MODEL APPLICATIONS OF DECISION SUPPORT SYSTEMS IN MEAT HYGIENE PROGRAMS

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ABSTRACT

Current systems to ensure safety of meat are to a large degree based on a "procedural" approach which specifies what inspection actions will be taken to protect human health. Both knowledge and disease priorities have changed substantially over recent decades, and moreover the scale of the problems created by any breakdowns in protection has escalated greatly, as food trading and consumption patterns have changed. It is now recognized that meat hygiene needs to focus primarily on ways by which the risk that product will represent a hazard to human health can be effectively reduced, rather than merely ensuring compliance with a defined set of procedures. In addition to human food safety, meat inspection has the potential to contribute information to improve animal health on a national and a local scale. This thesis examines example issues in order to identify possible approaches to the development of decision support systems which assist in protecting meat consumers and improving the health of livestock on farms. The main areas which were explored for this purpose were respiratory disease in lambs and chemical residues in slaughter animals.

A literature review of pneumonia and pleurisy in lambs showed that numerous factors have been proposed as predisposing causes for these diseases, but there was surprisingly little valid experimental or observational research evidence to support such statements. A hazard analysis was performed for the micro-organisms which have been isolated from pneumonic lungs. The major commonly detected organisms did not appear to cause a risk to healthy people. However there were a number of micro-organisms which are isolated on occasion from pneumonic and sometimes from healthy sheep lungs that might cause human disease.

A case-control study was carried out as an exploratory means to identify risk factors and to generate hypotheses about causal processes. A number of risk factors were initially identified at univariate level. At the second stage the importance of some of these risk factors was quantified in a logistic regression model. Finally a third stage analysis showed the interactions between the factors in a logistic path model, which consisted of three clusters. One cluster included characteristics of the farm and paddocks, one cluster included the yards and practices in the yards, and a third cluster included the types and number of animals on the farm.

Two intervention studies were subsequently carried out to evaluate the effect of making various management modifications on the prevalence of pneumonia and pleurisy at slaughter. One intervention study evaluated the time lambs spent in the yards after weaning and the use or oral or injectable drenches. The second intervention study evaluated the use of oral versus injectable drenches and the use of a shower dip versus a wand. The intervention studies showed an effect of time in the yards on pneumonia. There was some association between time in the yards and acute localised pleurisy but none between the other measures tested and respiratory disease. The studies showed clear temporal patterns with regard to pleurisy and pneumonia and enabled comparisons to be made between farms.

A study of inspection for pleurisy at slaughterhouses was analysed. The analysis identified the

temporal patterns of certain types of pleurisy. Comparisons were made between four participating premises. The sensitivity and specificity of meat inspection for the various types of pleurisy was analysed. The pleurisy data over an eleven year period of the entire country were analysed. Differences were shown between islands and regions.

The potential for development of components of a decision support system for pneumonia and pleurisy was illustrated with a number of examples. An important component was to determine how farmers could be assisted in improving the health of their lambs with regard to pleurisy. Ideas to improve farmer involvement were developed. The principles of a decision support system which evaluated the issue of cross-contamination due to handling of product by the inspector were developed.

Epidemiological principles of chemical residues in slaughter animals were investigated. A number of statistical quality control tests were applied to known data sets to evaluate what sample sizes would be required to detect changing trends or spatial paterns. Temporal simulations were performed to determine how well clusters in time could be detected. The Moving Average approach was used and it appeared that with the given data set sample sizes well beyond those feasible to achieve would be required. Spatial analyses with a number of different statistics were performed. In this case also, large sample sizes were required for reliable results.

It was concluded that use of a risk analysis model to define a risk-reduction strategy targeted to avoid any significant risk to the consumer offered a much more effective tool than a fixed sampling system. This model combines a range of possible risk reduction measures in various mixes, and determines whether or not each of the tested strategies achieves the goal of making it very improbable that a consumer would be exposed to sufficient levels of chemical residues in food to even constitute some minimal public health risk.

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CHAPTER 1

INTRODUCTION

Aim of Food Safety Programs

The aim of food safety programmes is to provide food which has a minimal risk of producing diseases or other adverse effects on the consumer, at a reasonable price.

This definition conveys the concept that the safety of food should be seen in the context of product price, ie additional safety can be bought at the cost of an increased price for the product. There should be an appreciation that there is a balance between the desire to eat various foods and the risk that is inherently taken in the process of eating each type of food. The definition is intended to express the idea that few human activities are risk-free, and that actions taken to reduce or eliminate risks must balance the additional benefit from safety programmes against the additional costs.

The critical components which should form the basis for designing meat safety programmes are explained below. They consist of process control, data collection, analysis/feedback, and risk assessment. In contrast, conventional food safety programmes rely on inspection of the carcasses of individual animals and are commonly called meat inspection systems.

Conventional Meat Inspection Systems

As an established concept 'meat inspection' has been central to efforts aimed at guaranteeing protection of the consumer. This term may have become counterproductive, because it emphasises what should be a component only of a more comprehensive food safety programme which includes process control and protection against chemical residues.

Inspection of animals in slaughterhouses is intended to protect the public health, but current inspection procedures support past disease priorities and outdated epidemiological understanding. They do not accurately reflect current concepts of product safety, and how it should be achieved. In New Zealand the vast majority of sheep, cattle, pigs, deer, goats and horses that are slaughtered for human consumption are subjected to an ante mortem and a post mortem inspection which are clinical and pathological evaluations. Usually both evaluations are carried out rapidly. However in case of abnormalities there is scope to detain live animals or carcasses and tissues for a thorough inspection, with laboratory backup if necessary. Those animals or parts of animals which are deemed unfit for human consumption will be condemned. Although the

intention is to remove product from the food chain which presents a public health hazard, in reality only tissues which display abnormalities are condemned. Many conditions of public health significance cannot be detected by current procedures. Only some of the animal diseases that are used to determine carcass disposition at meat inspection have public health significance.

Generally meat inspection systems in the Western world are 'procedure' driven. A product is inspected at the end of the production process. The systems are very occupied with compliance with their rules rather than with their more fundamental aims. These meat inspection systems are frequently based on the systems that were developed late last century. Many of the conditions that were considered to be significant at the time are no longer considered to be important. This is to some degree because the prevalence of a number of diseases in livestock has decreased. However there is an increasing awareness that some of the conditions which cannot be detected by conventional meat inspection are of great human health importance. These conditions include the presence of pathogenic micro-organisms and residues of chemicals.

There are other important functions which meat inspection already performs to some degree and which can be strengthened in new food safety programmes. They include surveillance of animal health and production, and defects of processing and marketing importance. These components are not concerned with food safety. Where these issues are addressed successfully farmers and meat processors will benefit from them. The ability to carry out post mortem examinations of large numbers of animals is a strong point of meat inspection. The role of meat inspection regarding animal health surveillance is explored below. This type of surveillance should not be considered as a stand-alone system. There are inherent weaknesses in the collection of the data, and it is biased in various respects. However an appreciation of the scope of these deficiencies will facilitate the incorporation of slaughterhouse data with other systems, ultimately leading to an overall animal disease surveillance system that will give a sufficiently accurate 'picture' of the situation. The removal of defects such as pleurisy, arthritic joints, and abnormally pigmented meat from carcasses is a cost to meat processing. Meat inspection systems can assist in quantifying the cost of labour and discarded product thereby providing valuable feedback to both farmers and meat processors. Such feedback is essential where diseases can be prevented on the farm, while the only remedy available to meat processors is trimming.

Process Control

The main tasks facing food safety programmes in relation to infectious diseases are to exclude pathogens from the food chain to a reasonable degree and to limit the opportunities for these pathogens to multiply during processing and storage. The phrases that have been coined to describe these concepts are 'pre-harvest food safety' and HACCP (Hazard Analysis Critical Control Point). In the case of pre-harvest food safety, risk factors which contribute to the existence of pathogens in farm animals are identified. Subsequently farmers can be encouraged to raise livestock in such a manner that the prevalence of pathogens in the livestock population is reduced. The HACCP principles can be applied to both farming and the meat processing industry. In the meat processing industry it focuses on the areas where processing can go wrong,

resulting in contamination of product and the multiplication of pathogens. Examples of such critical points are contact between skin, ingesta or faeces and the carcass, contact between meat and food handlers, and temperature abuse.

Risk Analysis

There is a need to develop risk analysis tools which will be able to assess the risk which pathogens, procedures and their interaction, pose to public health. This may be performed in a qualitative or a quantitative sense. There is a need to generate a greater appreciation among the general public and decision makers that with the present production and processing methods a nil-risk policy is not feasible. In fact it has never existed. The perception in the past that meat was "safe" if inspected and handled properly, was based on incomplete knowledge. New understanding which has become available over recent decades demonstrates the limitation of the inspection approach.

Risk analysis will need to be tied in with economic models. If unequivocally "safe" meat cannot be produced, then questions need to be asked about what are the economic options for producing meat of different degrees of "safety", to satisfy different market needs.

Animal Health Surveillance

Over the years the importance of disease surveillance has shifted from easily identifiable animal diseases to endemic diseases which are not clearly defined and which are strongly multifactorial. Monitoring for exotic diseases such as foot and mouth disease (FMD) through food safety programmes that are based at slaughterhouses is an important part of the overall national FMD surveillance programme. Most livestock will finally be slaughtered in a slaughterhouse. This provides an opportunity to assess the health of the national flock and individual farm flocks. The data for certain diseases can be acquired more easily and cheaply in a slaughterhouse than anywhere else. These data can then be used to assess the health status of the national herd and to monitor the effect of control or eradication campaigns. Especially in the case of endemic diseases such as pneumonia in sheep, and Johne's disease, slaughterhouse data will be invaluable to individual farmers.

Data Collection, Analysis and Feedback

The objectives of data collection have to be clearly defined before the actual data collection is started. It would need to be considered whether food safety, animal health or product quality

issues are considered.The success of process control, risk analysis and animal health surveillance depends heavily on the availability of data. These data need to be comprehensive and of good quality. Systems need to be in place to collect relevant data and to retrieve them on-line. In addition there is a need to analyse the data in a consistent, statistically sound manner. Computer hardware has reached the stage where it is able to perform the above functions in such a manner that data can be used for practical purposes. There is a need now to develop the appropriate software. Major problems still exist such as identifying geographical areas where animals have been raised before they were slaughtered. This problem may be of more importance in the cattle and sheep industry than in the pig industry.

Future Developments

There is a growing appreciation in the meat processing industry that control of the process of food production at all stages is as appropriate for them as it is for other industries. Issues such as human infections with *Salmonella* ssp., *Campylobacter* ssp., *Listeria monocytogenes*, verotoxigenic *E. coli* and *Toxoplasma gondii* in food of animal origin continue to make headlines in the media. They will not go away and the food processing industry is acutely aware of this. Bovine spongiform encephalopathy (BSE) has brought this subject even more clearly into focus at the worldwide level. Consumer rights have been clearly defined in law and are likely to be applied to instances of food poisoning more often in the future than is currently happening. In the case of *Salmonella* in eggs corrective action was taken by the poultry industry overseas. An increased awareness among farmers regarding their responsibility to supply healthy stock may seem desirable. However risk factors are usually poorly defined or not defined at all. Therefore the practices that farmers should comply with to supply animals carrying fewer pathogens are not very obvious. Improved identification systems of stock are currently being considered, especially for cattle. This will enhance systems such as AgriBase which is close to full implementation. In the near future the identification of stock may be less troublesome.

The ratification of GATT will have two major implications. Certification based on freedom of certain diseases on defined farms or in certain areas rather than in the whole of a country has become an accepted practice. Disease will be considered in relation to zones of varying size, rather than only in relation to a whole country. This will especially have an effect on the use of geographical information systems and statistical analysis. Any restrictions in trade will need to be scientifically based. This will especially have an effect on the development of risk analysis techniques.

Projects in the Thesis

This thesis takes two quite different issues as examples to explore how meat safety programs can adapt to the new priorities. First it explores how existing inspection findings could be used to

provide information to producers, based on the example of pleurisy in lambs.
Second, it examines the issue of chemical residue control, considering how a more effective surveillance system for preventing human health risks from such residues could be developed.

CHAPTER 2

PNEUMONIA AND PLEURISY IN LAMBS

Literature Review of Pneumonia and Pleurisy in Sheep

Introduction

Pneumonia and pleurisy can be detected during post mortem meat inspection. The various functions of meat inspection which were described in Chapter 1 apply to this disease. This section will review the literature on the disease in relation to these meat inspection functions. It focuses on the aspects which are not related to public health, while the next section will expand these findings into the public health area.

From an animal health perspective, acute fibrinous pneumonia can lead to a sudden death of affected lambs. In the case of chronic non-progressive pneumonia, a large number of lambs may be affected without showing clinical signs. Pneumonia and pleurisy in lambs is a costly disease for both farmers and the meat processing industry. The economic consequences of the occasional outbreaks of acute fibrinous pneumonia on individual farms are particularly visible to all involved. Sorenson (1976) reported a loss due to 21% mortality in a flock. The productivity effects in reduced growth rates of lambs that can arise from chronic non-progressive pneumonia have also been well documented (Kirton *et al.*, 1976; Alley, 1987b). The micro-organisms which are involved in pleurisy may be risks to the public health and the lesions can be unsightly. Removal of lesions is required for market access. Therefore inspection and removal of pleurisy lesions is required. Inspecting for pneumonia and pleurisy and the subsequent trimming of affected carcasses represent a considerable cost to the meat processing industry.

To properly assess the value of meat inspection with regard to pneumonia and pleurisy the literature on clinical signs, pathology, associated micro-organisms, and ways to prevent or treat the disease is reviewed. This is followed by comments on the costs of inspection and processing of affected carcasses and the use of this disease for the development of programs designed to return information to producers.

Clinical signs

Pneumonia and pleurisy in New Zealand sheep are seen in two main forms - an acute fatal

pneumonia, and a subclinical pneumonia which is detected when the sheep are sent for slaughter (Davies, 1985b).

Acute fibrinous pneumonia occurs in sheep of all ages but particularly in young animals. The outbreaks of acute pneumonia in lambs usually occur in January, February and March and especially in Northland (Davies, 1985b). Salisbury (1957) and Downey (1957) referred to an acute type pneumonia of ewes which occurred in October to December in Southland and Otago. Ewes at lambing are considered more susceptible (Rodger, 1989).

Acute fibrinous pneumonia is often diagnosed only after the animal has died, without signs being detected before. However the animals may have a mucopurulent nasal discharge and may cough from time to time. There may be respiratory distress and moist rales may be heard in the anterior thorax. They may stand apart from the rest of the flock. The disease is accompanied by fever, and animals usually die within 12 hours of the beginning of signs (Alley, 1991; Bruére and West, 1993).

Chronic non-progressive pneumonia affects sheep especially between 3 and 10 months of age. It tends to be more common in late summer-autumn to early winter, but can be seen in all seasons (Bruère and West, 1993).

Animals with chronic non-progressive pneumonia often show no signs. Weight gain may be reduced (Kirton *et al.* 1976; Alley, 1987b). Exercise tolerance may be poor and animals may cough when more than 25% of the lungs is involved (Alley, 1991).

The relationship between acute fibrinous pneumonia and chronic non-progressive pneumonia is uncertain (Alley, 1987; Bruère and West, 1993). Chronic and subacute lesions may result from mild forms of acute pneumonia turning into chronic lesions. One could also hypothesise that acute pneumonias are a result of superinfections on chronic non-progressive pneumonia. Alternatively it may be that these types of pneumonia represent the extremes lesion types rather than distinct disease entities (Davies, 1985a).

Pleurisy in lambs should not be considered a separate disease entity. Since adhesions are likely to arise after exudation in pneumonia, pleurisy should be regarded as part of the pneumonia/pleurisy complex (McGowan *et al.*, 1978).

Pathology

In the case of acute fibrinous pneumonia, consolidation occurs in the lungs and the rest of the lungs is congested. Mainly the antero-ventral areas of the lungs are affected but other areas of the lungs can become involved depending on the severity of disease. Blue-grey areas are sometimes present in cranioventral lobes which indicate necrotic lobules. Fibrinous pleurisy can occur and the parietal and visceral pleura may become attached to each other. There is fibrinous exudate. The character of the exudate changes with the duration of the disease from clear and

yellow tinged in peracute cases to thick yellow sheets in cases of a few days duration (Alley, 1991; Bruère and West, 1993).

A considerable variety of lesions are seen in the cranioventral lobes of lungs which are affected by chronic non-progressive pneumonia. Initially there are likely to be dull red, sunken areas of collapse. Different shades of red and grey can be seen in consolidated lesions. If the animal is severely diseased the cranioventral parts of the caudal lobes may also be pneumonic (Alley, 1991).

There is no reason to regard pleurisy as a separate entity because adhesions are likely to arise after exudation in any type of pneumonia. McGowan *et al.* (1978) noted a rise in the prevalence of pleural lesions closely followed by a rise in the prevalence of lesions of chronic non-progressive pneumonia in a survey of lambs killed at slaughterhouses. The pleurisy lesions may stay once the pneumonia has been resolved. They can accumulate over time.

The extensive pleural lesions which follow acute pneumonia are likely to be more costly than the small ones in the front part of the chest cavity (Alley, 1991).

Micro-organisms

A wide variety of organisms (including viruses, mycoplasmas, bacteria and helminths) have been implicated in pneumonia. Some of these have not been described by workers in New Zealand. Pneumonia is not caused by any species of micro-organisms in isolation but rather by an interaction of these micro-organisms and risk factors which is currently incompletely understood.

Parainfluenza virus type 3 (PI3), ovine adenovirus type 6, bovine adenovirus type 7, respiratory syncytial virus, and an unidentified cell-associated agent are viruses that have been commonly associated with pneumonia in lambs in New Zealand. Uncomplicated natural infections may cause mild upper respiratory tract disease (Davies, 1987a). PI3 is the virus which has been most commonly isolated from sheep with pneumonia in New Zealand (Davies, 1985b). Infection with the virus results in reduced levels of bactericidal activity of neutrophils after 6 days. This is believed to make lambs susceptible to *P. haemolytica* infection. Davies and Humphreys (1977), and Adair *et al.* (1982) described two adenoviruses which were isolated from New Zealand lambs.

Davies and Jones (1985) demonstrated the existence of titres to respiratory syncytial virus in lambs and it has been suggested that the virus might be involved in some outbreaks of respiratory disease in lambs (Davies, 1987a).

Alley and Clarke (1979) demonstrated that *M. ovipneumoniae* can colonise the ovine lung and produce mild pneumonic lesions. Compared with a pneumonic lung homogenate, this homogenate would more often produce severe lesions. They suggested additional agents are required to cause lesions as caused by natural disease. Ionas *et al.* (1985) isolated *M.*

ovipneumoniae from 89% of lungs from lambs from one flock and 80% from another flock. They also isolated *M. arginini* from 12% of the lambs of one of the flocks at slaughter but not from the other flock. *M. ovipneumoniae* can be recovered from the nasal tract of sheep and from both pneumonic and apparently normal lungs (Mew *et al.*, 1985). They compared a number of isolates by using bacterial restriction endonuclease DNA analysis and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The DNA analysis showed all 16 isolates having substantially different patterns. The protein analysis showed that each of 8 isolates tested had a unique pattern, but the isolates had the majority of bands in common. This work revealed the heterogeneity of the organism and it was suggested that heterogeneity may be linked to differences in pathogeneity.

P. haemolytica was isolated in large numbers from the nasal cavity of 73% of normal sheep and 78% of pneumonic sheep at slaughter (Alley, 1975). It was also frequently recovered from the trachea (54%) and lungs (59%) of pneumonic animals but considerably less often from the trachea (5%) and lungs (6%) of normal sheep. *P. haemolytica* is associated with both acute and chronic forms of pneumonia in sheep. *P. haemolytica* is commonly believed to be the main cause of lung damage in pneumonia (Alley, 1991). The serotypes of *P. haemolytica* are defined by the capsular polysaccharide. There are 15 serotypes of *P. haemolytica*. The serotypes are the main factor for determining immunity, but antigens which are not part of the capsule may have an influence as well. There are two biotypes: A and T. Serotypes 1,2, 5, 6, 7, 8, 9, 11 and 13 of the A type were identified from nasal isolates during a survey in New Zealand (Prince *et al.*, 1985). The authors mentioned that it would not be surprising if A12 and A14 could be detected in a wider survey. Bacteria of the T serotype were not isolated. These bacteria colonise the tonsils and are associated with septicaemia rather than pneumonia.

A number of other micro-organisms have been associated with pneumonia in sheep (Table 2.1). Alley (1975) isolated *N. catarrhalis* more often from pneumonic trachea (22%) and lungs (22%) than from normal trachea (1.6%) and lungs (1%). Both *E. coli* and *Staphylococcus* were isolated from normal and pneumonic lungs and trachea. Davies (1987a) mentions the common occurrence of *P. multocida* in association with pneumonia. *Bordetella parapertussis* has been identified as a potential pathogen (Chen *et al.*, 1988).

Dictyocaulus filaria and *Muellerius capillaris* are parasites of the sheep lungs. The importance of these parasites with regard to the development of pneumonia appears questionable (Bruère and West, 1993).

Table 2.1 Micro-organisms reported to have been isolated from the ovine lower respiratory tract in New Zealand and overseas with their references

Organisms	Isolation overseas	Isolation in New Zealand
Viruses		
Parainfluenza type 3	St. George (1972)	Davies *et al.* (1983)
Ovine adenovirus type 6		Davies and Jones (1985)
Bovine adenovirus type 7		Davies and Jones (1985)
Six species of ovine adenovirus and untyped adenoviruses	cited in Davies (1985a)	
Respiratory syncytial virus	LeaMaster *et al.* (1983)	Davies and Jones (1985)
Unidentified cytopathogenic agent		Davies and Jones (1985)
REO viruses (3 types)	cited in Davies (1985a)	
Influenza type A-2	cited in Stevenson (1969)	
Contagious ecthyma	cited in Stevenson (1969) Robinson (1983)	
Sheep pox	cited in Stevenson (1969)	
Rickettsiae		
Coxiella burnetii	cited in Stevenson (1969)	
Mycoplasmas		
Mycoplasma ovipneumoniae	Jones and Gilmour (1983)	Alley and Clarke (1977)

Mycoplasma arginini	Jones and Gilmour (1983)	Ionas *et al.* (1985)
Acholeplasma laidlawaii, *Mycoplasma conjunctivae*, *Ureaplasmas*	Jones and Gilmour (1983)	
Mycoplasma mycoides ssp. *mycoides*	Robinson (1983)	

Chlamydiae		
Chlamydia psittaci	Robinson (1983)	

Bacteria		
Pasteurella haemolytica	Stamp and Nisbet (1963) Gilmour and Brotherston (1963)	Alley (1975) Alley and Clarke (1977)
Pasteurella multocida	Robinson (1983)	Davies (1985a)
Bordetella parapertussis		Manktelow (1984) Cullinane *et al.* (1987)
Branhamella catarrhalis *Neisseria catarrhalis*	Robinson (1983)	Alley (1975) Alley and Clarke (1977)
Branhamella ovis	Jones and Gilmour (1983)	
Francisella tularensis	Robinson (1983)	
Salmonella spp.	Robinson (1983)	
Staphylococcus spp.	Robinson (1983)	Alley (1975)
Staphylococcus aureus		Alley and Clarke (1977) Thurley *et al.* (1977)
Streptococcus		Alley (1987a)
Streptococcus zooepidemicus	Stevenson (1974) cited in Gilmour (1978)	
Pseudomonas sp.		Alley (1987a)
Pseudomonas aeruginosa	cited in Stevenson (1969)	
Pseudomonas pseudomallei	cited in Stevenson (1969)	

Fusobacterium necrophorum		Alley (1987a)
Escherichia coli	Robinson (1983)	Alley (1975)
Corynebacterium pyogenes (*Actinomyces pyogenes*)	St. George (1972)	Alley (1987a)
Mycobacterium avium, *M. bovis* and *M. tuberculosis*	cited in Stevenson (1969)	
Actinobacillus lignieresi	cited in Stevenson (1969)	
Haemophilus spp. *Histophilus ovis*	cited in Stevenson (1969)	

Fungi	
Aspergillus fumigatus	cited in Stevenson (1969)
Cryptococcus	cited in Stevenson (1969)
Nocardia	cited in Stevenson (1969)

Micro-organisms in the upper respiratory tract

Alley (1975) isolated α -haemolytic *Streptococcus*, *Actinobacillus*, micrococci and *Bacillus* from nasal cavities. Malone *et al.* (1988) isolated a number of species from the nasal mucus and nasal swabs. They included *Mycoplasma ovipneumonia*, *Moraxella* ssp., *P. haemolytica*, *Staphylococcus albus*, *Bacillus* ssp., Haemolytic *staphylococcus*, non-haemolytic *E. coli*, PI3 virus, *Neisseria/Moraxella* ssp., *Staphylococcus pyogenes*, *Streptococcus* spp., *E. coli*, *Mycoplasma arginini*, *Acinetobacter* ssp., *Branhamella* ssp. *Micrococcus* ssp., *Pseudomonas* ssp. Queen *et al.* (1994) identified 93 bacterial isolates from nasal and tonsillar samples from ten clinically healthy domestic sheep. The organisms were: *Bacillus* spp., *Staphylococcus aureus*, *Staphylococcus sciuri*, *Staphylococcus simulans*, *Staphylococcus warneri*, *Staphylococcus xylosus I*, *Staphylococcus xylosus II*, Non-speciated *Staphylococcus*, Non-speciated *Streptococcus*, Non-speciated *Acinetobacter*, *Actinobacillus actinomycetemcomitans*, *Aeromonas hydrophyla*, Non-speciated *Aeromonas*, *Enterobacter agglomerans*, *Enterobacter cloaca*, Non-speciated *Enterobacter*, *Neisseria denitrificans*, Non-speciated *Neisseria*, *Moraxella (Branhamella) ovis*, *Pasteurella haemolytica* T, *Pasteurella haemolytica* 3 and *Salmonella arizonae*. The findings of these authors were important since these organisms are likely to be found in lungs of sheep that have been compromised by pneumonia.

Risk factors

Pneumonia is considered a multifactorial disease which is caused by the interaction of a wide variety of factors (Jensen and Swift, 1982). They include the causative micro-organisms (viruses, mycoplasmas and bacteria), the external environment of the animal (which includes farm management practices and the weather), and the immune status and general health of the animal. Below are discussed a number of the risk factors which have been identified in the literature and by expert opinion, although the body of research evidence supporting some of them is quite limited. It should be noted that the word "risk factor" has been used in this thesis in the sense which is common in epidemiology. It is not intended as a term with connotations to human health risk.

The nose plays a role in the defence mechanism of the body in that it warms and humidifies incoming air (Baskerville, 1981). If the animal is exerted and starts mouth breathing this defence mechanism is by-passed. Smith (1975) advises that driving of lambs should not take place over long distances too quickly. Temporary yards can be used to reduce the time that the animals are mustered. Mustering by motor bike may make the animals move too fast. In a similar vein one could expect that dogs that are inadequately controlled can contribute to the disease.

