(12,2), respectively, truncated 0-28) that were transformed into histograms of daily probabilities.

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

Another influential factor in the CSF models was the number of movements per day (MOVE). Although this figure can be obtained from surveys (see CHAPTER 2.5), the classification of the contacts in risk categories is subjective. It is even more difficult to estimate the probability of infection by any one of these movements (INF). Both these factors were particularly influential on the average number of IPs.

Not much information is available on the risk of local spread (LOCAL), partly because this is a conglomerate of risk factors acting over a relatively short distance around an infected farm and which is only poorly understood. More information could become available from analyses of field data currently under way in Europe. However, the results of our experiments suggest that this factor is of minor importance on the output of INTERSPREAD.

References

Cochran, W.G., and Cox, G.M. (1992). Experimental designs. 2nd Edition. Wiley Classics Library, John Wiley & Sons, New York.

Frank, P.M. (1978). Introduction to system sensitivity theory. Academic Press, New York.

Hunter, J.S. and Naylor, T.H. (1970). Experimental designs for computer simulation experiments. Management Science 16:422-434.

Jalvingh, A.W., Nielen, M., Dijkhuizen, A.A., and Huirne, R.B.M. (1996). Task B: Development of economic models for the integration into EpiMAN(EU), Final Report. In: EpiMan(EU): A computer system for the management of epidemiological data and prediction of risk and economic consequences during outbreaks of foot-and-mouth disease. Wageningen Agricultural University, Wageningen.

Kleijnen, J.P.C. (1979). The Role of Statistical Methodology in Simulation. In: Zeigler, B. P., Elzas, M. S., Klir, G. J., and Ören, T. I. (eds.) Methodology in Systems Modelling and Simulation North-Holland Publishing Company, Amsterdam, 425-445.

Kleijnen, J.P.C. (1987). Statistical tools for simulation practitioners. Marcel Dekker, New York.

Kleijnen, J.P.C. (1992). Verification and validation of models. Department of Information Systems and Auditing, Tilburg University, Tilburg, The Netherlands.

Kleijnen, J.P.C., van Ham, G., and Rotmans, J. (1992). Techniques for Sensitivity Analysis of Simulation Models: A Case Study of the CO2 Greenhouse Effect. Simulation 58:410-417.

Lindman, H.R. (1974). Analysis of variance in complex experimental designs. W.H. Freeman & Co., San Francisco.

Montgomery, D. (1997). Design and analysis of experiments. John Wiley & Sons, New York.

Sanson, R.L. (1993). The development of a decision support system for animal disease emergency. PhD Thesis, Massey University, Palmerston North.

Staubach, C., Teuffert, J., and Thulke, H.-H. (1997). Risk analysis and local spread mechanisms of classical swine fever. Epidémiologie et Santé Animale 13-32:06.12.1-06.12.3.

Stern, M.W. (1997). Interspread-Kernel User Guide. Unpublished manuscript, Massey University, New Zealand.