Manktelow (1970) suggests that yarding and mustering under dusty conditions is harmful to the animal. Recommendations have also been made to use concrete yards rather than dirt yards. If dirt yards are used it has been recommended to use a sprinkler system to dampen them down before animals are yarded. Smith (1975) regards the close confinement of stock in the yards as a negative risk factor. The time animals spend in the yards has been considered a negative factor as well.

Both cold and warm weather conditions have been considered of relevance. McIlroy *et al.* (1988) describe the use of linear modelling techniques including time series analysis. They detected a pattern with maximum lung condemnations occurring in the spring and the minimum in summer. The most important variable they found was the rain chill factor with a lag of two months. Alley (1991) commented that these findings may not be directly attributed to the weather but perhaps to other factors such as sheep huddled together. Cold shock following shearing may result in pneumonia (Bruère and West, 1993). Mustering and yarding under hot conditions may result in more mouth breathing. Robinson (1983) suggests that persistent heat stress may decrease resistance and predispose to respiratory infections. He considered shearing animals and providing adequate shade and water important. It has been recommended not to muster sheep during the heat of the day. Smith (1975) emphasises that a poor water supply is not acceptable and may increase the chance of infection.

Poor parasite control and/or undernutrition may increase susceptibility to pneumonia (Davies, 1985b). However drenching may also have negative aspects. Alley (1991) suggested that drenching procedures should be designed to avoid hand to nose contact of whole mobs, and Robinson (1983) mentions the importance of equipment functioning properly and using a correct drenching technique. The use of both shower dips and plunge dips has been associated with pneumonia. Robinson (1983) comments that pneumonia can follow inhalation of fluids during

dipping or spraying for ectoparasite control. In addition one could hypothesise that drenching and dipping procedures might be stress factors in their own right. The perceived role of *Dictyocaulus filaria* has been questioned by some (Bruère and West, 1993).

The environment in which the animals reside is important. Robinson (1983) considers respiratory disease a more significant problem in housed animals than those not housed continuously. Care should be taken that ventilation or air exchanges are adequate. The same would be true for the shipment of live sheep.

The mixing of animals at sale yards or shows followed by long transportation is deemed an important risk factor for respiratory disease by Robinson (1983). He recommends that animals be isolated for 2 to 3 weeks after transportation so that sick animals can be detected and treated. It would also reduce the risk of transmitting disease to stock that has been on the farm for a longer period.

Facial eczema has been mentioned by Sorensen (1976) as a likely stress factor.

McGowan *et al.* (1978) remark that the increasing prevalence of enzootic pneumonia-pleurisy complex in the lamb population as the season progresses may be a function of increasing exposure with age, without any change in the intensity of risk factors.

A number of the above risk factors such as the necessity to avoid yarding and mustering under dry, dusty conditions are currently widely accepted. Other risk factors such as the relevance of lungworm are often questioned by authors.

Vaccination and treatment

In the past vaccines have generally been of limited use. There were several possible reasons for this. PI3 was targeted in some vaccines while other viruses could still initiate the pneumonia. Although *P. haemolytica* is required for the disease, the variety of types reduced the efficacy of vaccines. A vaccine against one type of *P. haemolytica* does not necessarily work against another one. A different approach was taken by making a vaccine which was designed to stimulate antitoxin activity (Davies, 1987b). The protection was not serotype specific. The various vaccines that have been developed over the years illustrate the importance that is placed on immunity to prevent disease, although vaccines are not yet widely available in New Zealand or used, because research has so far failed to produce an adequately protective vaccine.

The treatment of individual ewes and lambs or hoggets with various antibiotics has generally been unsatisfactory (Bruère and West, 1993). The short clinical course of acute pneumonia makes treatment unlikely to be successful although preventative treatment of in-contact animals may still be economic in small flocks. Penicillin, ampicillin or oxytetracyclin can be administered although not all strains of *P. haemolytica* are sensitive to penicillin and ampicillin (Gilmour and Gilmour, 1989). The treatment with long acting oxytetracycline prevented lambs

that had been infected developing pasteurellosis (Gilmour *et al.*, 1988; Gilmour and Appleyard, 1989). Antibiotics prevented the transmission of chronic non-progressive pneumonia if administered parentally on the day of challenge (Alley and Clark, 1980).

Post mortem inspection and processing

All carcasses in abattoirs and meat export plants are inspected for pneumonia and pleurisy. Pleurisy in particular is a problem since all affected carcasses are diverted to the detain rail to have the lesions removed (Dysart, 1976). It is second only to contamination as far as reasons for retaining carcasses for trimming are concerned. In addition to this extra work, the disease may lead to downgraded and condemned carcasses. The extensive adhesions after acute pneumonic outbreaks are likely to be far more costly than the smaller adhesions associated with the chronic non-progressive lesions (Alley, 1991). In recent times it has become possible to save lungs for human consumption in New Zealand. As a result pneumonic lungs have become more important to the processing industry than in the past. Reduction of pleurisy and pneumonia in lambs would be beneficial to the meat processing industry. Since reduction and prevention of the disease, ie practices on the farm, are the key to increased profitability of meat processors as well as farmers, feedback of meat inspection findings is required to assist farmers in monitoring the prevalence of disease in their flock.

Pleurisy can be used as a model for food safety programs designed to return information to producers. The advantages are that a number of risk factors have already been established, at least provisionally. Some of these factors are related to farm management. Animals from many farms can be evaluated for the disease during routine meat inspection. The lesions can be detected by inspecting the carcass which makes it much cheaper than research involving culturing of bacteria. Only a small percentage of carcasses with pneumonia will show pleurisy. However, a similar problem may also arise with research into pathogens of human health significance where only a small percentage of carcasses from a farm can be sampled. Assumptions on the prevalence of a disease will be based on a limited number of animals from that farm. Pleurisy could be a result of chronic non-progressive pneumonia or of acute fibrinous pneumonia. The relationship between these two forms of pneumonia and the occurrence of downgraded carcasses is not clearly defined. The challenge posed by these issues will aid in more clearly defining restrictions for more effective use of slaughterhouse data.

Hazard Analysis of Pneumonia and Pleurisy in Lambs with Regard to Public Health

Introduction

The objective of this section is to review micro-organisms which have been associated with pneumonia and pleurisy in sheep with regard to their pathogenic potential in humans. Criteria which can be used for such an evaluation will be described and the micro-organisms will be assessed against these criteria as appropriate for each organism individually. Finally conclusions will be drawn as to the value of meat inspection of lungs and pleura to prevent human disease.

There are a number of micro-organisms that can commonly be found in the respiratory tract of ovines. They may predominate in the upper respiratory tract of healthy animals with few of them in the lower respiratory tract. However large numbers may be found in the lower respiratory tract if the animal has pneumonia. The occurrence of bacteria in the lower respiratory tract does not constitute proof of being a causative organism of pneumonia. Invasion of tissues may occur if weakened by disease.

Under current processing conditions meat and offal will contain micro-organisms after dressing. In the case of lungs, they may have been applied as contamination by meat handlers or they may have been present already. The ability of people to withstand a challenge by a micro-organism will depend on the micro-organism itself, the dose rate and the immunological and constitutional status of the person. A proportion of the micro-organisms that are usually harmless, can behave as pathogens if certain conditions are met. An example of such an opportunistic pathogen is *Pneumocystis carinii* in AIDS patients. In other cases the pathogenic potential of an organism may not be known yet. Certain micro-organisms are part of the ecological micro-flora of a tissue. People who eat this tissue regularly, will frequently be exposed to this micro-organism. A change in their immune status, the integrity of their skin or mucosal membranes or their general health status can provide an opportunity for the micro-organism to cause harm to the person.

For the purposes of this evaluation, if a micro-organism is described in both humans and in sheep and if no further classification is used, it is assumed to be one and the same organism. The possibility exists that some micro-organisms which are isolated from both man and sheep belong to different strains which do not cross the species barrier. Proof of this may be difficult. Serotyping or DNA analysis are able to differentiate between strains at times.

Materials and methods

Classification as food-borne zoonotic agent

The classification of the pathogenic potential of a micro-organism may differ depending on the

intended use of this classification. To evaluate the zoonotic potential of micro-organisms the questions below are pertinent.

1 Has the micro-organism been reported to occur in both sheep and humans?
2 Has the strain, subtype or serotype been reported to occur in both sheep and humans?
3 Is the organism/strain known to be a zoonotic pathogen?
4 Is the organism/strain known to cause food borne disease?
5 Does the organism/strain occur in both healthy and diseased sheep?
6 Does the organism/strain occur in the tissues that are ingested?
7 Does the organism/strain occur in higher densities in diseased than in healthy sheep?
8 Does the organism/strain occur in both healthy and diseased humans?
9 Does the organism/strain occur in higher densities in diseased than in healthy humans?
10 Are all people (eg >95%) exposed to the organism/strain early in life (eg <2 years of age)?
11 Do exposed people develop immunity to the organism/strain?
12 Is the organism/strain associated with disease in immuno-compromised people only?
13 Can the organism/strain be found and survive in the environment for extended periods?
14 Are there spatial and temporal aspects to the occurrence of the organism/strain?

Based on the answers some immediate conclusions may be drawn. There may be generally acknowledged pathogens or there may be organisms of no concern.

From a practical perspective it needs to be decided whether an organism should be addressed by a food safety programme or whether it should be ignored. For example *Campylobacter jejuni.* and verotoxigenic *E. coli* warrant concern while *Sarcocysts* ssp. in New Zealand sheep and cattle can be ignored. There are a number of micro-organisms where this decision is more difficult. This applies especially to those micro-organisms which are likely to cause disease if certain unfavourable conditions are met and those which occur infrequently.

The guidelines below are based on the concept that food safety need not address micro-organisms unless they have been described in the medical literature as causing disease in humans. Therefore as knowledge becomes available, certain organisms may be reclassified.

The assumption is also made that safety of product only applies:

- to people with fully functional natural defence mechanisms,
- for product which has not been subjected to temperature and/or other handling abuse,
- for product which has been prepared in accordance with conventional practices.

The concept of people with fully functional natural defence mechanisms should not be construed as a judgement of the value of life or of groups of people. It merely reflects reality that it is currently impossible to produce fresh meat that is indisputably safe for all people. Micro-organisms that can cause disease in the largest proportion of the population are considered the highest priority for general food safety programmes. At-risk groups will benefit from this too. Food safety programmes should address the needs of people at increased risk by identifying these

groups and providing advice as to how they can prevent disease in the most effective way. For instance food eaten by such people should be handled with extra care such as thorough cooking and certain foods should not be eaten or handled by them at all. Specific additional food safety services can be provided to such groups as a special case, beyond standard programs.

Criteria for deciding whether or not organisms should be included in a general food safety programme are listed in Table 2.2. As more information on the relationship between micro-organisms and human disease becomes available, the criteria can be amended.

Table 2.2 Criteria for including micro-organisms in or excluding from food safety programmes

Include micro-organisms in food safety programmes	Exclude micro-organisms from food safety programs
If they can cause disease in people who do not belong to an exceptionally high risk group.	If isolation from a human being has not been recorded in the literature.
This can be either relatively immediate or as sequelae detected a long time after the infection.	If it is considered to be a commensal to humans but occasionally becomes an opportunistic pathogen.
If there is a recorded suspicion of causing disease in people who do not belong to an exceptionally high risk group, at any time during or after the infection.	If it occurs in humans at a young age, without producing disease, and providing subsequent immunity.
	If it causes disease in people from exceptionally high risk groups only.
	If it has never been isolated from diseased persons and it has not been linked with causing delayed disease.
	If it is not believed to occur in a specific geographical area.

Interpretation of the literature

Descriptions of micro-organisms in the literature need to be interpreted with caution. Some organisms such as Parainfluenza type 3 have been isolated and described by many workers. Their existence in lungs can be accepted as commonly occurring. Other micro-organisms may have been isolated and described on a small number of occasions only. This may reflect that certain extraordinary events led to infections of sheep or humans with a very low probability of occurring. It is also possible that the culture may have been the result of contamination, or of incorrect identification in a laboratory. In conclusion there should be sufficient evidence from

various sources (both in the veterinary and the medical literature) to support the hypothesis that a specific micro-organism is a pathogen. Especially in the case of some older publications which have not been confirmed subsequently one should question their validity. On the other hand a recent publication with a 'newly' discovered organism in the lungs should lead to an intensified monitoring of publications for this organism.

Some micro-organisms may have been isolated from humans in certain geographical areas only. It should however be noted that at times micro-organisms are identified which people acquired in areas they visited some time ago. Examples are tourists and people working abroad. On the other hand it is also possible that certain micro-organisms have not been isolated because laboratories in a certain area may not perform tests for certain organisms.

The comments in the following sections are based on textbooks, articles and searches of the CD ROM database of the Commonwealth Agricultural Bureau. In the case of human disease consistent use has also been made of Benenson (1995) and Greenwood *et al.* (1992). The progressive pneumonia types do not occur in New Zealand and they are not discussed. Helminths have not been included either.

Results

Below comments are made on the organisms that are known to occur in the ovine lower respiratory tract. The type of comments vary from organism to organism and provide an insight into their public health significance. The conclusions at the end of each description indicate whether or not the organism should be considered important or not from a New Zealand food safety perspective. It should be noted that no attempt has been made to provide a comprehensive list of reasons for excluding micro-organisms as potential pathogens in New Zealand. For instance if an organism is not known to occur in New Zealand sheep, no further descriptions of its health risk are required to consider it unimportant from a New Zealand meat inspection and public health perspective. It should be noted that the organisms which are commonly found in the respiratory tract may be more likely to be transmitted by aerosols from animal to animal than by ingestion. The same consideration may at times apply regarding the transmission of micro-organisms in the ovine respiratory tract being transmitted to humans.

Actinobacillus lignieresi

A. lignieresi occurs rarely in New Zealand sheep and is associated with a purulent disease affecting soft tissue of the head and neck (Manktelow, 1984). Bisgaard *et al.* (1986) described the taxonomic difficulties regarding *A. lignieresi*, and reclassified some strains as *P. multocida*, *P. haemolytica* or as having phenotypic characters compatible with *Pasteurellaceae*. *A. lignieresi* is not described by Benenson (1995) or Greenwood *et al.* (1992) as a human pathogen.

Conclusion: *A. lignieresi* is not of importance as a zoonosis.

Actinomyces (Corynebacterium) pyogenes

St. George (1972) isolated *C. pyogenes* from pneumonic sheep. Alley (1975) isolated *Corynebacteria* from nasal cavities of normal and diseased sheep, and from normal lungs. He did not isolate *Corynebacteria* from normal trachea and from pneumonic trachea or lungs. Disease in humans as a result of this organism is not described in Benenson (1995) and Greenwood *et al.* (1992). However Hillin and Moncla (1991) describe that *Actinomyces pyogenes* can cause disease in humans. Since the normal habitat of *A. pyogenes* appears to be the oral cavity of humans and animals they consider this organism an opportunistic pathogen.

Conclusion: This species does not require specific attention from a food safety perspective.

Adenovirus

Madeley and Peiris (1992(a)) state that adenoviruses are endemic and a number of types spread readily among the population. The presumed way of infection for some types is by droplets but faecal-oral transmission also occurs They are of the opinion that generally vaccines are not required because of the many types, generally mild outcome and their endemic occurrence. The *Mastadenovirus* genus contains the mammalian adenoviruses. White and Fenner (1986) comment that the viruses in this genus are mammalian host specific.

Conclusion: Adenoviruses are specific for particular mammalian hosts and therefore the adenoviruses from the ovine respiratory tract are not relevant from a food safety perspective.

Aspergillus fumigatus

Aspergillus fumigatus is a common cause of aspergillosis in man. Especially patients on cytotoxic or immunosuppressive therapy are at risk. Since *Aspergillus* spp. are ubiquitous and the disease is usually a secondary infection healthy persons are considered to be resistant to a high degree. The mode of transmission is by airborne route (Benenson, 1995). Blackmore and Humble (1987) describe this organism as a non-zoonotic disease common to animals.

Conclusion: *Aspergillus fumigatus* occurs in man, animals and the environment. *Aspergillus fumigatus* from the respiratory tract of sheep does not appear to require specific attention from a food safety perspective.

Bordetella parapertussis

The disease caused by *Bordetella parapertussis* is not as severe as the one caused by *Bordetella pertussis*, which causes serious respiratory childhood disease (Preston, 1992). *B. parapertussis* is uncommon in most countries, but at times it causes outbreaks of whooping cough. Transmission is described as from person to person. Discharges from the (human) respiratory tract are considered responsible for transmission of the disease. *B. parapertussis* is commonly

isolated from pneumonic sheep lungs (Manktelow, 1984). Cullinane *et al.* (1987) isolated *B. parapertussis* from lambs with chronic non-progressive pneumonia and from healthy lambs. The relationship between ovine strains and human strains requires further clarification.

Conclusion: Food does not appear to have implicated as a reservoir for *B. parapertussis*.There does not appear to exist epidemilogical data suggesting a link of human disease with food ro animal handling.

Branhamella catarrhalis and *B. ovis*

B. catarrhalis (formerly *Neisseria catarrhalis* and now sometimes called *Moraxella catarrhalis*) is considered a common commensal of the upper respiratory tract (Fallon and Slack, 1992). It can cause disease of the lungs when the host defences are compromised. The organism may also be associated with conjunctivitis. Alley (1975) isolated this organism from both healthy and pneumonic animals. The percentage affected lungs in pneumonic sheep was considerably greater than in normal sheep.
B. ovis is not specifically mentioned by Benenson (1995) or Fallon and Slack (1992)

Conclusion: *B. catarrhalis* is a commensal that may cause disease in humans with health problems. The disease is of a respiratory nature which tends to exclude a food-borne origin. Both species do not seem to warrant attention from a food safety perspective.

Chlamydia psittaci

Chlamydia psittaci in humans is commonly associated with diseased birds. Infection usually occurs by inhalation. Person-to person transmission of the disease is rare (Benenson, 1995). Manktelow (1984) describes *Chlamydia psittaci* as the causative organism of infectious keratoconjunctivitis in sheep. The occurrence is common and up to 100% of a flock can be affected. Asymptomatic carriers exist and dry, dusty conditions may be predisposing factors. Manktelow does not mention lesions of the lower respiratory tract. Blackmore and Humble (1987) state that only strains causing psittacosis in birds and enzootic abortion in sheep are zoonoses. This abortion did not occur in New Zealand. Stanislawek and Thompson (1995) confirmed there was no evidence of the existence of the abortive strain of *C. psittaci*.

Conclusion: This organism does not seem to warrant attention from a food safety perspective.

Contagious pustular dermatitis

Benenson (1995) states that lesion are usually located on hands, arms and face but disseminated disease has been reported. Darbyshire (1961, cited in Stevenson 1969) describes a haemorrhagic pneumonia with necrotic foci in sheep. Contagious ecthyma which can occur on the fleece of sheep is a public health risk for people handling and dressing infected sheep. The average prevalence is 13% of infected lines of sheep at slaughter (Manktelow, 1984). Neither Manktelow

(1984) nor Bruère and West (1993) mention pulmonary lesions.

Conclusion Contagious pustular dermatitis is an organism which is of importance from a public health perspective. It does not appear to be likely that sheep lungs cause food-borne disease in humans.

Coxiella burnetii

Stevenson (1969) reports one article regarding *Coxiella burnetii* in sheep. *Coxiella burnetii*, the cause of Q-fever, does not occur in New Zealand.

Conclusion *Coxiella burnetii* does not warrant attention from a food safety perspective in New Zealand.

Cryptococcus

Cryptococcus has been diagnosed in a Merino wether in Queensland (Laws and Simmons, 1966, quoted in Stevenson 1969). Sporadic cases of *Cryptococcus neoformans* occur worldwide. The organism grows as a saprophyte in the environment and 5% - 10% of AIDS patients in the USA have developed Cryptococcosis (Benenson, 1995). Manktelow (1984) states that infections have been seen in humans, cats and dogs in New Zealand. It was believed that all acquired the disease from the environment.

Conclusion *Cryptococcus neoformans* is not of importance from a food safety perspective.

Escherichia coli

E. coli was isolated by Alley (1975) from 1% of pneumonic sheep lungs (2 isolations). He also isolated *E. coli* from 0.5% of normal lungs (1 isolation). There was also a description in 1987 (Alley, 1987a). No information of strains is available.

Conclusion: *E. coli* can occur both in pneumonic and normal lungs. The additional percentage of *E. coli* in pneumonic lungs is small. Since *E. coli* strains are common and their pathogenicity varies to a large degree, more information on this organism, especially in the case of chronic non-progressive pneumonia would be helpful.

Francisella tularensis

Francisella tularensis has not been reported in New Zealand by Manktelow (1984) or by Bruère and West (1993).

Conclusion: *Francisella tularensis* does not seem to warrant attention from a New Zealand

food safety perspective.

Fusobacterium necrophorum

F. necrophorum is not mentioned in Benenson (1995). However Bergey's manual of determinative bacteriology (Buchanan *et al.*, 1974) states that *F. necrophorum* can be detected in the natural cavities of man and other animals. In addition it can also be isolated from necrotic lesions, abscesses, and blood of man and other animals, particularly liver abscesses of cattle and pigs. Hardie and Shah (1992) state that *F. necrophorum* is regarded as an important animal pathogen. They do not mention human disease. Jousimies-Somer and Finegold (1991) describe *F. necrophorum* as an organism that may cause severe disease originating from pharyngotonsillitis

Conclusion: *F. necrophorum* may both occur in humans and in animals but it does not appear to be important from a zoonotic perspective.

Haemophilus somnus

Cheema *et al.* (1965, cited in Stevenson, 1969) isolated *Haemophilus* ssp. from pneumonic ovine lungs. Leamaster *et al.* (1983) observed that all adult sheep from one flock had a high seroprevalence of *H. somnus*. Stephens *et al.* (1983) established there was a close phenotypicc relationship between *H. somnus*, *H. agni* and *Histophilus ovis*. Howard (1992) mentions disease in humans caused by *H. influenzae*, *H. ducreyi* and *H. aegyptius*. He also mentions that various other species of *Haemophilus*, notably *H. parainfluenzae*, *H. aphrophilus* and *H. haemolyticus* are occasionally isolated from diseased humans.

Conclusion: There is no evidence to regard *H. somnus* (*H. ovis*) a human pathogen.

Influenza type A-2

Stevenson (1969) reported that the isolation of Influenza type 2-a by Romváry (Magy. Allatorv. Lap 17, 323-327) was the only isolation to date.

Conclusion Influenza type A-2 is not required to be included in a food safety programme .

Mycoplasmas

Mycoplasma ovipneumoniae and *Mycoplasma arginini* are the two most commonly reported mycoplasmas of the ovine respiratory tract. These two species of mycoplasmas, *M. conjunctivae*, and *M. mycoides* ssp. mycoides have not been reported by Taylor-Robinson (1992) or Benenson (1995) as occurring in human beings. Since the antigens of bovine, feline and avian *ureaplasmas* are different from those of the human strains, they have been placed in separate species (Taylor-

Robinson, 1992). *A. laidlawii* is rarely isolated from the human respiratory tract. Only *M. pneumoniae*, *M. hominis* and *U. urealyticum* unequivocally cause disease in humans.

Conclusion: The above tends to indicate that *Mycoplasmas* from the ovine respiratory tract are not likely to cause disease in humans.

Mycobacteria

References to the isolation of *Mycobacterium tuberculosis*, *M. bovis* and *M. avium* are reported in Stevenson (1969). Detailed post mortem inspection of New Zealand lambs did not reveal any tuberculosis. *M. bovis* lesions have been isolated from adult sheep (Cordes *et al.* 1981). Epidemiological evidence linking human tuberculosis to the consumption of meat seems to be lacking. Pasteurisation of milk lead to a reduction of human tuberculosis in many countries.

Conclusion: There is no firm evidence to link human disease to meat or offal consumption.

Nocardia

Nocardiosis is a sporadic disease in man and animals. The organism occurs in soil and is not considered directly transmissible from animals to man. Immuno-compromised people are at risk of opportunistic infections (Benenson, 1995). Stevenson (1969) mentioned that the article by Afzal, Ilahi, Ayaz & Sarwar (1966), *Pakist. J. Anim. Sci.* (1963/4), 33-360, was the only record of an infection in sheep.

Conclusion Nocardia is not of importance from a food safety perspective.

Parainfluenza type 3

Four types of parainfluenza viruses with antigenically distinct epitopes occur in humans. People are infected by inhalation and by person-to-person contact. Other parainfluenza viruses which are pathogens for cattle and other domestic species are not known to infect man (Madeley and Peiris, 1992(b)). White and Fenner (1986) describe that Parainfluenza 1 and 2 occur as autumn epidemics while Parainfluenza 3 and 4 occur endemically throughout the year.The age of acquiring Parainfluenza 1 and 2 infections is between 6 months to 5 years, and Parainfluenza 3 during the first 6 months. Parainfluenza 4 was described as occurring in children. Arguedas *et al.* (1990) suggest that disease of systems other than the respiratory tract is not commonly detected and report two immunocompromised children with neurological disease. Knott *et al.* (1994) also mentioned the existence of the disease in a number of cases with minimal or no respiratory signs.

Conclusion: There is no evidence that PI3 infections spread from animals to people. In addition the infections are so common in both animals and humans that trying to prevent the transmission of animal PI3 viruses to people seems futile.

Pasteurella haemolytica

Kaehler *et al.* (1980) provided evidence of species specificity in the cytocidal effects of *P. haemolytica*. *P. haemolytica* is apparently non-pathogenic for humans (Coghlan, 1992).

Conclusion: *P. haemolytica* is not of relevance from a food safety perspective.

Pasteurella multocida

P. multocida is rare in humans. Local cellulitis as a result of animal (cat or dog) bites is the most common disease manifestation of *P. multocida* in humans. Meningitis following head injury can occur. *P. multocida* may contribute to the severity of infections of the respiratory tract and *P. multocida* isolation in cases of appendicitis have been reported. *P. multocida* can be isolated from the nasopharyngeal region of many species. These animals can be healthy but some may also be septicemic or suffering from respiratory disease. (Coghlan, 1992). Beyt and Roosevelt (1979) stated that between 1946 and 1977 pulmonary infections with *P. multocida* have been reported more frequently in the medical literature. Men with an average age of 50 years with a clinical diagnosis of bronchiectasis were the most common group of patients.

Conclusion: *P. multocida* occurs in both healthy and diseased animals and is a zoonotic agent. The most common mode of transmission appears to be by bite wounds. Aerosols have been implicated too. People with impaired defence mechanisms seem to be at an increased risk of developing respiratory problems. The chance of healthy humans developing a food-borne *P. multocida* infection seems small and does not seem to warrant attention from a food safety perspective.

Pseudomonas aeruginosa

Ps. aeruginosa is described as an important agent for causing mastitis in cattle in New Zealand (Manktelow, 1984). *Ps. aeruginosa* is an opportunistic pathogen (Govan, 1992). A variety of disease conditions in humans have been described since it can infect almost any site and organ. *Ps. aeruginosa* is a saprophyte. Healthy human carriers can have the strain in the gastro-intestinal tract. Stiles (1989) states that *Ps. aeruginosa* can cause food-borne disease but that specific attention of its presence for healthy people does not seem justified.

Conclusion: Specific attention for this opportunistic pathogen does not seem warranted.

Pseudomonas pseudomallei

Pseudomonas pseudomallei does not occur in New Zealand

Conclusion *Pseudomonas pseudomallei* does not seem to warrant attention from a New

Zealand food perspective.

Reovirus

Orthovirus (reovirus 1,2,3), Orbivirus and Rotavirus are three genera of the Reovirus family which are pertinent to humans (Desselberger, 1992). Most people are exposed to reoviruses early in life and develop antibodies. The tree human serotypes of orthoreovirus are widespread in virtually any species of animal that have been carefully searched (White and Fenner, 1986). Davies (1985) states that only type 1 has been associated with ovine respiratory disease although all three reovirus serotypes have been isolated from sheep.

Conclusion The widespread occurrence in both animals and humans combined with the lack of evidence that animal REO viruses cause disease in humans tends to indicate that they do not warrant concern from a food safety perspective.

Respiratory Syncytial Virus

RSV will have infected most children by the age of 2 years (Talis and McIntosh, 1991). Immunity is acquired by infection but it is not complete and reinfections occur. RSV infections can result in a patient requiring hospitalisation. There are large outbreaks of RSV in humans every winter and RSV is the most common nosocomial infection on pediatric wards. Talis and McIntosh (1991) mention that human RSV can infect several simian and small animal species but infections of sheep by humans and *vice versa* are not mentioned.

Conclusion: RSV is a ubiquitous organism. If animal strains are capable of infecting humans, then this route of infection is of minor importance compared to the route of infection from other infected children. RSV in sheep does not seem to warrant attention from a public health perspective.

Salmonella

Robinson (1983) mentions the involvement of *Salmonella* ssp. in acute ovine pneumonia. Blackmore and Humble (1987) stated that the prevalence of asymptomatic infections in sheep is probably in the region of 1-5%. Information on the prevalence of *Salmonella* ssp. in both healthy and pneumonic lungs of New Zealand is not available. Manktelow (1984) lists gastro-enteritis, septicaemia and rarely abortion as signs of Salmonellosis in New Zealand sheep. Bruère and West (1993) list moderate to severe congestion of the lungs as a necropsy feature. *Salmonella* ssp. are not usually listed as pathogens associated with enzootic pneumonia in New Zealand.

Conclusion: Many *Salmonella* ssp. are of public health concern. The role of healthy and pneumonic lungs in causing human disease under New Zealand conditions cannot be fully evaluated from the data that have been sighted. The high rate of asymptomatic carriers of

Salmonella in the gastro-intestinal tract which can cause carcass contamination appears to be more important than the ocasional lung that may be infected.

Sheep pox

Sheep pox does not occur in New Zealand and is not considered a zoonotic disease.

Conclusion: Sheep pox does not require attention.

Staphylococcus

Alley (1975) isolated *Staphylococcus* from the nasal cavities, the trachea and the lungs of both healthy and pneumonic sheep. One percent of both the normal and the pneumonic sheep were positive. A larger percentage of pneumonic sheep (4%) had *Staphylococcus* than normal sheep (1%). Blackmore and Humble (1987) state that some animal strains of *S. aureus* are enterotoxigenic and have infrequently been associated with food poisoning in man. They consider this a non-zoonotic disease common to animals and man.

Conclusion: Both normal and pneumonic sheep have a low percentage of *Staphylococci*. Attention to pneumonic lungs for *Staphylococci* does not seem warranted especially in the light of the infrequent disease caused by animal *Staphylococci*.

Streptococcus ssp.

Alley (1975) isolated α-haemolytic *Streptococci* from nasal cavities of normal and diseased sheep, and from normal lungs. He did not isolate α-haemolitic *Streptococci* from normal trachea and from pneumonic trachea or lungs. Some α-haemolitic *Streptococci* are of public health concern..

S. zooepidemicus is associated with neonatal infections on lamb, where they can cause pneumonia and pleurisy. Outbreaks of human pharyngitis with nephretic sequela were attributed to unpasteurised milk (Barnham *et al.*, 1983)

Conclusion: α-haemolytic *Streptococci* occur in healthy sheep lungs. Pneumonic lungs need not pose a greater risk of containing *Streptococci.* However more information on *Streptococcus* ssp. in ovine lungs would be helpful in verifying the validity of the conclusion.

Discusssion

In the case of pneumonia and pleurisy, lesions occur which may be unacceptable to the consumer and the detection and supervision of removal can be considered one function of meat inspection.

Another function of meat inspection is the protection of the public health. The above hazard analysis has shown that many species of ovine respiratory pathogens are not likely to be of public health significance. A risk assessment approach with scenario sets and quantitative data is required to evaluate in detail the risk of pneumonic lungs and pleurisy to the public.

Usually pneumonia in sheep is caused by a small number of bacterial, viral and mycoplasmatic species, ie *P. haemolytica*, PI3, ovine Adenovirus type 6, bovine Adenovirus type 7, Respiratory Syncytial Virus and *M. ovipneumoniae*. There does not appear to be evidence that these organisms are pathogenic for healthy people. These findings are in agreement with Blackmore and Humble (1987). Also Stiles (1989) does not mention these common pathogens in his discussion of less recognised or presumptive foodborne pathogenic bacteria. Furthermore these organisms can also be found in healthy ovines. Especially the work by Alley (1975) which compared pneumonic with normal lungs was enlightening in this regard. On a number of occasions other species can be isolated from diseased lungs as well. As is the case with the commonly isolated species, some of these organisms can also be isolated from healthy sheep lungs.

The above hazard analysis identified a number of species which might be of public health significance. *Salmonella* ssp. and *E. coli* are examples of two species known to contain foodborne pathogens (Doyle, 1989). Their significance should be seen in the light of the probability of detection and the risk of cross-contamination. A meat inspection programme should be an integral part of a food safety programme. Such a programme should ideally be assessed on the number of diseased people as a result of food borne disease and in this regard palpation and subsequent removal of gross pathology may not necessarily have an overall positive effect. As an example, Alley (1975) described that 1% of pneumonic lungs contained *E. coli* and 0.5% of normal lungs contained *E. coli*. If it is assumed for the purpose of the argument that the *E. coli* isolates were all pathogens, that the sensitivity of the laboratory test was 100% and that the percentages apply to the whole of the sheep population.Then in a population of 1000 lambs with 10% pneumonia there will be 1000 * 0.1 * 0.01 = 1 pneumonic lamb with pathogenic *E. coli* and 1000 * 0.9 * 0.005 = 4.5 healthy lambs (no rounding is used in this example) with pathogenic *E. coli*. The sensitivity of detecting pathogenic *E. coli* by using gross pathology as a test would be 1/5.5 = 18.8%. It should also be considered that the sensitivity of detecting pathological lesions is not 100% as will be explored in a later section. This approach can be extended to all human pathogens in the ovine respiratory tract. The sensitivity would apply to detecting pathogens from (healthy and pneumonic) ovine lungs which could cause disease in humans.

It should be noted that pneumonic lungs may have higher levels of pathogens than healthy ones. In addition to this the risk of introducing pathogens onto the product should be considered at the same time. Meat inspectors may contaminate their hands after inspecting each of the 5.5 lambs. Depending on the frequency and effectiveness of hand washing, subsequent lungs and other offals will be contaminated. This issue will be explored in the section on Decision Support Systems. Also a meat inspector may be a carrier of pathogens which are not known to occur in sheep, such as Hepatitis A.

A food safety programme could prescribe the maximum acceptable number of lungs or other

tissues that may contain pathogens (eg *E. coli*). Based on this requirement measures should be taken to ensure that the number of affected lungs remains below this number. This need not necessarily apply to lungs which display gross pathology. Based on the above one may wonder whether a test with a low sensitivity such as the gross pathology test is useful for achieving these goals.

Case-Control Study of Pleurisy in Lambs

Introduction

The prevalence of pleurisy and pneumonia in lambs can to some degree be reduced by farmers when they are raising their sheep. At the time of slaughter meat processors and farmers will discover whether the animals have been managed successfully or not. During inspection the prevalence and severity of the disease can be recorded. Based on this information and on knowledge of risk factors, a Decision Support System (DSS) can be created to assist farmers in producing lambs with a lower prevalence of disease. The provision of feedback is clearly beneficial to both farmers and meat processors. A number of slaughterhouses have started to use computers for the recording and storage of data. This enables the creation of DSSs and their practical applications.

Risk factors for pneumonia and pleurisy have been described in the literature. However many of these risk factors are based on the opinio authors rather than being the result of research. Consequently it is not always known whether the perceived risk factors are correct or not. Moreover it is not known how important these risk factors are and whether interaction with other risk factors should be considered as well.

The most suitable exploratory approach to identify and quantify risk factors appeared to be a case-control study. This study tried to consider as many as possible of the risk factors mentioned by others, as well as factors which might be important but had not been reported yet in the literature. Because a retrospective study method was used, data already available from meat company records on carcass downgrading was used as the classification criterion in the case-control study, because it was the only suitable measurement available.

Materials and methods

Collection of data

Based on information provided by the AFFCO meat processing company, case and control farms were defined. Both types of farms had submitted lambs for slaughter between 1 January 1993 and 30 September 1993. The case farms had more than 1.21% of their lambs over this period downgraded for pleurisy whereas the control farms had none. Both the case and the control farms had all sent in a minimum of 20 lambs. Both types of farms were sent an identical questionnaire (Appendix I). The questionnaire contained questions which related to farming practices that are commonly seen as risk factors - such as type of

yards, drenching, fly strike treatment etc. In addition questions were included that related to the farm and farm management. Although some of these factors may not be considered to be relevant to pleurisy, they were included since the objective of the project was to serve as a hypothesis-generating exercise. The need to limit the questionnaire to a size that would not discourage farmers from participating was an important consideration. Farmers who did not respond initially were invited a second time by letter to participate. A total of 166 out of 400 questionnaires were returned. They were 92 cases and 74 controls.

Statistical analysis

The initial screening analysis of the data was done by univariate analysis. Variables were used for further analysis if $p < 0.15$ and <15 missing values. Also variables which were considered to have been largely represented by other variables were excluded from further analysis. One variable which was not considered biologically plausible as a causal or a surrogate variable was excluded as well.

Categorical data were analysed with the Chi-squared test. Continuous data were analysed with a two-sample t-test and with non-parametric tests (rank-sum test, median test, or Kruskal Wallis one-way ANOVA). Although the level $p < 0.15$ was considered to be a suitable screening value at the univariate level, this did not apply to the two subsequent stages ($p \leq 0.10$). Continuous variables were converted into dichotomous values for further analysis if statistically significant.

As a second stage the variables were entered in a logistic regression analysis. This was performed stepwise entering, the variable next in the equation which reduced the deviance most. Variables were only allowed to enter the logistic regression model if the deviance was reduced by at least 2.71. Since the distribution was a Chi-squared distribution this equated to $p \leq 0.10$.

The final third stage of the analysis was the construction of a path model. Initially a null hypothesis path model consisting of three clusters was constructed. The clusters included items related to (1) the number and types of animals, (2) the farm size and paddock-related issues and (3) yard-structure and yard-related management practices. The various putative paths in the null hypothesis model were analysed with step-wise logistic regression, similar to the logistic regression which was described as the second stage.

Results

Thirty risk factors which were identified at the univariate level were included in the subsequent steps. The codes and descriptions of the variables are listed in Table 2.3 while

the Chi-squared value, the p-value and the number of missing values per variable are listed in Table 2.4.

The logistic regression which completed the second step of the analysis consisted of nine variables (Table 2.9). These variables were distributed over the three clusters. The first cluster "Yards" included risk factors of the yards and issues related to the yards. The null hypothesis is displayed in Figure 2.1 while the cluster which appeared in the final path model is shown in Figure 2.2. It included the variables Lofre, Shorntw, Vac, and Tfarla which were part of the logistic regression at the second stage. If a farm drenches its lambs at a low frequency, then it is less likely to be a case farm. Also the factor Spwand appeared to have a protective effect. The risk factors of shearing or vaccinating lambs more than once (Shorntw and Vac) on the other hand were linked positively to being a case farm. The risk factors Timesaft, Stayaft and Dirt which were incorporated in the null hypothesis did not appear to have a direct association with case farms in the final path model. The factor of infrequently yarding (Timesaft) was negatively associated with lambs being shorn twice (Shorntw) while the existence of yards with dirt, clay, soil, earth, sawdust, grass or sand (Dirt) was negatively associated with the use of preventative flystrike insecticide application with spray-on or a wand (Spwand). The risk factor representing a low frequency of drenching (Lofre) was negatively associated with a large effective grazing area (Efarla) and positively with not yarding frequently after weaning (Timeafl). The variable which was associated with a flystrike problem on the farm (Ewealsofl) did not show an association with the use of preventive treatment for flystrike (Spwand) in a statistical sense.The existence of yards with concrete or cement (Concrete) was negatively associated with Dirt, while the number of permanent yards (Peryards) was positively associated with Dirt. Peryards was positively associated with Efarla, while this factor in turn was positively associated with a large total farm size (Tfarla). These two risk factors (Efarla and Tfarla) were also included in the next cluster.

The second cluster, "Farm", which pertained to the farm size and paddock-related issues included the risk factors for large average paddock size (Avpaddock), steep paddocks in which lambs had been kept (Steep) and Tfarla which were part of the logistic regression of the second step(Table 2.7). Figure 2.3 is the cluster of the null hypothesis while Figure 2.4 is the cluster of the final path model. Both Avpaddock and putting the rams out late (Daysout) were positively associated with case farms while docking the lambs at a relatively old age (Docking) did not have a significant association. Four factors were associated with Avpaddock, lambs having access to a lake (Lake) and Tfarla in a positive sense and lambs having access to a trough (Trough) and a large number of paddocks (Paddocks) in a negative sense. Daysout was positively associated with Steep and Paddocks. Steep was positively associated with Lake and Tfarla but there was a negative association with motorised mustering (Moto). Moto was positively associated with Paddocks.

The third cluster, "Livestock", addressed the number and types of animals which were

Table 2.3 Codes and descriptions used in the multivariate analysis

Variable	Description
Cattram	Ratio cattle / rams used for mating ≥ 12.00
Cattle	≥ 400 cattle on farm
Cattdens	≥0.31 cattle per acre (based on total farm size)
Conc	Farm has yards with concrete or cement
Coopworth	Farm had Coopworth or Coopworth cross rams
Daysout	Number of days rams were put out after the first farm put them out ≥ 85 days
Deer	Deer were kept on the fam
Dirt	Farm has yards with dirt, clay, soil, earth, sawdust, grass or sand
Docking	Average age of lambs ≥45 days when docked
Efarla	Large effective grazing area, ≥ 803 acres
Ewealsofl	Fly strike in ewes was a serious problem
Eweramdif	Different number of ewe and ram breeds
Lake	Lambs did have access to lake/pond/dam
Lambs	Number of lambs/hoggets on farm ≥ 420 (as at 1 July 1993)
Lofre	Low frequency of drenching, less than 1 time per 38 days
Ewes	Number of ewes used for mating ≥1,100
Moto	Motorised means were used for mustering in the majority of cases after weaning
Rams	Number of rams used for mating ≥18
Ewedens	≥2 ewes per acre of total farm size
Paddocks	≥ 60 paddocks on farm
Avpaddock	Average paddock size ≥ 25.00 acres (based on total farm size)
Peryards	Number of sets of permanent yards > 1
Shorntw	Lambs were shorn twice
Spwand	Prevention for fly-strike with spray-on or wand
Stayaft	On average stayed long in yards after weaning,≥ 4 hrs
Steep	Predominant topography where lambs kept was steep, at times flat or rolling as well.
Tfarla	Large total farm size, ≥ 904 acres
Timesaft	Not yarded frequently after weaning, ≤ 4 times
Trough	Lambs did have access to trough
Vac	Lambs vaccinated more than once

Table 2.4 Chi-squared values, p-values and number of missing values of statistically significant variables at univariate level

Variables	Chi squared value	p-value	Missing values
Cattram	3.23	0.0721	7
Cattle	6.63	0.01	4
Cattdens	2.43	0.1192	4
Conc	2.39	0.122	8
Coopworth	4.12	0.0424	8
Daysout	3.09	0.0789	7
Deer	2.76	0.0964	4
Dirt	3.69	0.0546	8
Docking	2.5	0.1136	11
Efarla	3.54	0.06	11
Ewealsofl	2.68	0.1018	0
Eweramdif	5.83	0.0157	10
Lake	4.97	0.0257	1
Lambs	8.96	0.0028	5
Lofre	12.38	0.0004	14
Ewes	5.55	0.0184	2
Moto	2.23	0.1352	8
Rams	9.12	0.0025	2
Ewedens	2.41	0.1206	4
Paddocks	5	0.0254	6
Avpaddock	3.39	0.0655	8
Peryards	3.65	0.056	1
Shorntw	6.89	0.0086	0
Spwand	4.28	0.0387	0
Stayaft	6.05	0.0139	11
Steep	5.38	0.0203	2
Tfarla	2.46	0.117	4
Timesaft	5.09	0.0241	8
Trough	2.27	0.1318	1
Vac	5.68	0.0172	0

Table 2.5 Beta coefficients, Standard errors (SE), Odds ratios and the 90% confidence intervals of variables included in the logistic regression model at the second stage

Variable	Coefficient	SE	5 %conf. lower	Odds ratio	5% conf. upper
Cattdens	2.3356	0.6937	3.3	10.3	32.2
Coopworth	2.02778	0.85905	1.9	7.6	31.1
Lambs	1.71175	0.67024	1.8	5.5	16.6
Lofre	-1.74519	0.59426	0.1	0.2	0.5
Avpaddock	1.58811	0.68107	1.6	4.9	15.0
Shorntw	1.23679	0.6738	1.1	3.4	10.4
Steep	1.23138	0.62357	1.2	3.4	9.5
Tfarla	-1.3297	0.74458	0.1	0.3	0.9
Vac	2.22376	1.1834	1.3	9.2	64.4

kept on the farm. It included the variables for high cattle density on the property (Cattdens), a large number of lambs on the farm in July (Lambs) and the use of purebred or crossbred Coopworth rams (Coopworth) which were directly associated with case farms. The direct association of risk factor of having deer on the farm (Deer) and case farms disappeared from the null hypothesis. However Deer and a large number of ewes which had been mated (Ewes) were negatively associated with Cattdens, while a high cattle to ram ratio (Cattram) was positively associated. Ewes was positively associated with Lambs while a different number of ewe and ram breeds (Eweramdif) was negatively associated. A high ewe density on the farm (Ewedens), Steep and Rams were negatively associated with Cattram, while a large number of cattle on the farm (Cattle) had a positive association.

Table 2.6 Associations, Beta coefficients, Standard errors (SE), Odds ratios (OR) and 90% confidence limits of significant paths in cluster "Yards"

Variables	Association	Coefficient	SE	5% lower	OR	5% upper
Pleurisy						
Lofre	-	-1.47269	0.44533	0.1	0.2	0.5
Shortw	+	1.3055	0.53619	1.5	3.7	8.9
Vac	+	1.45221	0.89087	1.0	4.3	18.4
Spwand	-	-0.8289	0.4783	0.2	0.4	1.0
Lofre						
Timesaft	+	1.15554	0.44666	1.5	3.2	6.6
Efarla	-	-0.78586	0.46654	0.2	0.5	1.0
Shorntw						
Timesaft	-	-1.73715	0.76419	0.1	0.2	0.6
Spwand						
Dirt	-	-0.97555	0.49216	0.2	0.4	0.8
Timesaft						
Efarla	-	-1.21251	0.53	0.1	0.3	0.7
Dirt						
Peryards	+	1.66597	0.54898	2.2	5.3	13.0
Concrete	-	-1.150425	0.55735	0.1	0.2	0.8
Peryards						
Efarla	+	2.07317	0.45265	3.8	8.0	16.7
Efarla						
Tfarla	+	6.31491	0.91891	122.5	552.7	2494.7

Table 2.7 Associations, Beta-coefficients, Standard Errors (SE), Odds ratios (OR) and 90% confidence limits of significant paths in cluster "Farm"

Variabless	Association	Coefficient	SE	5% lower	OR	5% upper
Pleurisy						
Daysout	+	0.72758	0.3	1.1	2.1	3.8
Avpaddock	+	0.79081	0.36092	1.2	2.2	4.0
Avpaddock						
Tfarla	+	3.06852	0.62391	7.7	21.5	59.8
Paddocks	-	-2.16803	0.82341	0.0	0.1	0.4
Trough	-	-1.76916	0.49808	0.1	0.2	0.4
Lake	+	1.01688	0.46578	1.3	2.8	5.9
Daysout						
Steep	+	0.78481	0.38435	1.2	2.2	4.1
Paddocks	+	1.74684	0.57051	2.3	5.7	14.6
Steep						
Lake	+	0.79442	0.41140	1.1	2.2	4.3
Moto	-	-0.73254	0.44116	0.2	0.5	1.0
Tfarla	+	1.45935	0.40114	2.2	4.3	8.3
Paddocks						
Efarla	+	2.23655	0.60273	3.5	9.4	25.2
Moto	+	1.33221	0.79396	1.0	3.8	13.9
Trough						
Tfarla	-	-1.28967	0.38933	0.1	0.3	0.5
Lake						
Tfarla	+	0.91183	0.37687	1.3	2.5	4.6
Efarla						
Tfarla	+	5.76488	0.78092	88.6	318.9	1147.8

Table 2.8 Associations, Beta-coefficients, Standard Errors (SE), Odds ratios (OR) and 90% confidence limits of significant paths in cluster "Livestock"

Variables	Association	Coefficient	SE	5% lower	OR	5% upper
Pleurisy						
Cattdens	+	1.25934	0.39905	1.6	3.5	6.8
Lambs	+	1.40066	0.39681	1.9	4.1	7.8
Coopworth	+	1.12276	0.54463	1.1	3.1	7.5
Cattdens						
Deer	-	-1.23734	0.60697	0.1	0.3	0.8
Cattram	+	2.27926	0.42029	4.9	9.8	19.5
Ewes	-	-1.35617	0.41771	0.1	0.3	0.5
Lambs						
Ewes	+	3.09268	0.46853	10.2	22.0	47.5
Eweramdif	-	-1.23498	0.59271	0.1	0.3	0.8
Cattram						
Ewedens	-	-2.7487	0.8028	0.0	0.1	0.2
Rams	-	-1.87191	0.5044	0.1	0.2	0.4
Cattle	+	2.37913	0.57992	4.2	10.8	27.9
Steep	-	-0.96908	0.48092	0.2	0.4	0.8

Discussion

The case-control study highlighted a number of risk factors for pleurisy in lambs. The definition of pleurisy was based on lambs being downgraded at slaughter for this reason. The results of the univariate analysis showed that the size of the farm and its physical attributes, and certain farming practices resulted in risk factors which were statistically

significant at this stage of the analysis. A number of these findings were consistent with risk factors which had previously been reported in the literature.

Alley (1991) suggested that drenching procedures with hand-to-nose contact of whole mobs was a risk factor. This study indicated that drenching the animals at a low frequency rather than a high frequency had a protective effect. Bruère and West (1993) mentioned that pneumonia may arise when climatic changes occur after shearing, although they consider it a less significant predisposing factor. This study showed that farms with lambs which had been shorn twice were more likely to be case farms. There were associations between these two factors (low frequency drenching and shearing twice) and the frequency of yarding after weaning. Infrequent yarding had a negative association with being a case farm which is consistent with attempts to keep stress to animals to a minimum (Smith, 1975). This study indicated a protective effect by using a spray-on or wand. This appears consistent with Bruère and West (1993) who mentioned that outbreaks of pneumonia have occurred after shower and plunge dipping. Manktelow (1970) alerted to risks which are posed by dusty sheep yards. This factor was confirmed in this study by the risk factor Dirt which had a negative effect at univariate level. In the final path diagram Dirt had a negative association with the use of a spray-on or wand which in turn had a protective effect. The use of yards of cement or concrete on the other hand had a protective effect.

Smith (1975) warned against the practice of mustering with a motorbike because animals are more likely to be driven too hard, thereby increasing the rate of breathing. The risk factor Moto which denoted the use of motorised means of mustering was positively associated with being a case farm.This study showed a negative association between Moto and Steep which may have indicated that farmers are less likely to use motorised means when animals are kept in steep paddocks. The proportion of steep paddocks on a farm may have lead to rams being put out later in the year. It may be that because lambs may not thrive well in steep paddocks they are more likely to have pneumonia. Alternatively the slower growth rate and possibly the fact that they will be submitted for slaughter later in the season if the rams had been put out late in the season might also have been contributing factors to the development of pneumonia-pleurisy. The use of half dried up and dirty dams was to be avoided if possible according to Smith (1975). This study showed that the risk factor Trough had a protective effect while the risk factor Lake was positively associated with being a case farm. However these two factors in combination with the risk factor Avpaddock which applied to a relatively large average paddock size and the risk factors which related to the size of the farm and the number of paddocks (Tfarla, Efarla and Paddocks) may have indicated that the degree of development of a property was of importance. Small properties may tend to be better developed, with smaller average paddocks, troughs and possibly other facilities which may be beneficial to lambs.

A large number of lambs on the property in July was associated with case farms. The number of lambs was associated with the number of ewes that had been mated in the previous year possibly indicating that large sheep properties are more at risk. At the same time the number of lambs was negatively associated with different numbers of ram and

time the number of lambs was negatively associated with different numbers of ram and ewe breeds. This may indicate that the offspring displayed a better health due to "heterosis superiority" (Minkema, 1973). Alternatively it may also be a surrogate variable for the management of the property. A number of factors were identified which suggested that properties that concentrated on cattle were more likely to have respiratory disease in the lambs. There were no articles in the literature referring to this issue. Similarly, the association between the use of purebred and cross-bred Coopworth rams and pneumonia has not been described previously.

The advantage of case-control studies is that they can help in generating hypotheses and in some cases provide new insights, and they do so in a relatively inexpensive manner, commonly using data which can be obtained rapidly. There are some inherent difficulties in the execution of a case control study as described above. The current study was based on data that were collected by a meat processing company for a different purpose and the data were therefore relatively crude, as one would expect. The trend towards enhanced data recording and retrieval systems by meat processing companies (such as the company which provided the data) would result in scope for separation of case and control farms based on more detailed information. The installation of computer terminals for the use of meat inspectors would enable the recording of more detailed data. The questionnaire was filled out by the farmers. Inevitably this will lead to variation in the quality of the data. An on-farm assessment by a trained interviewer is preferable but expensive.

Case-control studies do not provide strong evidence of causal links between risk factors and the occurrence of pneumonia - they merely show an association which may or may not be causal. However they can provide a starting point for investigations, from which other epidemiological methods can be used to further test whether or not relationships are causal.

A critical point is that because pneumonia and pleurisy are caused by multiple interacting factors, a field-based epidemiological approach is the only way in which valid assessments of the influence of various factors in combination can be made. One reason for the lack of progress in pneumonia control in sheep has been the lack of a multi-factorial approach to solving the problem.

This study has shown the benefits of recording the prevalence of disease at slaughter. The main beneficiaries of this approach were farmers since it provided them with information which they can use to improve the health of their flock. Also meat processors were beneficiaries since ultimately they should receive lambs with a lower prevalence of pleurisy. In this particular study the public health benefits seem small. The hazard analysis in the previous section concluded that pleurisy in lambs was of limited importance from a public health perspective. The study showed how useful data collected at inspection can be as a research tool.

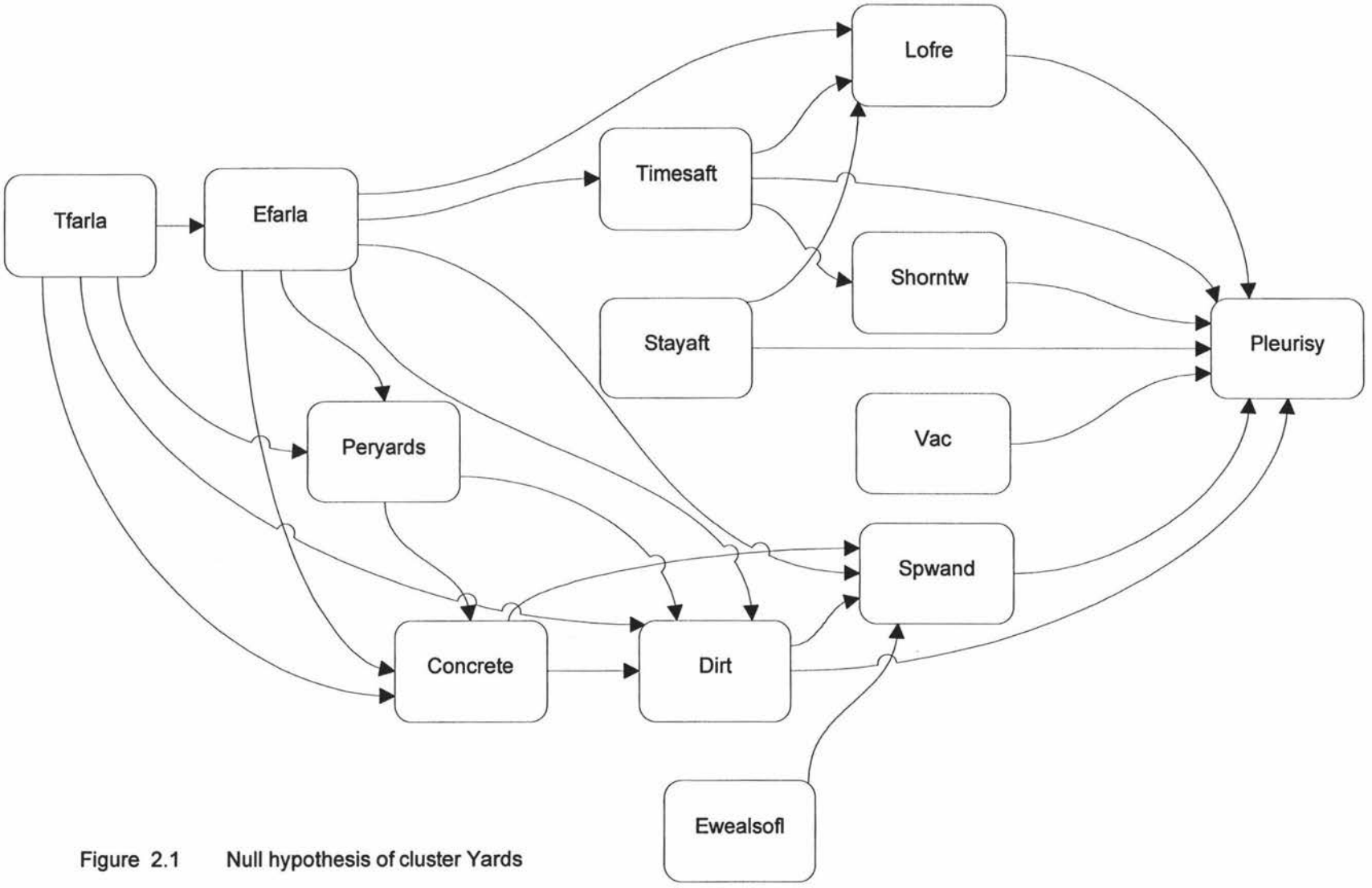


Figure 2.1 Null hypothesis of cluster Yards

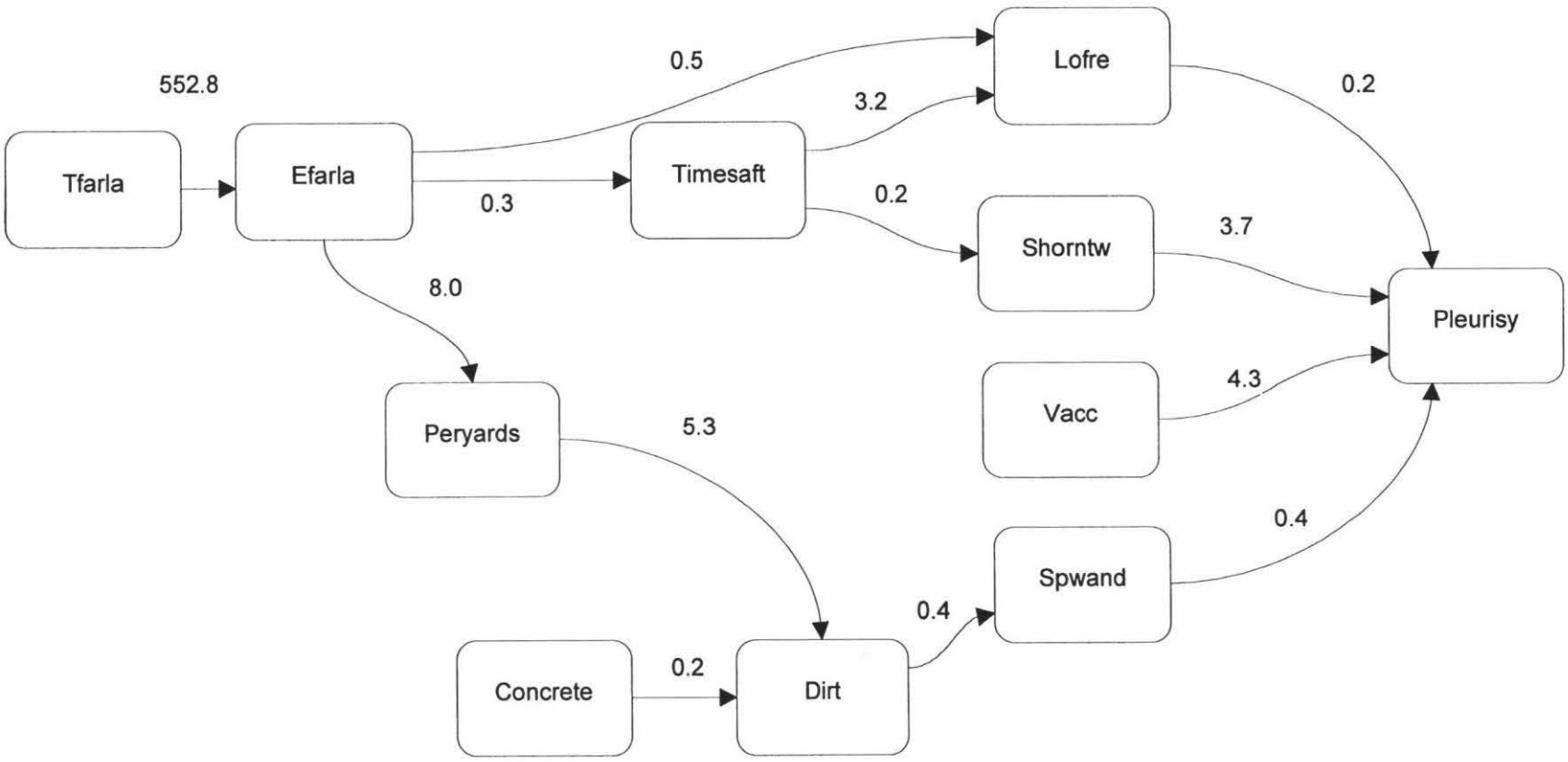


Figure 2.2 Final cluster Yards
p =< 0.1
Figures are values of Odds Ratios

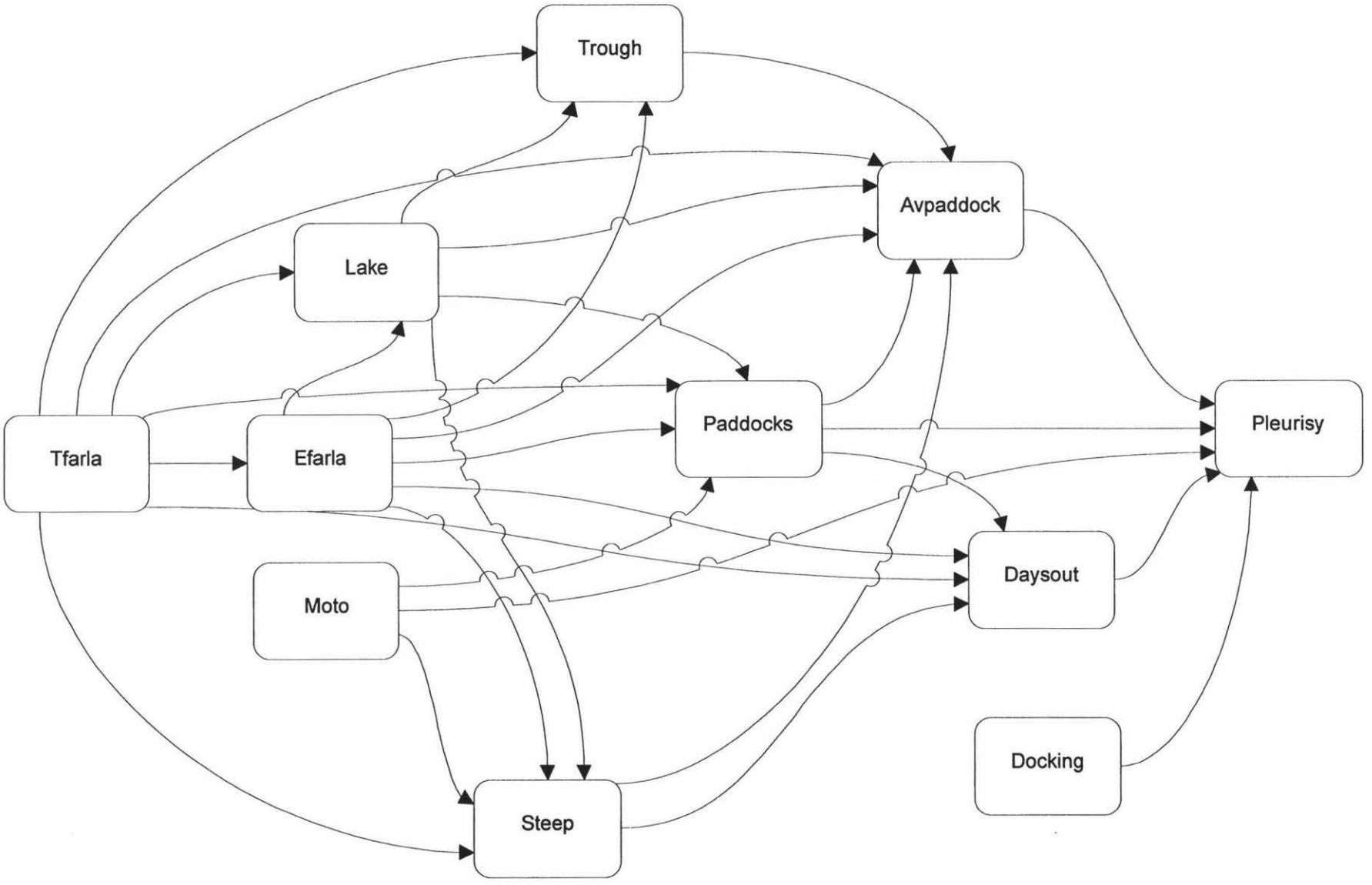


Figure 2.3 Null hypothesis of cluster Farm

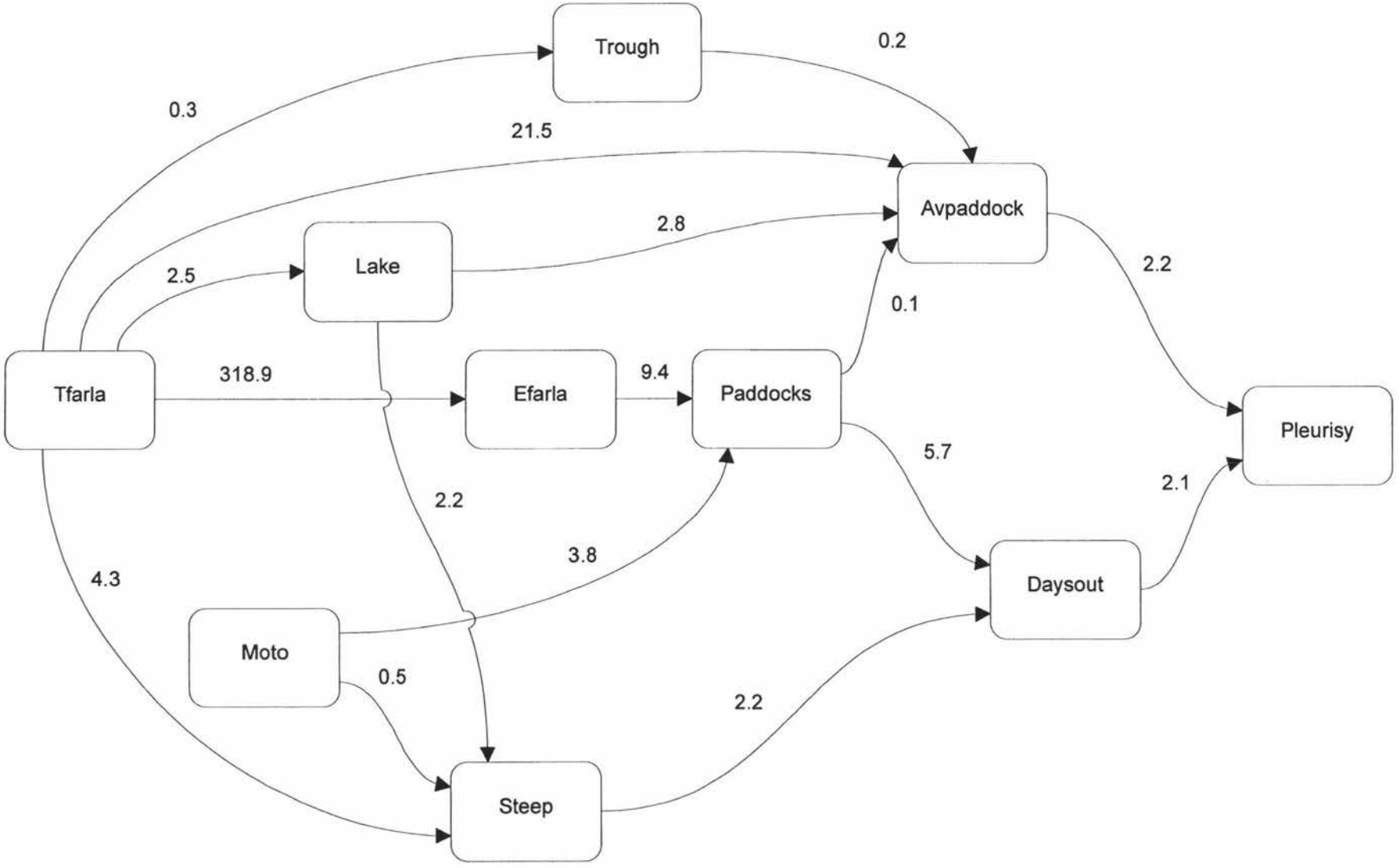


Figure 2.4 Final cluster Farm
p =< 0.1
Figures are values of Odds Ratios

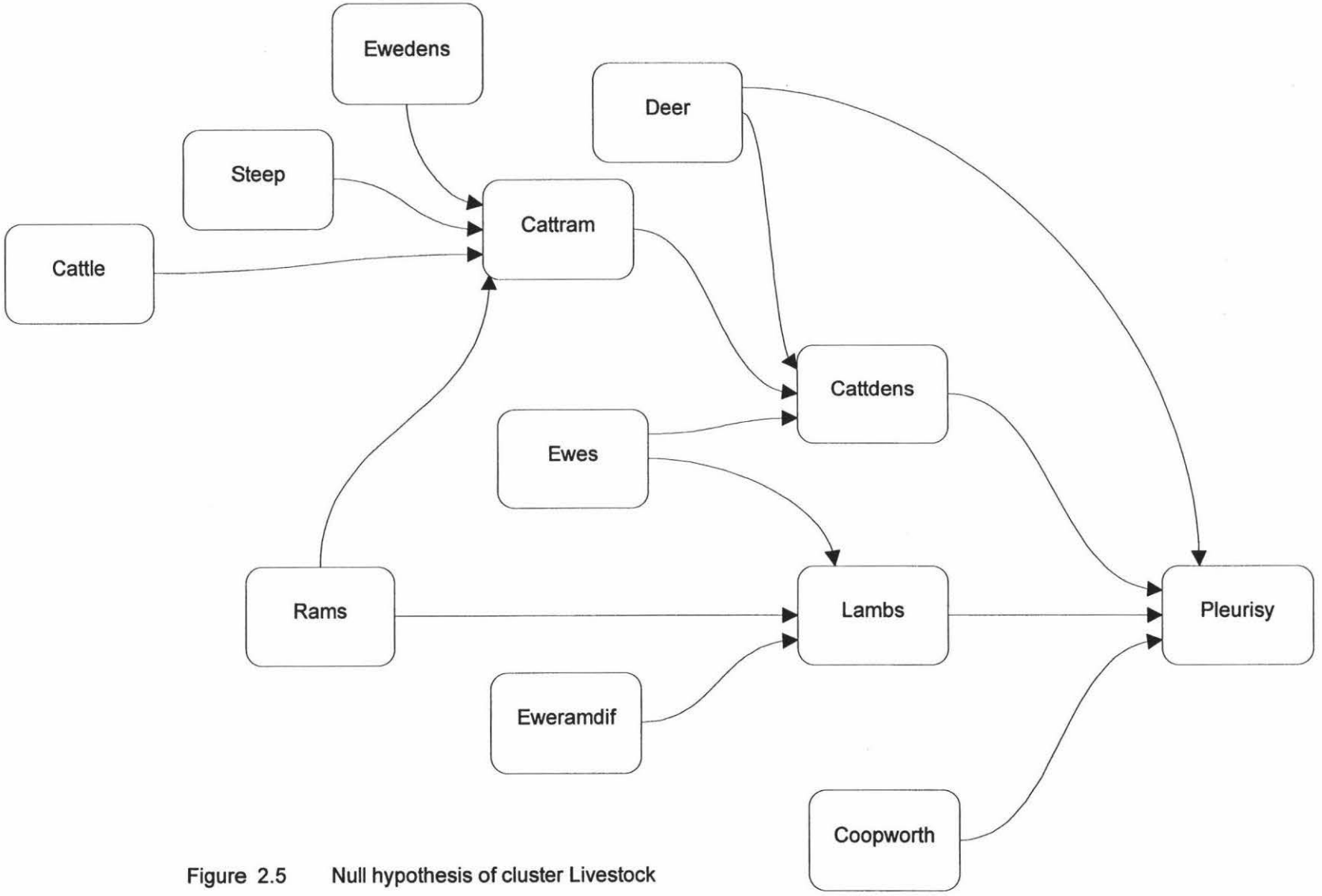


Figure 2.5 Null hypothesis of cluster Livestock

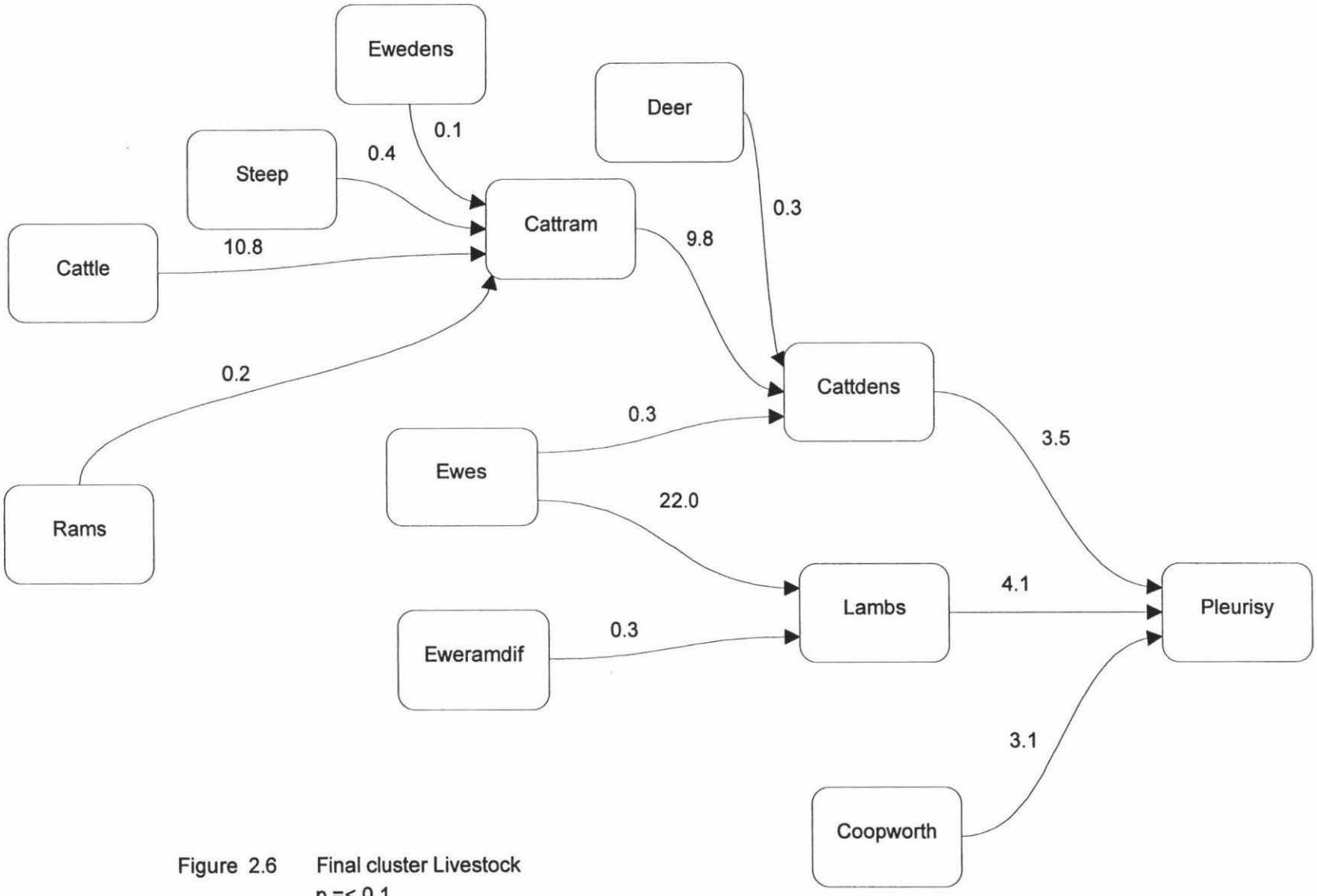


Figure 2.6 Final cluster Livestock
p =< 0.1
Figures are values of Odds Ratios

Intervention Study of Pleurisy and Pneumonia At Farm A

Introduction

A number of factors have been identified as being associated with the development of pleurisy and pneumonia in lambs. The objective of this trial was to further clarify and confirm the role of these factors. This would contribute to a better understanding of the possibilities and limitations of a Decision Support System (DSS) for pleurisy in lambs. During the previous case-control study, interaction was possible between many factors. In this intervention study the objective was to keep the other risk factors constant for exposed and unexposed lambs.

The factors under investigation in this study were:

- The time the lambs stay in the yards (ie one hour vs six hours).
- The effect of an oral drench versus an injectable drench.

A similar study was performed at a neighbouring farm (Farm B). Since the studies also differed in some important regards they are described in separate sections. Certain comparisons between the two farms are discussed in the section on Farm B. This study used the cooperation of farmers to compare management practices considered to have some potential for reducing the occurrence of pneumonia and pleurisy, in an effort to determine whether such changes would be justified.

Materials and methods

A total of 943 ram lambs (Romneys) participated in the trial which took place after weaning (23 November 1994) until the second draft of lambs was slaughtered (1 March 1995).

The allocation of lambs to treatment groups was performed in a manner which closely resembled random allocation. While the lambs were drafted through a race for treatment, the first lambs would receive an eartag of one colour, the second of another colour, and the third and fourth animal would receive either of the remaining two colours. All subsequent lambs would be allocated eartags in the same sequence of colours, ie the fifth lamb would receive the same colour as the first lamb, the sixth lamb the same colour as the second lamb, etc. The four groups consisted of lambs receiving either an oral (Nilverm, active ingredient 4% w/v levamisole HCL) or an injectable drench (Nilvax, active ingredient 6.8% w/v levamisole phosphate and combined clostridial vaccine), and staying for one or six hours in the yards during treatment. The label recommendation for Nilvax is for sheep greater than 20 kg. The following dose rates were considered suitable by the manufacturer, Mallinckrodt Veterinary Limited: 2.5 ml for lambs from 15 to 20 kg and 3.5 ml for lambs from 21 to 24 kg. Alternatively it was considered possible to use the dose rate of 2.5 ml for all animals between 15 and 24 kg. The heavy animals would

receive a low dose which would still be effective.

Approximately equal numbers from each of the four groups were going to be submitted for slaughter in two or three drafts. It was preferred that two drafts were submitted. In addition it was preferable that animals were slaughtered as late in the season as possible.

During the trials the animals were subjected to other interventions as part of the normal husbandry. Some of these treatments were potential risk factors for pleurisy/pneumonia. This could have had ramifications. To minimise the impact of other risk factors on the comparison between exposed and unexposed lambs, all lambs that participated in the trial were always be subjected to the same husbandry practices at the same time. They were grazed as one flock.

Weaning and first drench

On 23 November 1994, the day of weaning, the lambs were treated for flystrike and they were drenched. The animals stayed in the yards approximately the same time which was more than one hour. Zenith (active ingredient 250g/L difulbenzuron) was used in a shower dip for flystrike prevention.

The trial animals were separated into three groups of 236 lambs each (red, blue and brown eartag) and one group of 235 lambs (green eartag). This is when the allocation for treatments as described above occurred. During the first drench there were two groups of lambs (green and blue eartags) receiving the oral drench and two groups (red and brown) receiving the injectable drench. A sample of the lambs was taken and the average weight was 25 kg. The lambs with the green or blue eartags received 7 ml Nilverm and the animals with red or brown eartags received 3.5 ml Nilvax.

Second drench

On 19 December 1994, the lambs were drenched (orally or by injection) for the second time. The lambs stayed different times in the yards in accordance with the protocol. The animals with the red and green eartags stayed one hour in the yards and the lambs with the brown and blue eartags stayed six hours in the yards.

The four different groups as identified by their eartags were therefore:

Red eartag	One hour in yards and injectable drench	(Coded One_inject)
Green eartag	One hour in yards and oral drench	(Coded One_oral)
Brown eartag	Six hours in yards and injectable drench	(Coded Six_inject)
Blue eartag	Six hours in yards and oral drench	(Coded Six_oral)

The stocking density from weaning to the second drench was approximately 10 lambs per hectare. The number of lambs that were counted during drenching were 236 lambs from the One_inject group, 230 lambs from the One_oral group, 233 lambs from the Six_inject group and 234 lambs from the Six_oral group.

Third drench or slaughter

On 11 January 1995 the lambs were either drafted for slaughter or they were drenched for the third time and shorn. The lambs that were drenched, stayed approximately the same time in the yards. The number of lambs that were counted during drenching were 110 lambs from the One_inject group, 99 lambs from the One_oral group, 119 lambs from the Six_inject group and 109 lambs from the Six_oral group. The stocking density from the second drench to the third drench was approximately 14 lambs per hectare.

Fourth drench

On 30 January 1995 the lambs were drenched for the fourth time. The lambs stayed different times in the yards in accordance with the protocol. The stocking density from the third drench to the fourth drench was approximately 19 lambs per hectare.

Second draft for slaughter

On 28 February 1995 the remaining lambs were drafted for slaughter. The stocking density from the fourth drench until they were drafted for slaughter was approximately 5 lambs per hectare.

Inspection and procedures for diseased lambs

Selection for slaughter of both the exposed and the unexposed groups was based on a normal commercial assessment of their condition being suitable for slaughter. The second draft contained some animals which Farm A would normally have kept for a longer period to reach a better condition. They were included in the second draft to assist in the interpretation of the trial.

All lambs that entered the trial were to be subjected to a post mortem examination ie they were slaughtered if possible. This was because pneumonia in lambs even when not detectable clinically could still have resulted in the affected animals growing slower. It was therefore considered that animals with subclinical pneumonia were likely to be slaughtered at an older age than healthy animals. The only exceptions were lambs that were lost during the trial due to misadventure etc.

The inspection procedures at slaughter are described in Appendix II: "Protocol for post mortem inspection of lambs for pleurisy or pneumonia".

If during the trials animals became sick, they would be treated according to normal farming practices. Details of the category of lamb (drench/time in yards), signs, diagnosis and treatment for disease would have been recorded. If during the trial an animal died, the group to which the lamb belonged would have been recorded and a post mortem inspection of the lungs and pleura would have been carried out if possible and if fresh. The signs of pneumonia would have been

described if it occurred in the flock. None of the above was required during this trial.

Analytical methods

Each of the four groups had been subjected to one of four possible combinations of interventions (eg one hour in the yards and an oral drench). The four groups were aggregated into two groups in such a way that the interventions (time in the yards and manner of drenching) could be compared. This resulted in a comparison between lambs that had been kept in the yards for six hours with lambs that had been kept in the yards for one hour. The other comparison involved lambs which received an oral drench and those that received a drench by injection. The cumulative number of affected animals for the various pathology categories were calculated and the number of affected and unaffected lambs were placed in a two by two tables. The Chi-squared test was used to examine the hypothesis of independence whether the variables which examined whether the variables which were in the rows were independent from the variables defining the columns (Statistix, 1992; Dixon and Massey, 1969).

In order to evaluate the possibility of interaction between the time in the yards, type of drench and the date of slaughter log-linear modelling was used. The Log-linear model restates through logarithmic transformations the problem of analysing multiway frequency tables in terms similar to an ANOVA (Statistica,1993). Log-linear modelling was performed in Statistica (StatSoft, Inc). First the programme fits the model with no interactions between the factors. If the model does not fit it will fit a model with all two-way interactions. If this does not fit either the programme will fit all three-way interactions and so on.

The probability that a certain degree of pneumonia was accompanied by a certain degree of pleurisy was evaluated by comparing proportions. No statistical test was used.

The association between *Dictyocaulus viviparus* and respiratory disease was evaluated with Chi-squared tests.

Results

Information collected at the slaughter house

The number of lambs which were slaughtered and the following drafts were sent for slaughter and levels of disease were recorded by meat inspectors at post mortem inspection are displayed in Table 2.9.

Table 2.9 Number of lambs that were slaughtered and respiratory pathology that was recorded at Farm A

Intervention	Killed*	Not	plcl	plcf	plal	pnmi	pnmo	pnse
12/1/95								
Six_oral	121	70	2	1	1	47	2	
One_inject	125	76	1	1		46	3	
Six_inject	112	74	1	4	1	30	4	1
One_oral	125	84	1	1	1	38	1	
1/3/95								
Six_oral	105	33	10	4	5	32	33	6
One_inject	107	24	8	2	2	43	34	4
Six_inject	112	26	4	6	4	46	35	3
One_oral	95	18	5	5		47	25	4

*The code "Killed" describes the number of lambs that were slaughtered. Pleurisy categories are chronic large pleurisy (plcl), chronic flimsy pleurisy (plcf) and acute localised pleurisy (plal). The pneumonia categories are mild pneumonia (pnmi), moderate pneumonia (pnmo) and severe pneumonia (pnse) (See Appendix II for a description of the categories). Animals shown as "Not" had no respiratory tract lesions recorded.

On 12/1/95 one lamb from the Six_inject group died on the truck. On post mortem a mild pneumonia and a mild peritonitis were discovered. Only lambs inspected on the slaughterfloor are included in Table 2.9. This is to ensure that all lambs receive a similar standard of inspection.

One lamb from the Six_inject group had a pyogenic pneumonia on post mortem inspection. This pneumonia was not included in the analysis. On 3/1/95 one animal from the Six_inject group was condemned. This lamb had an enzootic pneumonia with secondary bacterial abscesses. During this trial no lambs were recorded in the category of acute systemic pleurisy.

Chi-squared tests to evaluate effect of treatments

Chi-squared tests were used to evaluate the effect of the two different treatment methods. Table 2.10 has comparisons of cumulative of various degrees of respiratory pathology on 1/3/95.

The only finding which was both biologically meaningful and statistically significant at $p < 0.05$ was the cumulative prevalence of acute localised pleurisy lesions where the groups of lambs that

had been in the yards for six hours had a larger proportion of affected animals than the lambs that had been in the yards for one hour only.

Log-linear modelling to evaluate interactions between treatments and time of slaughter

The log-linear model contained four coding columns which consisted of the variables for the time in the yards (six hours or one hour), type of drench (oral or injection), date of slaughter (12/1/95 or 1/3/95) and the status of animals (positive for specific pathology or not). The number of animals that were be part of a group of any combination of the previous four coding variables, were in placed in frequency columns as applicable for the specific pathology. The results of the various models are summarised in Table 2.11.

In the case of chronic large pleurisy, moderate pneumonia and severe pneumonia the best model consisted of 43, indication a higher prevalence of the conditions at the later date. In the case of any pleurisy, which denoted any degree of pleurisy, there were two interaction terms. These were time in the yards and being affected, and the data of slaughter and being affected. In a number of cases the best model was 4321, indicating a saturated model. No conclusions of practical use can be drawn from such findings. There were also two situations were the best model was 4,3. This means that the best model was explained by the main effects date of slaughter and diseased or not. This model does not have a meaning in the context of the objectives of the study.

Table 2.10 Chi-squared tests comparing the effects of treatments by using cumulative prevalences of respiratory pathology on 1/3/95

Treatment categories		p-value	No. cells <5.0[a]
Chronic flimsy pleurisy			
six hours	one hour	0.2104	
oral	injection	0.7198	
Chronic large pleurisy			
six hours	one hour	0.7093	
oral	injection	0.4331	
Acute localised pleurisy			
six hours	one hour	0.0305 *	
oral	injection	0.9667	
Pleurisy of any degree			
six hours	one hour	0.2745	
oral	injection	0.6212	
Mild pneumonia			
six hours	one hour	0.2063	
oral	injection	0.8547	
Moderate and severe pneumonia			
six hours	one hour	0.2213	
oral	injection	0.3193	
pneumonia of any degree of severity			
six hours	one hour	0.7422	
oral	injection	0.5642	
Pleurisy or pneumonia			
six hours	one hour	0.9522	
oral	injection	0.4829	

* indicates statistically significant value at $p<0.05$

[a] Number of cells with expected value less than 5.0

Table 2.11 Log-linear model which evaluates the interactions between treatment and the time of slaughter for Farm A

Dependent variable with count data	Best model*	Chi-squared value	df	p-value
Any pleurisy or pneumonia	4321	0.000000	0	1.0000
Any pleurisy	43, 41	4.149469	10	0.9403
Any pneumonia	4321	0.000000	0	1.0000
Chronic large pleurisy	43	6.252169	12	0.9028
Chronic flimsy pleurisy	4,3	13.03801	13	0.4449
Acute localised pleurisy	4,3	13.23866	13	0.4296
Mild pneumonia	4321	0.000000	0	1.0000
Moderate pneumonia	43	5.437662	12	0.9417
Severe pneumonia	43	4.631432	12	0.9692

* Codes which were used for best model were: 1 = Time in the yards, 2 = Drench, 3 = Date of slaughter and 4 = Diseased (pleurisy/pneumonia) or not

Relationships

The table below shows combinations of pneumonia and pleurisy which were recorded in individual animals.

	pnmi	pnmo	pnse
plcl	3	21	4
plcf	7	9	0
plal	0	10	3

Table 2.12 shows the number of animals, and the proportions of the total number of animals which were found to have both pleurisy and pneumonia or only one of the two conditions on post mortem inspection.

Table 2.12 Combinations of pathology of lungs and pleura at Farm A

disease codes	total	Pleurisy and pneumonia			
		Both pleurisy and pneumonia		Either pleurisy or pneumonia	
		Number	(Proportion)	Number	(Proportion)
plcl	32	28	(0.88)	4	(0.12)
plcf	24	16	(0.67)	8	(0.33)
plal	14	13	(0.93)	1	(0.07)
pnmi	329	10	(0.03)	319	(0.97)
pnmo	137	40	(0.29)	97	(0.71)
pnse	18	7	(0.39)	11	(0.61)
total *		57		440	

* 405 animals did not display any signs of pleurisy or pneumonia and one animal displayed pyogenic pneumonia.

Large proportions of lambs with either chronic large or acute localised pleurisy had pneumonia recorded as well. In the case of chronic flimsy pneumonia this percentage was lower. A large number of lambs had mild pneumonia. However only a small proportion of these animals had pleurisy as well. As the degree of pneumonia increased the percentage of lambs with pleurisy increased as well.

Relationship with Dictyocaulus viviparus

The involvement of *D. viviparus* involved in the development of pneumonia and/or pleurisy was examined by evaluating the association of this parasite with pneumonia with the help of Chi-squared tests. There were two cases with *D. viviparus* on 1/3/95 which had both pleurisy and pneumonia. The occurrence of this parasite in relation to respiratory disease is displayed in Table 2.13. The overall chi-squared values for 12/1/95 and 1/3/95 were 0.07 and 0.59, and the p-values were 0.7907 and 0.4423 respectively.

Table 2.13 Relationship of pleurisy and pneumonia with *Dictyocaulus viviparus*

D.viviparus	No pleurisy or pneumonia	Pleurisy and/or pneumonia
21/1/95		
Signs of *D.viviparus*	17	9
No signs of *D.viviparus*	287	170
1/3/95		
Signs of *D.viviparus*	4	19
No signs of *D.viviparus*	97	300

Discussion

The case-control study discussed in the previous section identified the frequency of drenching and the time lambs stayed in the yards as potential risk factors. Robinson (1983) placed emphasis on using equipment that was in proper operating condition and the animals should be adequately restrained as well. Alley (1991) suggested that hand to nose contact of all animals in a flock might play a role. It could also be hypothesized that the transfer of micro-organisms from one animal to the next one by the drenching gun might be another reason for the frequency of drenching being a risk factor. Since it was not possible to drench two groups less often than two other groups for practical reasons, a solution to test this hypothesis was found by drenching two groups of animals by subcutaneous injection and the other two groups orally.

The trial did not prove that drenching by injection resulted in less pneumonia or pleurisy than an oral drench. Several reasons could be considered as an explanation. The case-control study evaluated this effect in interaction with other risk factors. Also it also cannot be ruled out that other aspects of drenching were of importance which was not simulated in this trial. Therefore, although the trial did not show a difference it should not be construed as comprehensive evidence that the frequency of drenching is unimportant.

The case-control study identified the time lambs stayed in the yards on average after weaning as a risk factor at the univariate level. Smith (1975) stated that one must avoid holding lambs in sheep yards for a period, since it combines various risk factors including close confinement. Bruère and West (1993) mention that the stress of mustering and yarding are risk factors for the outbreak of acute fibrinous pneumonia. This intervention study showed that the groups of lambs that had been in the yards for a longer period (ie six hours) had a higher cumulative prevalence of acute localised pleurisy.

Temporal patterns of respiratory disease have been described by several authors (McGowan *et al.* 1978, Davies, 1985b). The log-linear modelling demonstrated the increase of various types of respiratory pathology at the later slaughter date.

The correlations showed that if pleurisy was detected, pneumonia was often detected as well. The reverse was not the case. In particular when mild pneumonia was detected, only in 3 % of the animals pleurisy was detected as well. These findings should be considered when advanced DDSs are created since lambs with pleurisy only constitute a small proportion of lambs with respiratory disease. A discussion on this will occur at the end of this chapter in the section on DSSs.

Although lungworm has been incriminated as a possible risk factor for respiratory disease, authors have had reservations about this possibility (Sorenson, 1976, Bruère and West, 1993) . An association between *D. viviparus* and pneumonia or pleurisy did not appear to exist in this trial.

Intervention Study of Pleurisy and Pneumonia At Farm B

Introduction

A number of factors have been identified as contributing to the development of pleurisy and pneumonia in lambs. The objective of this trial was to further clarify the role of some of these factors. The factors under investigation in this study were:

- The effect of two different types of flystrike treatment.
- The effect of an oral drench vs an injectable drench.

A similar study was carried out at Farm A (see previous section) and the same objectives apply. Certain aspects of the post mortem findings at Farm A and Farm B will be compared in this section.

Materials and methods

A group of 1000 lambs participated in the trials. The trials started on 25 January 1995 (fourth drench) and finished on 30 August 1995 when the last draft of lambs was sent for slaughter.

During the trials the animals were subjected to other treatments as part of the normal husbandry. Some of these treatments are risk factors for pleurisy/pneumonia. This could have affected the outcome of the trials. To minimise the impact of other risk factors on the comparisons of the various treatment groups, these groups were always subjected to the same husbandry practices at the same time. The lambs were grazed as one flock at commercial stocking rates.

Treatments before the trials started

The lambs were weaned on 14 November 1994. At this time the lambs were drenched orally (first drench) with 4 ml Albezol (active ingredient albendazole) and received a shower dip with Zenith (active ingredient difulbenzuron). All lambs received the same treatment. The different treatments which were to be investigated did not start at this stage. The second drench with Albezol (5 ml) took place on 7 December 1994. This oral drench was the same for all lambs. The third drench with Albezol (6 ml) took place on 28 December 1994. This oral drench was the same for all lambs.

Fourth drench

The fourth drench took place on 25 January 1995. At this stage the following procedures were carried out.

- The lambs were sexed.
- The male lambs that were going to be slaughtered by Farm B were eartagged. There were four different colour eartags. The eartags and therefore the various treatments were allocated to the lambs in the drafting race in a manner which resembles randomness as described for Farm A.
- The lambs were drenched. The lambs with a white or black eartags received an injectable drench (Nilvax) and the lambs with a purple or orange eartags received an oral drench (levamisol).
- The lambs were shorn.

Fly strike treatment and fifth drench

The fifth drench took place on 23 February 1995. The lambs were also treated for flystrike. This was the first time the lambs received a different treatment for flystrike. Lambs with white and purple eartags were treated with a wand while lambs with black and orange eartags were treated with a shower dip.

The four different groups as identified by there eartags were therefore:

White eartag	Injectable drench and wand dip	(Coded Inject_wand)
Black eartag	Injectable drench and shower dip	(Coded Inject_shower)
Purple eartag	Oral drench and wand dip	(Coded Oral_wand)
Orange eartag	Oral drench and shower dip	(Coded Oral_shower)

Subsequent drenches

Subsequent drenches took place on:

24 March 1995	7 ml oral drench or 3.5 ml injectable drench (Nilvax)
21 April 1995	7 ml oral drench or 3.5 ml injectable drench (Nilvax)
19 May 1995	8ml oral drench or 3.5 ml injectable drench (Nilvax)

Inspection and procedures for diseased lambs

It was considered preferable that all lambs that entered the trial were going to be subjected to a post mortem examination, ie they were to be slaughtered if possible. This is because pneumonia in lambs, even when it could not be detected clinically, could still result in affected animals growing slower. Therefore animals with subclinical pneumonia were likely to be slaughtered at an older age than healthy animals.

If animals became sick during the trials, the animals would be treated according to normal farming practices. Details of the category of lamb, symptoms, diagnosis and treatment would be recorded. If during the trials an animal died, the category of lamb would be recorded. If pneumonia occurred in the flock the symptoms would be described and the number of affected animals would be estimated.

Lambs were to be slaughtered when their condition was considered appropriate. It was attempted to send approximately the same numbers of animals per different groups (eartag colour) for slaughter.

The inspection procedures at slaughter are described in Appendix II: "protocol for post mortem inspection of lambs for pleurisy or pneumonia".

Analytical methods

The analytical methods are described under Methods and Material of the Intervention Study of Pleurisy and Pneumonia at Farm B.

Results

Observations of diseased stock

A large lamb from the Oral_shower group had wasted away. The animal was found dead on 20 March 1995. There was pus in the lungs and the lungs stuck to the rib cage. The right hand side of the lungs was affected.

Information collected at the slaughterhouse

The number of lambs which participated in the trial, the slaughter dates and the pathology that was recorded is listed in Table 2.14. There were no lambs with acute systemic pleurisy.

The details of the gross pathology of the draft which was slaughtered on 12 May 1995 were not recorded. This draft consisted of 54 lambs of the Oral_wand group, 56 lambs of the Inject_wand group, 75 lambs of the Inject_shower group and 55 lambs of the Oral_shower group. In total 15, 13, 12 and 22 lambs of the previous groups respectively were lost on the farm and were consequently not inspected.

There was one animal (Oral_wand 7/3/95) which had two different types of pleurisy lesions plcl and plal. There was no animal with more than one type of pneumonia lesions.

Effects of treatment

The different treatments which were applied to the lambs of Farm B were examined with Chi-squared tests to evaluate their effect on pneumonia and pleurisy. Tables 1,2 and 3 of Appendix III show the p-values which resulted from the various comparisons of the cumulative prevalences of the different types of respiratory pathology.

No significant findings were detected in the category of chronic flimsy pleurisy and there were no lambs with acute systemic pleurisy. Acute localised pleurisy occurred in a number of lambs but there were no significant results in this category.

On 10/4/95 a relatively large number of lambs from the Oral-wand group was affected by chronic large pleurisy lesions and there were no lambs from the Oral_shower group with these lesions. The cumulative prevalence of the groups treated with a wand was significantly higher ($p<0.05$) than that of the groups treated by a shower on 13/6/96.

Mild pneumonia was the most frequently detected defect. However at no date was the cumulative prevalence of one of the intervention groups (drench or flystrike treatment) significantly higher than the other one at $p<0.05$.

The categories of moderate pneumonia and severe pneumonia were combined because the number of lambs with severe pneumonia was small. On 13/6/95 and on 30/8/95 the cumulative prevalence of the groups treated with a wand was higher than the groups treated with a shower. When the cumulative prevalence of pneumonia of any severity (which included included mild, moderate and severe pneumonia) was considered, the treatment by wand had a higher and shower a lower cumulative prevalence than expected on 30/8/95.

Pleurisy and pneumonia were combined to compare lambs with any signs of respiratory disease and lambs without any signs of respiratory disease. There were no significant findings with regard to the cumulative prevalences.

Table 2.14 Number of lambs that were slaughtered and respiratory pathology that was recorded at Farm B

Treatment	killed*	not	plcl	plcf	plal	pnmi	pnmo	pnse
7/3/95								
Oral_wand	25	8	2	1	1	15	1	1
Inject_wand	26	14	1	2		9	2	
Inject_shower	20	7		2		10	2	
Oral_shower	23	12	2	1		10		1
10/4/95								
Oral_wand	81	32	8	5		39	6	1
Inject_wand	83	19	5	5	2	43	17	1
Inject_shower	67	30	4	1		31	5	
Oral_shower	82	27		9	1	44	11	
13/6/95								
Oral_wand	57	10	8	8	3	32	10	1
Inject_wand	51	7	9	4		41	2	
Inject_shower	53	16	4	8	2	32	1	
Oral_shower	44	7	6	7	3	30	3	
30/8/95								
Oral_wand	18	12	5	1		2		
Inject_wand	21	10	10	1		1		
Inject_shower	23	12	9	2		1		
Oral_shower	24	14	5	5				

*The pleurisy categories are chronic large pleurisy (plcl), chronic flimsy pleurisy (plcf), acute localised pleurisy (plal) and acute systemic pleurisy (plas). The pneumonia categories are mild pneumonia (pnmi), moderate pneumonia (pnmo) and severe pneumonia (pnse).The various pleurisy and pneumonia categories are described in Appendix II.

Log-linear modelling to evaluate interactions between treatments and time of slaughter

The procedures which are used below are explained in the section on Farm A.The code 43 illustrates a temporal pattern with regards to the various forms of respiratory pathology. In the case moderate and severe pneumonia an interaction between the dip and pathology was established as well (Table 2.15).

Table 2.15 Log-linear model which evaluates the interactions between treatment and the time of slaughter at Farm B

Dependent variable with count data	Best model*	Chi-squared value	df	p-value
Any pleurisy or pneumonia	4321	0.000000	0	1.0000
Any pleurisy	43	12.25907	24	0.9768
Any pneumonia	4321	0.000000	0	1.0000
Chronic large pleurisy	4321	0.000000	0	1.0000
Chronic flimsy pleurisy	4,3	24.35034	27	0.6108
Acute localised pleurisy	4,3	16.58516	27	0.9408
Mild pneumonia	4321	0.000000	0	1.0000
Moderate pneumonia	43	25.14689	24	0.3979
Moderate and severe pneumonia	42, 43	20.33948	22	0.5618

*Codes which were used for best model were: 1 Drench, 2 Dip, 3 Date and 4 Status

Temporal Analysis of pleurisy and pneumonia

The tables which apply to the analysis of the temporal pattern are displayed in Appendix III. The statistical significance of the data was evaluated with Chi-squared tests. A high prevalence of chronic flimsy pleurisy lesions was recorded on 13/6/95 and it declined on 30/8/95. However the prevalence of chronic large lesions increased during the trial and the highest prevalence occurred on 30/8/95. The cumulative (prevalence) of acute localised lesions was relatively low throughout the trial, peaking on 13/6/95. The prevalence of pleurisy of any degree, which was a combination of the previously described types of pleurisy was high on 13/6/95 and especially on 30/8/95 where the prevalence was 0.44 and the cumulative prevalence was 0.22.

There was a high (cumulative) prevalence of mild pneumonia on 13/6/95 and lower (cumulative) prevalence on 30/8/95. There was a high prevalence of moderate and severe pneumonia on

10/4/95 and a lower (cumulative) prevalence on 30/8/95.The highest cumulative prevalence of pneumonia of any degree, which was a combination of the previously described types of pneumonia, was recorded on 13/6/95 and a lower cumulative prevalence was seen on 30/8/95. The prevalence decreased from 0.74 to 0.05.

Relationships

The table below shows combinations of pneumonia and pleurisy which were found in individual animals. See Table 2.14 for disease codes.

	pnmi	pnmo	pnse
plcl	25	12	4
plcf	34	9	1
plal	6	5	0

The number of animals, and the proportions of the total number of animals which were found to have both pleurisy and pneumonia or only one of the two conditions on post mortem inspection are shown in Table 2.16. A large number of lambs had mild pneumonia. However only 19% of these animals had pleurisy as well. In the case of pleurisy, more than 50 percent of the affected animals were affected by pneumonia as well.

Table 2.16 Combinations of pathology of lungs and pleura at Farm B

disease codes	Total	Pleurisy and pneumonia			
		Both pleurisy and pneumonia		Either pleurisy or pneumonia	
		Actual	(Proportion)	Actual	(Proportion)
plcl	78	41	(0.53)	37	(0.47)
plcf	62	44	(0.71)	18	(0.29)
plal	12	11	(0.92)	1	(0.08)
pnmi	340	65	(0.19)	275	(0.81)
pnmo	60	26	(0.43)	34	(0.57)
pnse	5	5	(1)	0	(0)
total *		95[a]		366[a]	

* 237 animals did not display any signs of pleurisy or pneumonia

[a] One animal displayed both chronic large and acute localised pleurisy as well as moderate pneumonia.

Comparison between Farm A and Farm B

During March 1995, lambs from both Farm B and Farm A were slaughtered on 7/3/95 and 1/3/95 respectively. A comparison which was made between the two farms is shown in Table 2.17.

Table 2.17 Comparison of pathology between two farms early in March 1995

	Farm A		Farm B	
Status	pleurisy	pneumonia	pleurisy	pneumonia
Affected	55	312	12	51
Not affected	364	107	82	43

An examination with Chi-squared tests of the values of the two farms for both pleurisy and for pneumonia showed that although the prevalence of pleurisy was similar ($p=0.9253$), the prevalence of pneumonia at Farm B was significantly lower than at Farm A ($p=0.0001$).

Discussion

The case-control study identified the use of a spray-on or wand as a protective risk factor with regard to the development of pleurisy. Bruère and West (1993) suggested that shower and plunge dipping may be a precipitating factor but they also state that its role in causing pneumonia is uncertain. Since the levels of stress might be of importance it was decided to compare two commonly used interventions for the prevention of flystrike, the use of a wand dip and the use of a shower dip.

There was one significant finding with regard to the cumulative prevalence of pleurisy and this occurred on 13/6/96 where the wand treatment resulted in a higher prevalence than the treatment with a shower. However it should be noted that these comparisons applied to lambs with chronic large lesions vs lambs without chronic large lesions but including lambs with other forms of pleurisy. The results should therefore be interpreted in conjunction with the results of pleurisy of any degree. No significant results were detected when comparisons were made based on pleurisy of any degree. Furthermore this result was not repeated for the last draft which was slaughtered on 30/8/95.

On 13/6/95 and on 30/8/95 the cumulative prevalence of moderate and severe pneumonia of the groups treated with a wand was higher than the groups treated with a shower. However there

were no cases of moderate or severe pneumonia on 30/8/95 and only four cases with mild pneumonia. Despite this, also the category of pneumonia of any severity was significant on this date. The log-linear model had a similar result by providing a best model consisting of dip and status for the combination of moderate and severe pneumonia. The significant findings with regard to the dip and pneumonia should be considered with caution. For example in the case of the log-linear model, despite only a very small number of lambs with severe pneumonia (five in total, of which four had received a wand treatment) they were sufficient to change the non-significant findings of moderate pneumonia and wand treatment into a significant finding of moderate and severe pneumonia and wand treatment.

There were no findings recorded in Appendix III on the cumulative prevalence with regard to drenching which were statistically significant. The issue of drenching was discussed in the previous section.

In addition to the findings that were discussed above, there were other statistically significant findings at $p<0.05$. Some of these applied to the prevalence and also some findings applied to combinations of groups which did not have a biological meaning. This was because each of the two groups of lambs which were compared contained animals from all four different groups of treatment (oral and injection drench, and wand and shower dip).

The various significant findings were not always measuring different findings. A large number of chi-squared tests were performed and at the level of $p<0.05$ a number of statistically significant results could be expected. The analyses did not appear to provide strong evidence for an effect of treatment (anthelmintics applied by drenching or injection, fly strike prevention by wand or shower dip) on the development of pleurisy or pneumonia

McGowan (1978) referred to the existence of temporal patterns of respiratory disease in lambs. The log-linear model showed evidence for an association between the date of slaughter and pleurisy or pneumonia. Temporal analyses by chi-squared tests showed that chronic large pleurisy was highest at the last date of the trial. Acute localised pleurisy had low prevalences and showed a pattern which was similar to pneumonia. There was a lower prevalence at the end of the trial. Mild and moderate/severe pneumonia showed reductions in prevalence towards the end of the trial. Mild pneumonia peaked on 13/6 while moderate/severe pneumonia peaked at 10/4. This suggests that after reaching a peak in time, pneumonia in lambs becomes less and pneumonia lesions resolve. In the case of pleurisy the lesions may not resolve or not to the same degree and pleurisy lesions resulting from pneumonia are detected at a later stage.

The comparison between pleurisy at Farm A with Farm B showed no difference while there was a considerable difference with regard to pneumonia. This is an important finding since it shows that the recording of pleurisy without considering pneumonia as well may provide imprecise impressions. This issue will be further discussed in the section on DSSs.

Analysis of Lamb Pleurisy Inspection Trials

Introduction

The MAF Disease and Defect database provides information on pleurisy in lambs by premises on a monthly basis. However this database provides limited information because on a routine basis the speed of the dressing chain makes it difficult to make detailed notes of findings. Inflammatory lesions of the pleura are recorded as pleurisy regardless of their size and whether they are acute or chronic. The system does have a special category for carcasses which are condemned for pleurisy. Inconsistencies of inspection and judgement are likely to occur. The sensitivity and specificity of meat inspection are not known.

The objectives of these trials was to acquire a better appreciation of gross pleural pathology by degree of severity during a full slaughter season, to compare these findings between premises and to establish the sensitivity and specificity of inspection procedures. Once Decision Support Systems (DSSs) become available on a wider scale, knowledge about these performance characteristics becomes increasingly important. Extrapolation of the performance characteristics from premises where this has been examined to other ones where this has not occurred may be inappropriate and might to lead to unsatisfactory performance of the DSS at the latter premises.

Material and methods

Trials were carried out in four slaughter houses: ME17 (Timaru) and ME20 (Bluff) were in the South Island while ME42 (Wairoa) and ME47 (Moerewa) are in the North Island. The data spanned a consecutive 13 month period, but each premises supplied data over a smaller number of months due to seasonal plant closures.

The pleurisy findings of all lambs were recorded on a weekly basis. On one occasion the monthly figures were provided.

Four disease categories were used during the trials.

- Chronic pleurisy Type I (Coded as "Major pleurisy")

  Description: (i) Fibrous adhesion to pleura
  Colour: Grey or white
  Extent: Usually >50mm
  Consistency: Fibrous, thickened

  Description: (ii) Thickened and/or purulent pleura
  Colour: Variable

Extent: Variable
Consistency: Variable

- Minor Pleurisy Type II pleurisy ("wipe outs") (Coded as "Minor pleurisy")
  Colour: White
  Extent: <50mm
  Consistency: Flimsy, fibrous, very little thickening of pleura.
  Indistinguishable upon drying of pleural surfaces.

- Acute pleurisy (Coded as "Acute pleurisy")
  Acute hyperaemic fibrinous with no systemic involvement.
  Colour: Pink, yellow or cloudy
  Extent: Usually > 50 mm
  Consistency: Velvety, and thickened, or gelatinous fibrin

- Septicaemia (Coded as "Septicaemia")
  Hyperaemic pleura with obvious signs of systemic involvement.

Temporal aspects of pleural pathology were examined by graphs with percentages prevalence. Pearson correlation coefficients were used to compare premises over time. These correlations indicate a degree of linear association between variables (Statistix, 1992). The underlying assumption of using these coefficients is independence of observations. The analysis of the prevalence was based on half-monthly figures. ME20 had six missing values because of its short slaughter season and this could have influenced the Pearson correlation coefficient considerably. Therefore two Pearson correlation coefficients were calculated, one with ME20 included and one without ME20.

Results of analysis of prevalence

A total of 2,504,327 lambs participated in this trial. The prevalence of pleural lesions over the entire trial period is shown in Table 2.18 on a premises basis. There are considerable differences between the four premises. ME 17 and ME 20 (South Island) have a cumulative prevalence of Major pleurisy that is much lower than the cumulative prevalences recorded at ME42 and ME 47 (North Island). The cumulative prevalence for the category Minor pleurisy is more similar. There were large differences in the category Acute pleurisy with ME47 having a cumulative prevalence which was 12 times as high as ME20. The large number of Acute pleurisy at ME 47 included 251 cases in one particular month. The number of lambs with Septicaemia was small at all premises, with no lambs recorded in this category by ME20.

Table 2.18 Prevalence of pleural lesions and the ratio of Minor pleurisy / Major pleurisy

Premises	Major pleurisy	Minor pleurisy	Minor pleurisy/ Major pleurisy	Acute pleurisy	Septicaemia (actual number)
ME17	3.68%	2.20%	0.598	0.09%	51
ME20	3.59%	1.77%	0.491	0.03%	0
ME42	9.01%	1.96%	0.218	0.11%	3
ME47	9.48%	2.87%	0.303	0.36%	48
TOTAL	6.75%	2.20%	0.326	0.15%	102

All premises showed low levels of Major pleurisy in November and December after which the prevalence starts to rise (Figure 2.7). In this Figure and the following ones, June is Month 1.

The cumulative prevalences of Major pleurisy of ME17 and ME20 at the end of the trial are considerably lower than the cumulative percentages of ME42 and ME47. However the pattern of disease on a half-monthly basis shows high Pearson correlation coefficients between all premises, suggesting similar trends at all premises (Appendix IV). The graph shows that although ME42 and ME47 are consistently higher, the high values occurred especially during the early months of the trial. This is consistent with an increase in prevalence as the season progresses. The lambs that were slaughtered early in the trials were likely to be older than the lambs slaughtered towards the end of the year and in the beginning of the next year.

The prevalence of Minor pleurisy is shown in Figure 2.8. There is a trend of increasing prevalences from January onwards. On a number of occasions the prevalence of ME47 was higher than at the other premises. ME47 displayed high levels of Minor pleurisy in the early months of the trials which was similar to Major pleurisy. The correlation coefficients of Minor pleurisy showed a high degree of correlation between all premises (Appendix IV).

The combination of Minor pleurisy and Major pleurisy shows a high degree of correlation between the premises. This is similar to the correlations when the pleurisy categories are examined individually (Appendix IV).

The ratio of Major pleurisy to Minor pleurisy was evaluated to detect differences in patterns of the chronic pleurisy categories between the premises. Figure 2.9 shows that ME17 and ME20 display a similar trend. However, whereas the ratio of these two premises increases after November-December, the opposite occurs at ME47. The correlations between ME47 on the one hand and ME20 and ME17 were negative. Throughout the season the ratio Major pleurisy/Minor pleurisy is high at ME42 and ME47 (North Island) in comparison with ME17 and ME20 (South Island).

A low level of Acute pleurisy was recorded at ME20 at all times (Figure 2.10). ME47 in contrast

consistently had the highest prevalence. A relatively high degree of correlation could be detected between ME42 and ME47 (Appendix IV). Low correlations existed between ME17, ME20 and the other two premises.

No Septicaemia was recorded for ME20 (Figure 2.11). This is consistent with its low number of Acute pleurisy lesions. The numbers of animals with Septicaemia was small at all premises which may make the graph with regards to trends misleading. In a number of cases there were not sufficient lambs to calculate correlation coefficients (Appendix IV).

When Acute pleurisy and Septicaemia were combined there appeared to be a relatively high degree of correlation between ME42 and ME47.

Discussion of analysis of lesion types

McGowan *et al.* (1978) reported an increase in pleurisy in lambs from January onwards. These findings are confirmed in this study. This study showed that most of the pleurisy lesions in slaughter lambs are of a chronic nature. The increasing trend applied both to Major pleurisy and to Minor pleurisy. McGowan *et al.* (1978) also reported that lambs in the North Island had a higher prevalence than lambs in the South Island. Although only two premises from each island were included in this study a similar pattern emerged. It is of interest to note that although the prevalence at the premises differed, especially with regard to Major pleurisy, similar trends could be seen at these premises by calculating the correlation coefficients.

Davies (1985b) states that acute fibrinous pneumonia usually occurs in January, February and March and most commonly in Northland. Sporadic outbreaks and individual cases also occur throughout New Zealand. Farmers are discouraged from submitting diseased stock for slaughter and the number of septicaemic lambs was very low. However lambs that recovered will have contributed to the various pleurisy lesions at a later stage during the season. The extent of this remains unknown.

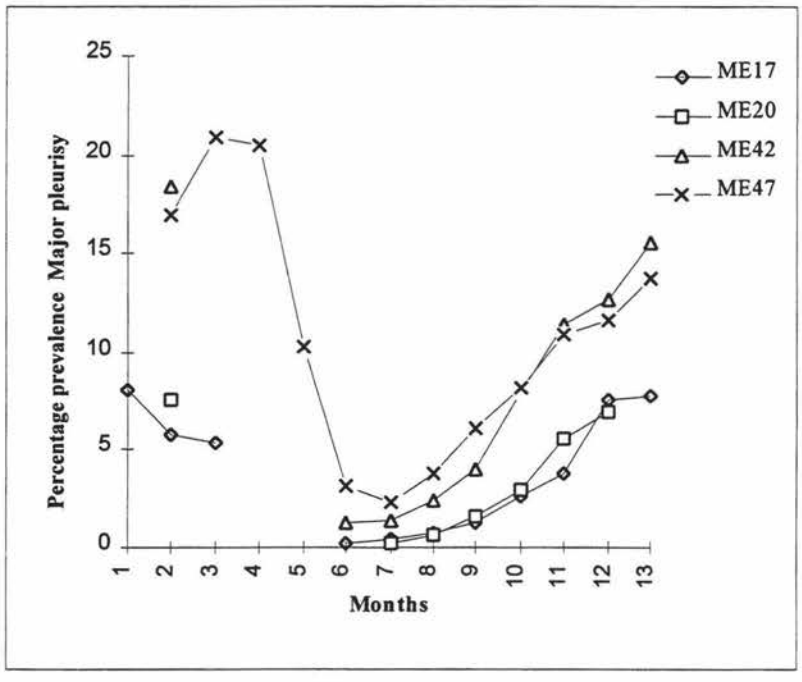


Figure 2.7 Percentage prevalence of Major pleurisy at four slaughterhouses

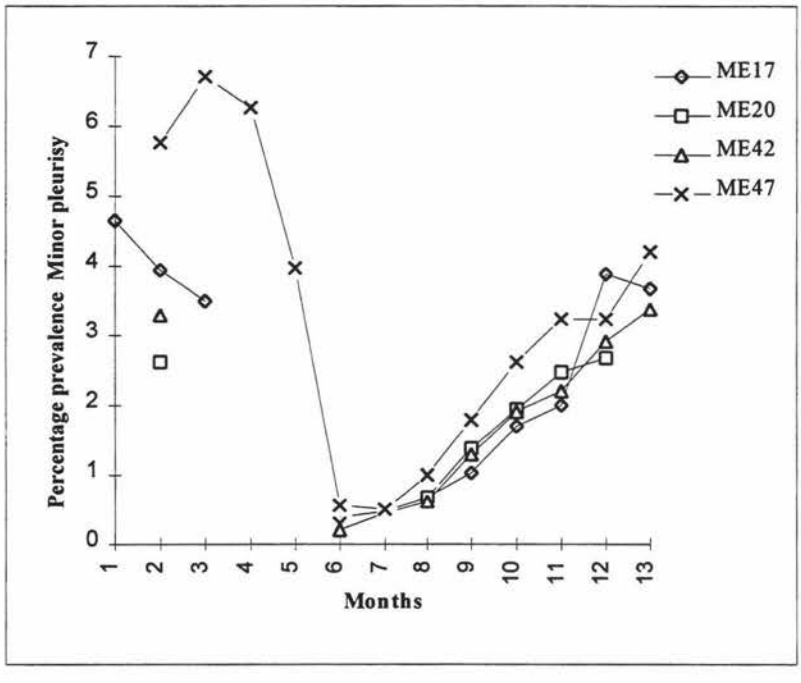


Figure 2.8 Percentage prevalence of Minor pleurisy at four slaughterhouses

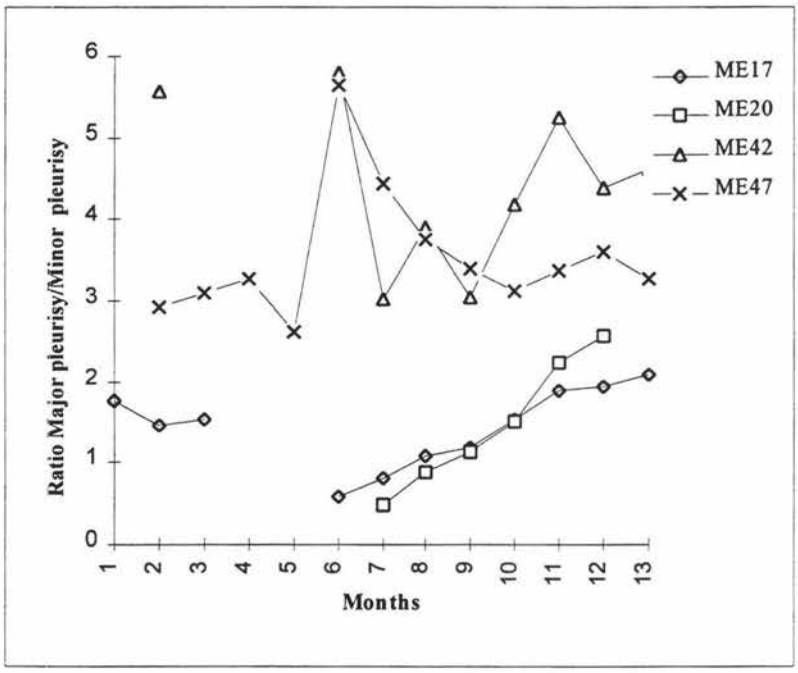


Figure 2.9 The ratio of Major pleurisy/Minor pleurisy at four slaughterhouses

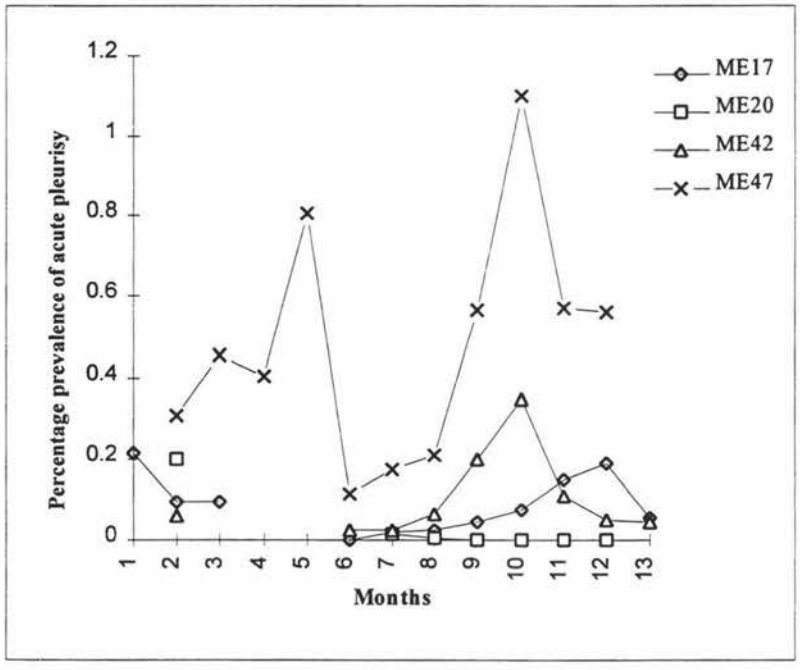


Figure 2.10 Percentage prevalence of Acute pleurisy at four slaughterhouses

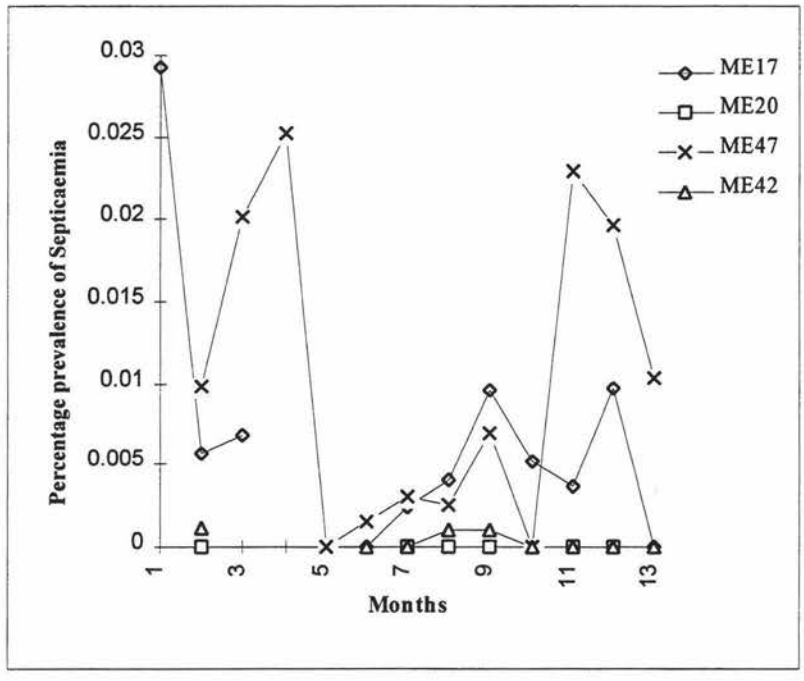


Figure 2.11 Percentage prevalence of Septicaemia at four slaughterhouses

Materials and methods for sensitivity and specificity

See materials and methods above for the description of the lesion types.

In addition to routine inspection, detailed inspection of 30 lambs was performed on a daily basis at premises ME17, ME20, ME42 and ME47 during one season. At times a smaller or a larger number of animals were inspected for the purpose of these trials. The sensitivity and the specificity of meat inspection for pleurisy were established by comparing the results of routine and detailed inspection. The groups of 30 carcasses were selected at the inspection stand. They were detained as a group. A recorder noted the tickets that were applied on routine inspection and then removed them so that the results would not be known on detailed inspection.

The following meat inspection performance indicators were calculated:

- Sensitivity (%) a / a+c
- Specificity (%) d / b+d
- Non detection rate per 1000 carcasses inspected 1000 (c / c+d)
- Wastage rate per 1000 carcasses inspected 1000 (b / c+d)

The codes (a, b, c and d) are explained in Table 2.19.

Table 2.19 Codes used for determining meat inspection performance characteristics

Test result	Disease present	Disease absent	Total
Disease detected	a	b	a+b
Disease not detected	c	d	c+d
Total	a+c	b+d	a+b+c+d

The wastage rate is the number of carcasses which are incorrectly diagnosed per 1000 inspected carcasses and is similar to the concept of false positives. The non detection rate is similar to the concept of false negatives.
In a number of carcasses, pleurisy was initially detected by the inspector on routine inspection but it may have been classified in a manner which was deemed incorrect by the evaluator (eg Minor pleurisy was considered Major pleurisy by the evaluator) . For the purpose of pleurisy detection these carcasses will be considered as having been correctly identified as carcasses with pleurisy. However for the purpose of the various categories (eg Major pleurisy) these carcasses will be considered as if the disease had not been detected.

Results of sensitivity and specificity analysis

A total number of 14,417 lambs received intensive inspection.

A number of statistics were calculated to evaluate inspection performance with regard to the various pleurisy categories. The meat inspection performance characteristics for pleurisy regardless of the category (eg Minor pleurisy) are shown in Table 2.20. Pleurisy in this case includes all categories. Where the initial inspector and the evaluator had a different opinion regarding the category of pleurisy it was still considered 'pleurisy' for the purpose of this analysis. The sensitivity varied between 0.8018 and 0.9696. The specificity was greater than 0.9950 in all cases. The maximum non detection rate per 1000 carcasses was 32 carcasses.

Table 2.20 Meat inspection performance characteristics of pleurisy

Premises	Sensitivity	Specificity	Non detection rate per 1000	Wastage rate per 1000
ME17	0.9696	0.9959	19	4
ME20	0.8050	0.9997	12	0
ME42	0.8018	0.9956	32	4
ME47	0.8842	0.9972	28	2
Total	0.9225	0.9974	22	3

The sensitivity for minor pleurisy was lower for all premises (Table 2.21). At one premises the sensitivity decreased to 0.3960. The specificity remained high.

Table 2.21 Meat inspection performance characteristics of Minor pleurisy

Premises	Sensitivity	Specificity	Non detection rate per 1000	Wastage rate per 1000
ME17	0.9058	0.9979	7	2
ME20	0.6040	1.00	1	0
ME42	0.3960	0.9960	26	4
ME47	0.5556	0.9992	35	1
Total	0.7821	0.9985	20	1

Table 2.22 Meat inspection performance characteristics of Major pleurisy

Premises	Sensitivity	Specificity	Non detection rate per 1000	Wastage rate per 1000
ME17	0.9394	0.9993	15	1
ME20	0.8201	0.9997	6	0
ME42	0.8348	1.00	17	0
ME47	0.8812	0.9987	22	1
Total	0.9035	0.9994	14	1

Table 2.23 Meat inspection performance characteristics of Acute pleurisy

Premises	Sensitivity	Specificity	Non detection rate per 1000	Wastage rate per 1000
ME17	.9027	.9927	2	0
ME20	1.00	1.00	0	0
ME42	.2857	1.00	2	0
ME47	.7500	1.00	1	0
Total	.8571	.9999	1	0

The sensitivity for Major pleurisy was higher than for Minor pleurisy (Table 2.22). At all premises the specificity of inspection remained high. The wastage rate per 1000 carcasses also remained low for all categories. It should be noted that the actual number of cases with Acute pleurisy (Table 2.23) was extremely small for ME20.

Discussion of sensitivity and specificity analysis

Generally the specificity of inspection was very good. It should however be noted that the "gold standard" consisted of another inspector who took more time and effort to make a definitive diagnosis. This was for practical reasons.

The sensitivity for Minor pleurisy varied considerably from 39.6% to 90.6%. The sensitivity of Major pleurisy varied from 82.0% to 94.0%, reflecting the larger size of this lesion. The sensitivity of Acute pleurisy displayed variation between 28.6% and 100%. It should be considered that these percentages applied not only to the detection of the defect but also to the correct classification of the defect. The sensitivity of pleurisy of any size or degree of acuteness

varied between 80.2% and 97.0%. In conclusion, the sensitivity of pleurisy regardless of the category was higher at each premises than for the specific categories.

Although sensitivity and specificity are commonly used in many serological tests, examples in the literature of performance characteristics of inspection procedures are scarce. Hathaway and McKenzie (1991) emphasised the necessity of establishing these characteristics for the different inspection procedures for all tissues from each class of slaughtered livestock for validating current national codes of meat inspection. The use of DSSs places an additional dimension to the urgency for establishing these characteristics. As this trial has shown, the sensitivity for a certain defect in a wide sense (pleurisy) may be satisfactory, but the sensitivity may become unsatisfactory when a classification is considered at the same time (eg Minor pleurisy at ME42).

There are currently moves underway within the New Zealand Ministry of Agriculture to routinely compare prevalence figures between premises. Standard queries of databases can be used for this purpose and they can become part of a DSS. Differences in prevalence can occur due to conditions such as the climate or farm management, or due to inspection standards. Once differences are detected between premises attempts should be made to establish in the first place whether these differences occur due to differences in inspection standards or for other reasons.

Analysis of Pleurisy Data of the MAF Disease and Defect Database

Introduction

The MAF Disease and Defect (D&D) database collects prevalence and condemnation figures for specified gross pathological diseases including pleurisy. The data are gathered from all premises that slaughter animals for sale for human consumption. Since it has been in place for an extended period of time, analyses of the significance of differences in pleurisy levels can be made. The D&D database only addresses the prevalence of pleurisy and, if generalised, the condemnation of carcasses. A proportion of the lambs which were condemned for pleurisy is likely to have had acute fibrinous pneumonia. However more information on this relationship is required.
In the previous section a trial was described at four premises where detailed inspection data was collected. For this reason the analysis below of the pleurisy from the D&D database concentrated on these four premises. The objectives of this section are to examine whether or not similar temporal patterns occur at various premises, regions and the two main islands, and what the extent is of the differences of their levels of prevalence and percentage condemnations. It has been suggested at times that a single sentinel premises can be used to evaluate the prevalence of a disease in a geographic area instead of all premises in that area. This section will examine the consequences of such an approach with regard to pleurisy.

Materials and methods

The analyses applied to lambs which were slaughtered in meat export slaughter houses over the period 1/10/81 - 30/9/92. The premises of major interest were ME047 (Moerewa) and ME042 (Wairoa) in the North Island, and ME017 (Smithfield, Timaru) and ME020 (Ocean Beach, Bluff) in South Island.

For comparative reasons four regions were created. They consisted of premises which were relatively close to the above four premises and these premises themselves. The region "M" is close by ME047, region "W" close by ME042, region T close by ME017 and region "O" close by ME020 (Table 2.24). In addition "N" denotes the North Island and "S" the South Island.

The difference in percentage prevalence between the islands was examined by considering their percentages and the different levels were quantified with a linear regression which contained the 132 months as cases.

The prevalence and the percentage condemnations for pleurisy in regions M, W, O, T were calculated. They were also calculated for the North Island excluding regions M and W (coded as "N Rest") and the South Island excluding regions O and T (coded as "S Rest"). The percentages of the pleurisy prevalence and of lambs condemned for pleurisy were compared.

Table 2.24 Premises of which pleurisy data were compared

Region M	Region W	Region T	Region O
ME035	ME008	ME014	ME020
ME047	ME010	ME016	ME021
ME063	ME029	ME017	ME022
ME103	ME042	ME018	ME050
	ME058	ME026	ME080
	ME065	ME034	ME112
	ME087	ME037	
		ME041	
		ME069	
		ME078	

Similar calculations and comparisons were performed for the four premises of interest (ME017, ME020, ME042 and ME047), and their neighbouring areas.

The degree to which two neighbouring premises could predict the prevalence of pleurisy at trial premises was evaluated with Pearson correlation coefficients and with linear regressions. The data points consisted of the monthly prevalence figures during the period 1/10/81 - 30/9/92.

Linear regressions were performed to evaluate the different levels of pleurisy prevalence of the four trial premises, and between these premises and two of their neighbouring premises.

Linear regressions were performed to evaluate the importance of the month of the year (October, November etc.) and the actual year (1982, 1983, etc.) to predict the actual prevalence for a particular month (eg October 1982). The average of the respective months (October, November, etc) was calculated and the average of the respective years (1982, 1983, etc.) The dependent variables consisted of the actual prevalences as recorded at the four premises (ME017, ME020, ME042, ME047) while the independent variables consisted of the monthly and yearly averages of these respective premises as appropriate. Therefore the actual percentage for a month contributed to both the monthly and the yearly average of a premises. The Kruskal-Wallis one-way ANOVA performes a non-parametric one-way ANOVA. In practice it is typical to assume that if the null hypothesis is rejected, the differences are due to differences of the central values of the groups (Statistix, 1992).

Results

Comparison between islands

The number of lambs slaughtered, the number of carcasses with pleurisy, and the carcasses condemned for pleurisy are shown in Table 2.25. The ratio of the prevalences of pleurisy for the North Island and the South Island equals 1.76. However, the ratio of the percentages of condemned lambs for the North Island and the South Island equals 1.

Table 2.25 Number of slaughtered lambs and percentage prevalence by island

Island	Slaughtered	Pleurisy	%Pleurisy	Condemned	%Condemned
North Island	146,513,129	11,967,792	8.17%	15,456	0.011%
South Island	196,988,017	9,142,200	4.64%	21,234	0.011%
Total	343,501,146	21,109,992	6.15%	36,690	0.011%

The different prevalence levels of the North Island (PrN) and the South Island (PrS) as quantified with a linear regression was:

$$PrN = 0.01 + 1.52 * PrS \quad (R\text{-squared} = 0.77)$$

This regression illustrates the large difference in prevalence between the two islands over the 11 year period.

Comparison between regions

The number of slaughtered lambs, the number of lambs with pleurisy whether condemned or not are shown in Table 2.26. The three areas created this way in the North Island each had a prevalence greater than 7.8% while the three areas in the South Island were each smaller than 5.0%. This reflected the differences seen in the previous section. The condemnation percentages showed large differences which were not evident in the comparison between the islands. The bottom part of the South Island (O) showed a very low percentage while S rest (ie South Island without area O and T) showed a high level. The ratio between the two areas equalled 0.007/.020= 2.9.

Table 2.26 Number of slaughtered lambs and percentage prevalence by region

Area	Slaughtered	Pleurisy	%Pleurisy	Condemned	%Condemned
N Rest	73,835,548	6,159,254	8.34%	6,728	0.009%
M	12,941,619	1,016,741	7.86%	1,872	0.014%
W	59,735,962	4,791,797	8.02%	6,856	0.011%
S Rest	20,593,518	869,115	4.22%	4,100	0.020%
O	68,216,985	3,345,935	4.90%	4,444	0.007%
T	108,177,514	4,927,150	4.55%	12,690	0.012%

Comparison between premises and their regions

The four premises were compared with other premises in their region (Table 2.27). Both ME042 and ME047 had considerably higher prevalences than the other premises in their area (W and M respectively). This may be a reflection of the areas chosen being large or diverse with regard to risk factors. Another possibility is that meat inspection standards may vary between premises. The percentage condemnations showed large differences between premises and other premises in their vicinity. Area W had a condemnation percentage which was four times as high as that of ME042. It should be noted that these comparisons included periods over which some premises have been closed down. However the lambs could have been slaughtered by other premises in the vicinity.

Predictions of the prevalence of pleurisy based on values of other premises

The Pearson correlation coefficients and the coefficients and p-values of linear regressions which were used to evaluate to what degree two neighbouring premises could predict the prevalence of pleurisy of the trial premises are listed in Appendix V. This appendix also contains a number of simple linear regressions which model the relationship between the four trial premises and between each of these premises and two of the neighbours.
ME042 showed a better correlation with other premises, both in its own area and in the rest of the country. The higher prevalence of ME042 than premises in the South Island was reflected in the higher coefficients. ME017 had R-squared values which were similar to ME042. The R-squared value of ME020 with ME022 as the independent variable was 0.57 only.

Table 2.27 Percentage prevalence by slaughterhouse and neighbouring area

Area	%Pleurisy	%Condemned
ME017	4.86%	0.005%
T excluding ME017	4.53%	0.012%
ME020	4.99%	0.003%
O excluding ME020	4.88%	0.007%
ME042	10.78%	0.003%
W excluding ME042	7.64%	0.012%
ME047	10.52%	0.007%
M excluding ME047	6.05%	0.019%

Effect of month and year

The importance of the effect of the month of the year (eg October, November) and the actual year (eg 1982, 1983) on the prevalence of pleurisy were examined with linear regressions (Table 2.28). The regression coefficients of the analyses of the months and of the years varied between 0.99801 and 1.0000 (data not shown). ME047 had two outlying values (prevalence > 0.35). If these were removed, but average month or year were not corrected, the R-squared values for the month, year and both became 0.5096, 0.0151 and 0.5317 respectively. The above analyses showed the importance of the month for prediction purposes. Although the years were statistically significant, their impact on the actual prevalence was relatively unimportant.

The effects of the years (eg 1982, 1983) were also assessed by using Kruskal-Wallis one-way non-parametric ANOVA (Table 2.29). This test suggest that in the cases of ME017, ME020 and ME047, the means of the years based on the monthly prevalences are not different. This means there are no years with months that are consistently higher than the months in other years. In the case of ME042, after the removal of one year with the highest values, statistically significant figures could no longer be detected at the $p < 0.15$ level.

A similar approach as above was taken, for comparing the means of the 11 years of two premises in the lower half of Table 2.30. The yearly means of pleurisy prevalences of ME017 and ME020 (both South Island plants) are not significantly different. The same applies to ME042 and ME047 (both North Island plants). However combination of a North Island and a South Island plant leads to significant differences.

Table 2.28 P-values and R-squared values of linear regressions to evaluate the month and year factor

Factor	ME017	ME020	ME042	ME047
Month	p 0.0000 r^2 0.8065	p 0.0000 r^2 0.7903	p 0.0000 r^2 0.7411	p 0.0000 r^2 0.3321
Year	p 0.05 r^2 0.0416	p 0.0836 r^2 0.0384	p 0.0000 r^2 0.1617	p 0.0036 r^2 0.0650
Month and year	r^2 0.8220	r^2 0.8215	r^2 0.8496	r^2 0.3946

Table 2.29 Kruskal-Wallis one-way non-parametric ANOVA to analyse year effects

Premises	Kruskal-Wallis statistic	p-value
ME017	3.6395	0.9621
ME020	3.5191	0.9401
ME042	18.9201	0.0413
ME042 excluding 1990	12.7298	0.1752
ME047	5.2799	0.8717
ME017 ME020	7.7450	0.9934
ME017 ME042	48.9693	0.0005
ME017 ME047	63.3122	0.0000
ME020 ME042	50.0161	0.0002
ME020 ME047	63.0944	0.0000
ME042 ME047	26.7534	0.1792

Discussion

McGowan *et al.* (1978) reported a substantial difference in the prevalence of pleurisy between the North Island and the South Island, but also mentioned that they only analysed one year's data. The data of 11 years are analysed above and it confirms the difference. However the percentages prevalence in this study were considerably higher. McGowan *et al.* reported only 4.5 percent in the North Island and 1.9% in the South Island. It is not clear what causes the difference. McGowan *et al.* were not able to draw any conclusions regarding pleurisy within each island. The issue of shifting animals from region to region and from island to island as mentioned by them, will also have occurred in this study. However the large number of lambs that were inspected in each of the six areas gives confidence that conclusions can be drawn from this. All three areas in the North Island had a prevalence more than 7.5%. All three areas in the South Island had a prevalence of less than 5%. It is appreciated that the way the region "N rest" and "S rest" had been created was to some degree arbitrary.

Davies (1985b) states that acute fatal pneumonia is most common in Northland in January, February and March, but sporadic outbreaks and individual cases occur throughout New Zealand. Bruère and West (1993) assumed that the majority of pleural lesions reported from slaughter surveys are a consequence of subclinical pneumonia. This leaves the challenge to explain the difference between the prevalences of pleurisy of the North Island and the South Island. The case-control study looked at management and farm-related risk factors to explain large pleural lesions. It seems plausible that factors related to the geography and/or climate also play a role. Manktelow (1984) suggested that dry dusty conditions might be initiating or exacerbating factors for chronic non-progressive pneumonia. McIlroy *et al.*(1989) showed an association between pneumonia, and wind chill and rain. Alley (1991) suggested that the association could have been due to sheep behaviour such as huddling together or housing. However this study did not show large variations in pleurisy levels between the various years at individual premises. One might expect this to occur if pneumonia and pleurisy are related to the weather. This might indicate that climate plays at most a minor role. However the comparison between Farm A and farm B in an earlier section lead to caution when trying to extrapolate the prevalence of pleurisy to the prevalence of pneumonia.

A number of analyses were done to evaluate how representative a premises can be for a region and whether other premises can be used to predict the prevalence for this premises. The premises in the South Island had prevalence levels which were relatively close to those of the areas around them. However large difference existed for the North Island premises. Also the correlation between ME047 and ME063, two neighbouring premises was very low. These findings should be seen as a warning against using individual slaughterhouses as being representative for a large area without further research. Several reasons for the differences could be considered. A number of risk factors apply and the regions that have been chosen based on proximity of premises may have had a different degree of homogeneity with regard to risk factors. Premises can source their animals from a large area and the risk factors which apply to parts of an area could be relevant. In the previous section differences in sensitivity of meat inspection were demonstrated.

The issues that have been raised above are of particular importance when DSSs are used. There

will be a need for much data to make a DSS operational and it will be tempting to make use of available data, for instance from neighbouring premises. At times this approach may be appropriate, but it will be important to verify first that the data are appropriate for the premises from which the DSS operates.

Decision Support Systems to Evaluate Pleurisy in Slaughter Lambs

Introduction

Data regarding findings of post mortem inspection are becoming more readily available in many premises. In some cases automated advice is provided to farmers if their animals are displaying levels of a disease which are considered too high. In order to use this data correctly, certain conditions need to be fulfilled otherwise incorrect conclusions might be drawn. In this study pleurisy and pneumonia in lambs will be used as an example for the development of a Decision Support System (DSS) which can be used in the meat industry to maximise benefits for all parties involved.

Currently all pleurisy lesions are recorded by a meat inspector. These lesions can result from acute fibrinous or chronic non-progressive pneumonia. In the case of acute fibrinous pneumonia many farmers will have been aware of the existence of the disease in their flock because of its dramatic signs. The existence of chronic non-progressive pneumonia is usually not clear to farmers and a problem is frequently only suspected if expected weight gain does not occur.

Several stakeholders will benefit from a DSS and for different reasons:

Farmers

Is there going to be a problem?
Has there been a problem?
What are the options for prevention and how do the costs and benefits compare?

Meat processors

What is the cost of processing?
Who causes the problems?

Consumers

How effective are food safety procedures and how can the ratio of benefits to costs be maximised?

The components of the DSS which are outlined below draw on the knowledge that is currently available. In the case of carcasses which have been downgraded for pleurisy some risk factors are known and some predictions can be made. There are still considerable gaps in the knowledge regarding pneumonia and pleurisy, especially when they are not severe. The case-control study identified factors which were associated with the development of pleurisy and subsequent downgrading of product. This knowledge has been used to predict problems of carcasses being downgraded. Certain aspects of the case-control study warrant consideration. Due to circumstances (receivership of a meat company) no data are available from the South Island. Furthermore the questionnaire had to be phrased in such a way that farmers were not discouraged

from responding. The questionnaire contained six pages, which was the maximum size considered acceptable to most farmers. A number of potential risk factors are difficult to quantify or might be seen as an insult or embarrassing. For instance the control of dogs by shepherds is important but the evaluation of this procedure by questionnaire is difficult. As a result, the DSS's ability to predict pleurisy problems will be limited at this stage in its progressive formulation.

Work by Alley (1987) has shown that chronic non-progressive pneumonia results in a lower growth rate. A clear seasonal pattern with regard to pneumonia and pleurisy in lambs has been shown in the intervention studies of this thesis, the analysis of the MAF Disease and Defect database and in other studies. The analysis of the MAF D&D showed differences between the North and the South Island which have not been adequately explained yet. Between regions and premises differences can also be distinguished. The differences may be to some degree be explained by such local factors as the climate and differences in sensitivity of meat inspection. This could be seen as an incentive to ensure that DSSs have data as shown to be applicable to a location. The DSS for the existence of pleurisy will have two main benefits to farmers. It will identify whether their level of pleurisy (of all degrees of severity) is higher than 'expected'. Also it will predict how the levels of pleurisy will increase as slaughter is postponed. Therefore this component of the DSS will mainly be of commercial interest to farmers and meat processors. An example of the effect of food safety procedures is explored in a section which models the effect of cross-contamination and hand washing.

DSS for pleurisy of any degree of severity

A DSS can alert a farmer that pleurisy in his lambs is higher than expected. This may trigger off an evaluation of factors at the farm which may affect pleurisy levels. Currently the following factors could be included in this component of the DSS.

- the month of slaughter,
- the performance of other farmers from the same area,
- pleurisy prevalence in previous years.

The relationship between the time of the year (ie the season) when the lambs are slaughtered and their age at this time is not clear. These two factors may have a different impact on the development of pleurisy.

Procedures

- Establish the size of the farm which will be investigated, eg 800-1000 lambs slaughtered per season. The size of a farm may influence its slaughter pattern. Therefore it was decided to make the farm size an input factor of the model.

- Determine the area with which the farm in question should be compared. This will initially take place based on the geography and climate of a region.

- An evaluation is made subsequent to the above, to ensure that the farms in the area which was established above can be considered as one relatively homogeneous population. A simple way of doing this is to calculate the cumulative pleurisy prevalence percentages of all participating farms and display this in a histogram. The same will be done for farms of the various regions of the above area. Histograms are created again. The histogram of the whole area is then compared with the histograms of the various smaller areas A comparison between Farm A and Farm B in a previous section showed that comparisons between pleurisy levels and pneumonia levels of farms need not lead to the same conclusions. The DSS outlined below will only use pleurisy data since pneumonia data are not available.

It has been a common procedure to compare the point prevalence of pleurisy of a farm in one particular month with the prevalence of all farms of a region that slaughtered lambs in the same month. Below, the use of cumulative and of actual figures will be explored. The number of lambs slaughtered by a farm may be relevant when determining the confidence one can have in the degree of disease. When comparing the cumulative percentages of a farm and its area, farms with pneumonia problems are more likely to slaughter their lambs later in the season than other farms. Therefore a comparison of cumulative or actual figures may also lead to incorrect conclusions if the number of animals that have been slaughtered are not included in the DSS. Below examples are given of various approaches. Some of the procedures have been provided only to illustrate differences in approach and they may be unsatisfactory.

The objective of the discussion which follows is to consider various levels of complexity and data requirements for decision support tools which might be used in a slaughterhouse. Taking the example of pleurisy, a range of approaches from the very simple (but quite imprecise) up to the statistically more precise (but more demanding) are examined to illustrate the principles of developing such tools. Within the scope of this thesis, it is not possible to develop a working version of such a DSS, but the principles are illustrated using a spreadsheet model to demonstrate the processes - though not the nature of the user interface which would be adopted.

Over time it is envisaged that a series of DSS tools for different diseases could be developed. Some would be at the simple end of the range, others would use research to produce much more powerful guides to farmer action. At this stage, it will be necessary to take some practical examples and use them to decide which level of sophistication of DSS tools have the best sensitivity and specificity in diagnosing herd and flock problems from slaughter data.

Comparison of the point prevalence and cumulative prevalence

A set of fictitious data which is displayed in Table 2.30 will be used to illustrate components of the DSS. The performance of Farm A in 1993 was evaluated by comparing its point prevalence with that of other farms from the same area (Appendix VI, Figures 1-5). The prevalence of a month of Area X is multiplied by the number of animals killed by Farm A to acquire the figures for "All Farms". The actual slaughter pattern of farms in Area X is not considered at this stage. These tests showed that there were no statistically significant findings ($p<0.05$). The performance of Farm A was similar to the average performance of All Farms.

Table 2.30 Fictitious data of pleurisy and slaughtered lambs of Farm A and Area X

Month	Farm A				Area X			
	Pos. 1992	Kill 1992	Pos. 1993	Kill 1993	Prev. 1992	Kill 1992	Prev. 1993	Kill 1993
10					0.0990		0.0780	
11					0.0530		0.0230	
12					0.0200		0.0120	
1			6	100	0.0210		0.0170	300
2	11	300	14	200	0.0340	600	0.0300	300
3	20	300	20	300	0.0680	300	0.0510	300
4	25	200	20	200	0.0870		0.0780	
5	11	100	25	200	0.0970		0.0950	
6					0.1100		0.1100	
7	24	100			0.1180		0.1230	
8					0.1120		0.1230	
9					0.1310		0.1220	

Month month of the year

Farm A

Pos. 1992 the number of lambs of Farm A with pleurisy in 1992
Kill 1992 the number of lambs slaughtered by Farm A in 1992
Pos. 1993 the number of lambs of Farm A with pleurisy in 1993
Kill 1993 the number of lambs slaughtered by Farm A in 1993

Area X

Prev. 1992 mean prevalence of pleurisy in Area X of farms which slaughtered 800-1000 lambs in 1992
Prev. 1993 mean prevalence of pleurisy in Area X of farms which slaughtered 800-1000 lambs in 1993
Kill 1992 slaughter pattern of farms in Area X in 1992
Kill 1993 slaughter pattern of farms in Area X in 1993

Based on the information in Table 2.30 the cumulative prevalence figures were calculated (Appendix VI, Table 1). Another assessment was made by comparing the cumulative numbers of affected and not affected lambs. It was assumed other farms of the same size would submit the same number of lambs per month and their prevalence was calculated as the average prevalence of the region. Therefore the tests to evaluate the performance of Farm A (Appendix

VI, Figures 6-10) do not consider the slaughter pattern of Area X. This shows that the approach based on cumulative prevalence has significant values ($p<0.05$) whereas the point prevalence does not.

A component of a DSS has been developed in Excel. This requires the entry of the number of lambs slaughtered from a farm, its number of lambs with pleurisy and the (cumulative) percentage of lambs overall with pleurisy in a region. The chi-squared value and the p-value will be returned (Figure 2.12). The bold figures are the figures that need to be keyed in. All other figures will appear automatically.

		Evaluation of the existence of pleurisy problems					
	Chi-squared		INPUT			Regardless of kill pattern	
			Pleurisy	Negative	Total		
Percentage region		Farm A	2	298	300	0.5	
2		Region	6	294	300	0.5	
			8	592	600	1	
			0.013333	0.986667	1		
			EXPECTED VALUES				
			Pleurisy	Negative			
		Farm A	4	296		chi-sq	2.027025
		Region	4	296		p value	0.154523

Figure 2.12 Example of a component of a DSS to evaluate the existence of pleurisy problems regardless of slaughter pattern

Comparison of cumulative prevalence including consideration of stock slaughter pattern

The above two approaches have the disadvantage that farms that slaughtered lambs late in the season may do so because of pneumonia problems. As a result the initial results during the season will appear to be favourable on spurious grounds. The inclusion of slaughter patterns of lambs may alert farmers to problems.

Based on the example of Table 2.30, the average lamb slaughter pattern for farms in Area X that slaughtered 800-1000 lambs was 300 in Month 1, 2 and 3 of 1993. The number of lambs with pleurisy was five, nine and 15 and the cumulative number of lambs with pleurisy was five, 14 and 29 respectively. The chi-squared tests (Appendix VI, Figures 10-15) will again compare the performance of Farm A with other farms in Area X, but now having regard to the slaughter patterns.

Since farm A may keep diseased animals until later in the season, the healthy lambs that were slaughtered early may provide a biased impression. This would become obvious at the end of the season (in this example Month 5), but not early in the season. The figures that show the numbers of lambs slaughtered on a monthly basis may assist in acquiring more accurate knowledge of the disease status of the animals. Therefore the chi-squared tests of slaughter patterns only

(Appendix VI, Figures 15-20) in addition to the previous tests will enhance the understanding of the problem.

A component of a DSS was developed in Excel which requires the entry of the number of lambs slaughtered from a farm and from a region, the farms number of lambs with pleurisy and the cumulative percentage of lambs with pleurisy in a region. The chi-squared value and the p-value will be returned. The component will also advise whether the pleurisy and slaughter performance of the farm are good. The bold figures need to be keyed in. All other figures and comments will appear automatically

			Evaluation of the existence of pleurisy problems							
		Chi-squared		INPUT		Different kill pattern				
				Pleurisy	Negative	Total				
Cumulative percentage region			Farm A	**12**	688	**700**	0.7			
	5		Region	15	285	**300**	0.3			
				27	973	1000				
				0.045	1.621667					
				EXPECTED VALUES						
				Pleurisy	Negative					
			Farm A	19	681		chi-sq	8.629878		
			Region	8	292		p value	0.003307		
			Pleurisy performance		Good performance					
			Slaughter performanc		Good performance					
			To be read with graphs to assess statistical significance of slaughter performance.							

Figure 2.13 Example of a component of a DSS to evaluate the existence of pleurisy problems with consideration of the slaughter pattern

Instead of comparisons with chi-squared tests, a graph with cumulative figures for both the number of slaughtered lambs and the number of lambs with pleurisy can be created for an area (Figure 2.14). The top line shows the mean cumulative percentage of pleurisy to which three standard deviations have been added. The bottom line shows the mean cumulative percentage of the slaughter figures minus three standard deviations. For both lines, the values for the last month have been set to one and the cumulative percentages of the previous months have been amended accordingly. A farmer will make two entries each month, one of the number of lambs slaughtered and one of the number of lambs affected by pleurisy. If both cumulative values of a farm fall between the lines, its pleurisy and slaughter patterns will be considered normal. If one or both values fall outside the lines, the farm may be performing better or worse than other farms. The recording above is based on a slaughter season starting in October/November. The lambs over this period usually show a high level of pleurisy and it may be more appropriate to start recording in December each year. This way the top line will start at a lower level.

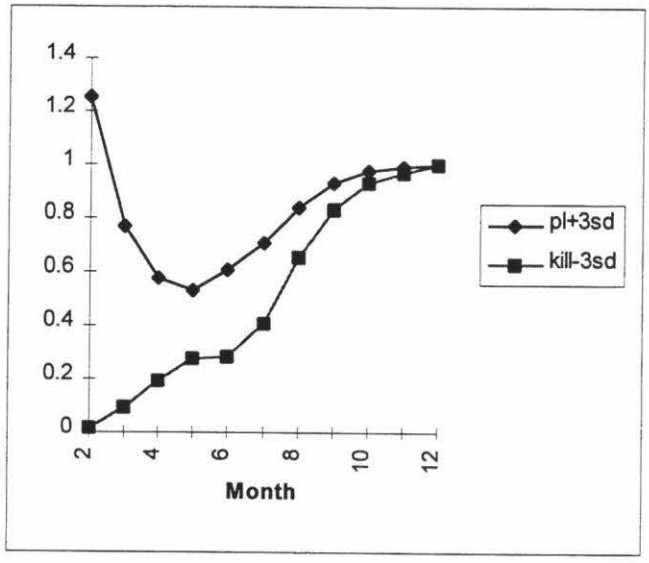


Figure 2.14 Graphical assistance for farmer to evaluate his performance with regard to pleurisy and slaughter of his lambs

Performance of a farm over time

The performance of Farm A in comparison with the other farms in Area X over time was evaluated by using ratios. Table 2.31 contains the figures for the numerators and denominators of these ratios. In Month 1 of 1992 no lambs were slaughtered and therefore no comparison could be performed. The pleurisy situation on Farm A was worse in Month 2 of 1993 than in Month 2 of 1992 when compared with other farms in Area X. The ratio increased from 1.33 to 3.5. The slaughter ratio remained the same over the 2 years. During both years, Farm A still had more lambs on the farm than the other farmers in area X. The Pleurisy ratio of Month 3 of 1993 (2.33) was better/lower than the pleurisy ratio of Month 2 of 1993 (3.5) which was the previous month. Therefore Farm A was getting closer to its target of being an average farm in Area X. However it was worse/higher than Month 3 of 1992 (1) which was the same month one year ago. The slaughter ratio increased from 0.45 (Month 2) to 0.6 (Month 3) in both 1992 and 1993. Note that it is preferable for this farm to increase the slaughter ratio (ie slaughter animals as early as possible) to decrease the pleurisy ratio.

Based on the above a farmer can evaluate whether or not the pleurisy levels of the lambs are satisfactory compared with other farmers this year. He can also determine whether he is improving on last year. The size of the ratio will provide an indication as long as the range of animals slaughtered (800-1000) remains unaltered. No p-value has been used to indicate the significance of the ratios. Statistically significant results were provided in the previous section while this section enables a farmer to evaluate the degree of difference between his farm and the other farms.

Table 2.31 Evaluation of farm performance over time

	Farm A proportions	Area X proportions	Ratio Farm A/ Area X
Pleurisy in Month 2			
1992	11/300= 0.04	20/600= 0.03	0.04/0.03= 1.33
1993	20/300= 0.07	14/600= 0.02	0.07/0.02= 3.5
Slaughter in Month 2			
1992	300/1000= 0.3	600/900= 0.67	0.3/0.67= 0.45
1993	300/1000= 0.3	600/900= 0.67	0.3/0.67= 0.45
Pleurisy in Month 3			
1992	31/600= 0.05	41/900= 0.05	0.05/0.05= 1
1993	40/600= 0.07	29/900= 0.03	0.07/0.03= 2.33
Slaughter in Month 3			
1992	600/1000= 0.6	900/900= 1	0.6/1= 0.6
1993	600/1000= 0.6	900/900= 1	0.6/1= 0.6

This Decision Support System cannot evaluate (yet) which risk factors on the farm contribute to the problem (or perhaps good performance). Feedback from farmers on their management practices would be required for this.

DSS to evaluate the probability of developing severe problems

The case-control study which was described in one of the previous sections identified a number of risk factors for pleurisy which could lead to downgrading of carcasses. The second step of the analysis of the data consisted of the development of a logistic regression. A number of important risk factors were identified and also their relative importance. Entry of the relevant data (eg have certain lambs been drenched frequently or shorn twice) will provide the probability that the farm will be part of the high pleurisy prevalence group.

Formulas have been developed in Excel to evaluate the risk of developing severe problems (Figure 2.15). The bold figures are the figures that need to be keyed in. The expected value {E(Y|x)} will appear automatically.

Prediction of severe problems					
coeff	SE		yes/no		
-2.73762	0.83888	constant	1		3.718573
1.23679	0.6738	shorntw	1		4.718573
-1.74519	0.59426	lofre	1	E(Y|x)	0.788072
2.02778	0.85905	coopworth	0		
2.22376	1.1834	vac	1		
2.3356	0.6937	cattonp	1		
1.58811	0.68107	padtotnp	0		
1.71175	0.67024	lambsm	0		
1.23138	0.62357	steep	0		
-1.3297	0.74458	tfarla	0		

Figure 2.15 Component of a DSS to evaluate the probability of a farm developing problems

The development of a feedback system

Pleurisy and pneumonia occur in various degrees of severity depending on a number of risk factors. A feedback system with a component of active farmer involvement may increase the effectiveness of the systems which are described above.

As an initial step a number of farms will be selected to be the test farms. The test farms will be asked to complete a questionnaire when they are submitting their lambs for slaughter. This approach is different from the approach taken during the case-control study. Farms will be asked the age of the lambs at slaughter, since the age categories will be important components of the system. In addition a number of risk factors which have been identified above will be asked each time when a draft of lambs is slaughtered. The number of questions will be smaller than during the case-control study.

Multivariate regression analysis is then used to quantify the degree of association between respiratory disease and the risk factors including the age of the lambs. The most important risk factors will then be used for feedback to other participating farmers.

The actual scheme will ask farmers when they submit lambs for slaughter to complete a simple tick sheet with the small number of questions and advise the age of the slaughtered lambs. The system then calculates the expected level of respiratory disease in the submitted flock of lambs based on regression analysis of the submitted data. The farmer will be advised of the outcome and whether he is doing better or worse than expected or whether he is within the normal range. Suggestions may be made how changes in farming practices can affect his disease level.

The feedback from farmers has several advantages. They will be more involved and will therefore be more likely to value the information. Since feedback is ongoing, improvements to the system can be made. In addition questions can be added or deleted from the list to evaluate other risk factors.

DSS for food safety

The second area in which DSS tools could be very useful would be in helping to adjust slaughter and meat processing procedures in order to reduce the risk of exposing the consumer to microbial and chemical hazards in food of animal origin.

As a risk-based and HACCP approach becomes the standard approach to food safety, it will become essential to make more refined decisions on meat handling practices in slaughter plants, since it will be largely these practices rather than inspection procedures which will determine the risk faced by the consumer. Since the risk to the final consumer will be probabilistic in nature, and since the protective value of any action taken will also be probabilistic in effect, it will be necessary to move away from fixed procedures to one which adjusts practice to the "real" risks associated with a particular product or set of circumstances. For example, it may be necessary to increase the frequency of hand-washing by inspectors in hot weather or when they are handling meat which should have a reduced bacterial load (eg hospital or airline food). The following example illustrates how a probabilistic approach can be used to formulate optimum handling practices.

The most commonly occurring organisms which are associated with ovine pneumonia are not considered to be of public health significance. However there are some organisms of public health significance such as *E. coli* and *Salmonella* spp which can be detected in pneumonic animals at times. Since these organisms are also associated with healthy animals and also because the sensitivity of post mortem inspection is not 100 percent, precautions to prevent cross-contamination by the lung-hand-lung route should be considered if lungs are used for human consumption.

A probabilistic approach has been used to evaluate the interaction of a number of factors which

contribute to the bacterial quality of lungs before they leave the slaughterfloor. They include the prevalence of a type of organism on a lung set, the probability of cross-contamination to lungs which are subsequently palpated through contact with hands, and the effectiveness of hand washing. The principles which are used in this example will equally apply to other examples of cross-contamination. The data which are used in the example are fictitious and only serve to explain the principles of this model. The spreadsheet model which is shown in Appendix VII was developed in Excel/@Risk and the cell and column references refer to this appendix.

A lung can be positive for a certain organism for two reasons. The lung may have come from an animal which was infected by this organism on arrival at the slaughterhouse (termed "original infection"). Alternatively it may have acquired the organism through cross-contamination, ie originally another animal was infected and now two animals appear infected (termed "total infection"). In this example cross-contamination at the inspector's position will be considered and the cross-contamination which will have occurred before the inspector handled the product, ie cross-contamination by a butcher, will be ignored. This has no major impact on the principles of this model.

The prevalence of original infection will determine the probability of a set of lungs carrying an infection when it arrives at the inspector's position. Each lung set (Cells A12:A36) of the 25 lungs that have been modelled will have the same probability of being positive as a result of the original prevalence. The original prevalence of infection is modelled by a distribution in the 25 cells (Cells B12:B36). The example shows different values for each of the 25 different lung sets although they all have the same underlying distribution. A binomial distribution is modelled in each of the 25 cells of Column C12:C36. As an example, the distribution in Cell C15 is "=RiskBinomial (1, B15)". This means that n=1 (ie one lung set) and the probability of the lung set being positive for an infection due to the prevalence in the population depends on the distribution in Cell B15.

If a lung set is positive it may contaminate the hands of an inspector. The inspector may then contaminate the next set that he palpates. The probability of this occurrence can be established in a trial. For the purpose of this explanation the (fictitious) value 0.9 has been chosen (Cell D11). A formula is entered in each cell of the column D13:D36 which enters this value (0.9) in the cell, if the cell in the previous column (D12:D35) which was one row up equalled 1. For example if C13 was 1, then D14 becomes 0.9. Otherwise D14 will be 0. A binomial distribution is entered in all cells of column E13:E36 with n=1 and p=the probability which was displayed in the cell to the left in the same row. For example E14 contained the formula "=RiskBinomial (1, D14)".

The cross-contamination resulting from a lung set which was originally positive (eg lung set one) is not necessarily limited to the next lung set (eg lung set two). The lung set (eg lung set three) which follows after this lung set (eg lung set two) may become contaminated too. Trials would be required to establish the probability of this as well. It would seem reasonable to assume that the probability decreases as the difference between the lung sets increases, but this is not essential for this model. For the purpose of this explanation a fictitious value (0.8) has been chosen. Similar formulas apply to columns F14:G36 as were used for columns D13:E36. The only difference is instead of referring to one row up, the formula will now refer to two rows up.

For example if C13 was 1, then F15 becomes 0.8. Otherwise F15 will be 0.

The same philosophy can be used for subsequent lungs sets. This example considers up to three lung sets back. In a practical situation a higher order may be required.

In order to establish whether a lung set has been contaminated, the Cells from columns E, F and I are summed across a row. For example Cells E15, H15 and I15 will be summed, and if the sum is greater than zero, the lung set will have been cross-contaminated by one of the previous lung sets. This is shown by a "1" in Cells J12:J36. To determine whether a lung set was positive, regardless of the reason (ie original prevalence or cross-contamination, Cells C12 and J12 are summed and if greater than zero the lung set was positive, denoted by "1".

By comparing the sum of the originally positive lung sets (Cell D37) with the sum of the lung sets that are contaminated by either the original prevalence or cross-contamination (J15), the effect of lung handling can be assessed.

Several interventions are possible to reduce the probability of cross-contamination. In this case the washing of hands has been chosen. If it is (temporarily) assumed that hand washing is 100% effective in removing contamination from the inspector's hands, it is possible to calculate the average proportion of lung sets that are contaminated. Each cell in column M12:M36 denotes that washing occurs after a number of lung sets have been palpated as indicated in the cell immediately left to it in column L12:L36. For example Cell M17 denoted that washing occurs after the palpation of the number of lung sets which are described in Cell L17, which is 6. Cell M17 will return the proportion of lung sets which were infected.

Under practical conditions hand washing is unlikely to be 100% effective. Cell O10, Q10 and S10 are used to denote the reduced probability of cross-contamination after hand washing. They show that if hands are washed, the lung sets that are palpated subsequently will have a lower probability of being infected by the lung sets which were palpated before the hand wash than if hand washing had not occurred. The figure which is to be entered in Cell P7 represents after how many lung sets the hands are washed. Columns O12:O36, Q12:Q36 and S12:S36 symbolise the cross-contaminated lung sets. In the case of O12:O36, only O12 uses the probability of Cell O10. In the case of column Q12:Q36, the top two cells (Q12 and Q13) use the probability of Cell Q10. In the case of column S12:S36, the top three cells use the probability of Cell S10. Under normal circumstances one would expect hand washing to occur regularly (eg after 6 lung sets). The cell in Column W12:W36 which signified this routine level would provide an indication of the average proportion of contaminated lung sets.

This model has been designed in @Risk which is a software package that is used for probabilistic modelling. It is the intention that after entering the appropriate information a large number of iterations are run to decide on the mean numbers of infected lung sets and their distributions. Based on these distributions and on the sensitivity, decisions can be made as to the appropriateness of certain procedures.

Discussion

A variety of DSSs are currently in use or are being developed. EpiMan, based around a Geographic Information System is an example of a DSS which can be used in the area of animal health, in case of an outbreak of foot and mouth disease (Sanson *et al.*, 1991). APACHE III is an example of a DSS which is used in the area of human health area (Nowak, 1993). This system makes predictions of survival of critically ill patients in intensive care units. The main function of meat inspection is the protection of the public health. However, it has other functions as well including monitoring of animal health and production, detecting aesthetic defects and monitoring animal welfare. Work in these various areas can be assisted by the development of DSSs. A system which is designed to improve the national health status of pigs by using slaughterhouse data has been described by Willeberg *et al.* (1984/5). This chapter has shown that there is considerable scope for expanding the use of DSSs in meat inspection.

There are certain conditions which need to be fulfilled for DSSs in meat inspection to be successful and this chapter has addressed some of these. Harbers *et al.* (1992) reported errors in identification and registration of animals and abnormalities. The intervention study at Farm B suffered a set-back when a draft of animals was inspected without the detailed recording of their respiratory pathology. Systems which focus on ease of use for inspectors and which are an integrated part of their daily routines are required for an successful implementation of a DSS. The sections on sensitivity of meat inspection of different categories of pleurisy showed differences between premises.

In order to get acceptance of a DSS by stakeholders, the DSS must address issues that they consider to be of real concern. The stakeholders must accept that the information that is provided to them is necessary to have. This thesis used pleurisy because a large number of animals are affected as was demonstrated in the section on the MAF Diseases and Defects Database. Costs can be calculated from these figures and both farmers and meat processors will benefit from a reduced prevalence of respiratory disease.

Research is required to determine the variables that will be used in the DSS. A number of sections of this thesis addressed research into areas of animal disease which were suitable for the development of DSS components. In some cases the actual results could be used as direct components such as the case-control study. In other cases areas were explored which would have an impact on the functioning of the DSS such as the sensitivity and specificity analysis.

The information which is provided to the stakeholders must be such that it includes practical solutions to their problems. The case-control study produced a mix of risk-factors and enabled the provision of potential advice in this regard. Advice on management practices can lead to a re-evaluation of the running of a farm. On the other hand the comment that the keeping lambs in steep paddocks may not be amenable to change. Nevertheless, it does have the advantage of explaining to a farmer why he has a problem and that efforts to reduce the problems by other means may be futile and a waste of resources.

Meat companies can only trim carcasses if affected by pleurisy. However by providing feed-back

to farmers, they too can influence the quality of the livestock they are buying. By telling farmers how they perform compared to the average of their area, they may have provided the farmer the incentive to improve the health of his stock. One would suspect that most farmers would not like to be among the 'worst' farmers of an area. This approach may be particularly useful if it is extended to other conditions which are amenable to improved farm management practices.

This study has shown that there is a need for ongoing research into risk factors and subsequent validation of putative risk factors. The intervention studies at Farms A and B were examples of this. Ongoing validation and establishment of 'new' risk factors can be done as suggested in the section where farmers are requested to fill out check sheets with a small number of questions.

There is a need for the DSS to be sufficiently accurate at the farm level to be of practical use. Certain risk factors may exist if one looks at the population of all farms in New Zealand but the impact at the level of an individual farm may be so low that attempts to rectify it becomes pointless.

The information must be presented in an easy to understand format. This last section has illustrated various ways of explaining and showing to farmers how well they performed. Ratios, p-values and graphs were used. This approach ensures that both farmers who like to see figures and those who prefer a graphical display will remain motivated to make use of the DSS.

The system must have a favourable benefit-cost ratio. Already computer systems are used for a variety of functions but a DSS will be an added cost. At some stage it needs to be evaluated whether the added cost is worth the benefits.

Information gathering must be non-threatening and should not require much effort from the farmer. The case-control illustrated the difficulties of reducing the number of questions without loosing too much information. Once research has identified and validated a number of risk factors, a small number of questions can be asked which will be a small effort. Farmers should also receive the assurance that any information they provided will be used in a confidential way, as was done in the case-control study.

In conclusion this chapter has addressed a number of components which can be used for the successful development of a DSS by looking at pleurisy and pneumonia in lambs. Meat inspection has a number of functions and components of a DSS can be created for the various stakeholders.

CHAPTER 3

CHEMICAL RESIDUES IN SLAUGHTER ANIMALS

Introduction

Objectives and priorities of sampling for residues

Sampling for chemical residues has several objectives:

- To verify that the population of animals that are submitted for slaughter do not have non-complying levels of residues and to provide this assurance to both domestic and overseas consumers.
- To deter people from submitting animals for slaughter that have non-complying levels.
- To alert decision makers to the existence of unacceptable situations for their corrective actions. For instance they may stipulate a longer withholding period for a certain chemical which may reduce the number of non-compliances of that particular chemical.

In theory, detection of an excessive level of a particular residue in a randomly sampled animal could reduce the quantity of non-complying product if that animal is condemned. In practice, this does not happen and moreover this is not the purpose of a residue sampling programme. In contrast with routine post mortem inspection in slaughterhouses where all animals are inspected, a residue sampling programme usually only takes samples of a relatively small number of animals and inferences regarding the population are drawn from these samples.

The approval of chemicals and the production of slaughter animals can be seen as integral components of a processing system. Satisfactory performance of this system is monitored by sampling. Some overseas markets are demanding a formal residue testing system to ensure that meat that enters their country does not contain residues which exceed Maximum Residue Levels (MRLs). Sampling for residues supports the assurances given by certification and allows some quantification of the degree of compliance to be introduced into certification. Unequivocal demonstration that non-compliances do not occur may not be feasible nor is it desirable. The residue system currently in place in New Zealand is to a some degree influenced by overseas requirements to ensure market access for particular countries.

A formal priority setting mechanism could be developed to provide the best possible assurance of protecting public health within the constraints of the financial resources available for residue monitoring. A variety of theories and techniques including risk analysis, cost-benefit analysis and decision theory can contribute to a transparent cost-effective system. There are several components that should be considered when setting sampling priorities.

- The likelihood that a certain chemical will be made available to animals, both in correct and in excessive levels, inside and outside the withholding priod, or to livestock classes for which it was not approved.
- The likelihood of detecting non-complying levels.
- The risks to human health, which is caused by either acute or chronic toxicity.
- Knowledge of the numbers of non-compliances of specified chemicals in the past. If there has been a low level of non-compliances for a chemical in the past, then extensive testing is not warranted (if the adverse effects are not severe). The issue of clustering is closely related to this.

It should be appreciated that at times overseas markets may require testing which is appropriate for product from their domestic market but which may seem futile for an exporting countries where other conditions apply. Nevertheless, these demands will have highest priority because meeting them ensures market access.

The objective of this chapter is to evaluate some sampling plans and what conclusions may be drawn with regard to temporal and spatial clustering. The framework for a DSS is drawn which can be used to provide assurances. This system is based on quantifying the use of specific chemicals at all its stages and consequently sampling is one component only of assuring that the product has acceptable levels of non-compliances.

Non-compliances

Maximum Residue Levels (MRLs)

Expermental studies are carried out on test animals and No Observed Effect Levels (NOELs) are established based on the acute, subacute and chronic effects in test animals. A safety factor (which is often 100) is applied to the most sensitive animal species for determining the Acceptable Daily Intake (ADI). This is used as the amount of chemical a person can ingest on a daily basis throughout his/her life without harmful effects. Based on certain assumptions of dietary intake, MRLs are calculated. Work has been carried out by Codex to establish MRLs for international trade, but individual governments usually set their own levels for national use. Since there are differences in approach, various countries can have different MRLs. MRLs have been regarded by some as levels of residues in slaughter animals that should not be exceeded. On the other hand, it has also been argued that since large safety factors have been applied, product which exceeds an MRL is not necessarily a true public health risk. Although this may sometimes be correct, this view is not considered relevant in this thesis.

Notwithstanding the above, frequently it will be more useful to look at all animals that have tested positive for a chemical rather then only those that show non-complying levels. There are two main ways to evaluate residue data in animals. One can look at the levels and determine means and other statistics. Another approach is to categorise the data of a particular residue as to detection or not, or being non-complying or not. One should be mindful that with the

improving sensitivity of analytical techniques, more "positive" tissues may be discovered in the future even if no changes occur in farming practices which might increase the occurrence of residues. Therefore if levels of detected chemicals are compared over time, the limit of detection of the analytical methods need to be taken into account.

Reaction to non-complying levels

Frequently when problems occur, increased sampling is demanded by the public or overseas authorities. An increase in the number of samples may provide the following benefits:

- A better appreciation of the situation.
- An increased deterrent effect, although the chance of offenders getting caught may still remain small in reality.
- More offending tissues may be condemned. However this is not the purpose of a sampling programme and therefore questionable as a benefit.

A drawback may be that the detection of more non-compliances may lead to a vicious circle of more sampling resulting in the detection of more non-compliances and so on. Without a defined objective for the testing, a practical solution may not always be available to resolve this situation. It is important to clarify in advance what actions should be taken should certain levels of non-compliances be exceeded. These actions could include campaigns to improve awareness by users, changes in withholding periods, or withdrawal of chemicals from the market.

Categories of chemicals that can leave residues

There is a multitude of chemicals that can leave residues in tissues. Sampling is commonly performed for chemicals that do not occur naturally in the environment and which leave detectable residues in animal tissues. New chemicals could be placed on a residue sampling list when they are approved for application to animals or for release in the environment. In addition to these chemicals, naturally occurring chemicals with potentially harmful effects on human health if exposure is of sufficient level and duration have been included in residue sampling programmes. Chemicals can be placed in three categories for the purpose of residue sampling.

Animal remedies

Animal remedies are registered for particular livestock classes but may be used off-label for other livestock classes.

Environmental contaminants

Environmental contaminants include invertebrate pesticides, vertebrate pesticides, herbicides, Chemicals can also be applied to the land as fertilisers or as inadvertent components of fertilisers. Industrial waste which is being disposed of, may intentionally or accidentally be distributed to animals.

Chemicals that occur naturally in the environment

A number of chemicals have been identified which occur naturally in the environment, can be detected in animals, and which are deemed to be harmful to humans.

Chemical compounds that are discussed in the thesis

The four types of chemical compounds which have been chosen as examples in this thesis, cover various aspects of chemical residues. Both avermectin/milbemycin and levamisole are chemicals which are intentionally applied to animals to reduce their parasite burdens. The interpretation will focus on whether animals have non-complying levels or detectable levels of these chemicals or not. Cadmium occurs at low levels in the environment but high doses result from environmental contaminantion. Cadmium is not supplied to animals intentionally. Finally copper is a metal that occurs in every animal as a physiological component. Grazing and other management factors can affect the levels in each animal. In addition copper is applied to some animals as an animal remedy.

Analytical Methods

Analysis of data needs to consider a number of issues such as livestock class, temporal aspects, geographical aspects, risk factors and their interaction. After evaluation of these issues, the validity of assurances about the residue status of products can be assessed. This section discusses a number of analytical methods that are relevant to sampling for chemical residues. Some of these methods are explored in depth in the next section.

Baselines

A baseline for a residue is a set of data for a livestock class over time and space which models the levels of chemical residues which have been detected within the sampling program, and the degree of compliance with legal requirements. Baselines can be used to evaluate whether current results of residue testing and consequently the levels of residues in meat are acceptable. If residue testing has taken place over a period of time, knowledge about the occurrence of a chemical in the past is available.

As a first step the livestock classes for the various chemicals are defined. Once it has been established that a livestock class is sufficiently homogeneous, the distribution of the chemical based on data from previous years is calculated. Data on a chemical which occurs physiologically in each animal can be used to fit to a distribution. The statistical distribution of chemical residues in a population will depend on the physiological attributes of the population itself, the type of residue and the type of information that is of interest. For instance, a binomial distribution could be considered if the issue is whether or not a non-compliance occurred. Based on the above approach baselines are established for various chemicals in classes of livestock.

The number of non-compliances that can be expected will be determined by the baseline levels (eg mean and standard deviation) and the maximum permitted residue level (MRLs). MRLs are set by procedures that are based on toxicological risk assessment for human beings. Baselines and MRLs do not necessarily bear much relationship to each other. An estimate of the expected number of non-compliances (all other aspects kept unchanged) can be calculated from the distribution of the chemical in the slaughter population and the MRL.

When deciding on baselines, changes in husbandry procedures need to be considered. For instance a chemical may be removed from the market and an existing, other chemical may absorb the market share of the chemical that was withdrawn. This could result in the other chemical causing more non-compliances, perhaps to an unacceptable level. The new sampling system should be able to detect the emerging trend within a 'short' period. Alternatively, the existence of an additional monitoring system that follows farming trends would assist in amending sampling programmes as appropriate.

The establishment of a baseline implies that the level of non-compliances which is included in

this baseline distribution is considered acceptable from a public health perspective. MRLs have been set to protect the consumer, but at the same time non-compliances are known to occur. The fact that the non-compliances are occurring without any large scale remedial action supports this view. Equally, a data set should not be used for a baseline where a level has been considered unsatisfactory and where remedial action has been taken. The establishment of acceptable levels of non-compliance does not imply that non-compliance by individual farmers should be condoned. Rather it reflects the reality that a certain level of non-compliance is likely to occur and this level cannot be reduced unless additional resources are allocated to remedy this problem. Instead of considering each non-compliance a breakdown of the processing system which is to provide 'safe' food, a certain level of non-compliances could be considered acceptable or at least tolerable. Assurances regarding the distribution of a chemical in a population may be more appropriate than concentrating on individual non-compliances.

A reduction of the samples taken could be considered once baselines have been set. Programmes to verify that current levels reflect historic baselines are sufficient, rather than each year carrying out a full scale sampling programme. There are several sampling programmes that might allow this reduction. These sampling programmes are continuous and share a background of being developed for industrial manufacturing. CUSUM is widely used in the meat industry. It alerts to violating a predetermined level, ie if the programme is not under statistical control. Another approach is sequential sampling. If a temporal pattern has been detected several options could be considered to amend sequential sampling schemes. These are, sampling at temporal peaks of non-compliances, or changing the sampling scheme and acceptable levels based on temporal patterns.

Livestock classes

A livestock class can be defined by:

- species
- sex
- age
- breed
- purpose for which it is farmed (eg dairy)
- risk factors such as chemicals or practices to which a group of animals is likely to be subjected (eg growth promotants)

Sampling of a population for chemical residue purposes is usually based on the premise that the population in question (livestock class) is homogeneous for this particular purpose. The degree of homogeneity of a population is determined by the diversity of its genetic pool and the variety of its environment. Therefore absolute homogeneity of a population is not a practical possibility. Rather a 'considerable degree' of homogeneity should be evaluated. At times, livestock classes for the purpose of residue testing may contain groups of animals which are usually considered very different. For instance it may be adequate to use lambs and older sheep as the only two

ovine livestock classes for one chemical while for another chemical the division of sheep into lambs, hoggets and older sheep may be more appropriate.

Aggregation of livestock classes beyond what would commonly be seen as normal (eg grouping sheep and cattle together) might be required for cost reasons but would have to be done with caution, because it implies that the underlying distribution of residue values is the same, and produced by similar causal influences. The more that groups are aggregated, the less defensible this assumption becomes. It might be better to test one well-defined population rather than taking small numbers from various populations. In the case of environmental contaminants, the levels of a chemical in one livestock class may serve as an indicator for the levels in other livestock classes, even though it may be inappropriate to combine the data.

Due consideration has to be given in advance to the management practices to which various groups of animals are subjected. A problem of interpretation arises if for instance a chemical starts to be used in one livestock class of cattle but not another one.

Residue data can be analysed as categorical or continuous data. Data will be analysed as categorical if the objective is to estimate the proportion of animals which exceed certain levels. This includes non-compliances, detectable levels or arbitrarily set levels. A suitable statistical test can be performed to determine if a group of animals is significantly different from other ones. This test will show whether or not there is a difference between classes of livestock and whether or not aggregation of livestock classes for testing purposes could be considered. It is possible to make comparisons between one livestock class and an aggregation of the other livestock classes. Alternatively all livestock classes can be classified in their respective groups and then a test can be carried out. An example of such a test is the chi-squared test. It is a low power test, and very susceptible to variation in numbers of animals. It is the simplest but not always the best test. Log-linear modelling can be used where a number of relevant variables need to be included in the analysis. Based on the results, a discrete distribution such as the binomial distribution can be used to fit the data. The resulting distribution can be used as a baseline when further sampling in the future takes place of the various livestock classes to explore whether changes (eg levels of non-compliance) have occurred.

If chemicals are detected, they are usually measured as continuous values. The data can be evaluated as to their fit to normal, lognormal or other distributions. An example of continuous data are copper levels where all animals are positive ie they all have a detectable content of copper but the levels will vary between animals. Examples of data which can be analysed as categorical data are levamisole and avermectin. Some animals may be positive and some may not be positive when tested. Levamisole and avermectin can be analysed as continuous data as well since the levels per positive animal will vary.

In the case of continuous data, an ANOVA (Analysis of Variance) can be performed to compare the means of livestock classes. If the means are not significantly different, aggregation of livestock classes could be considered. The assumption for an ANOVA is that the populations from which the observations were drawn are independent, normally distributed, and have a common population variance (Larsen and Marx, 1986). Histograms are created of each livestock class with a normal curve superimposed on the histogram. In the case of a normal distribution

the histogram should not deviate considerably from the normal curve. The Wilk-Shapiro/Rankit plot and the Wilk-Shapiro normality statistic (Shapiro and Francia, 1972) can also be used to evaluate normality.

If the data do not fit the assumptions of standard parametric tests, nonparametric tests can be used. The Rank Sum two-sample (Mann-Whitney) test combines all data and converts them to ranks (Statistix®, 1992). For each group the ranks are summed. This sum is the rank sum statistic for each group. The test actually compares the distributions of the two groups. However if they are similar it is commonly concluded that the central values of both groups are similar. The Median test first establishes the median for two data sets. Then the number of values above and below the median in each group are counted and a p-value using chi-squared approximation is calculated. In the case of the Kruskal-Wallis One-Way ANOVA the data are ranked and then a parametric ANOVA is applied.

The tests listed above are to be used to decide which group of animals are to be used as livestock classes. In addition these tests will also be of use to detect temporal and spatial patterns.

Random sampling

Existing sampling programmes for chemical residues typically attempt to give each animal in a livestock class an equal chance of being selected, through the use of random sampling. This is based on the assumption that each animal in a given livestock class would be expected to have an equal chance of having a violative level or the same mean value of a residue.

However random selection for residue sampling purposes can apply at various levels:

- Individual animals.
- Individual lines (ie animals from one farm slaughtered on the same day).
- Individual farms.

A key question is whether individual animals, lines or farms should be randomly selected for residue sampling purposes. The answer depends on the purpose of the sampling: eg assessment of the likely exposure of consumers to chemical residues or monitoring of farm compliance with specifications such as dose rates or withholding periods.

Under current commercial circumstances, once an animal has been slaughtered it is boned out into small cuts. At retail level the size of portions of the meat and offal that are sold to individual consumers is usually small. It seems reasonable to assume that generally each consumer has an equal chance of eating a small part of any animal. If one wishes to base sampling on human exposure each animal should have an equal chance of being selected regardless of factors such as the size of the farm of origin.

A slaughter animal is part of a group of animals that have come from a farm where they have

stayed a certain period (up to their entire life) before being slaughtered. Animals from the same farm have been exposed to the same husbandry practices to varying degrees. Especially animals which are slaughtered on the same day will have been subjected to the same risk factors to a large degree. Therefore if one of these animals is positive for a chemical, the other animals in the line are more likely to be positive than would be expected based on chance. Sampling by line would be based on these considerations. It takes more time to slaughter and to inspect a large line than a small line. Therefore if during a day an equal number of small and large lines is slaughtered and sampling occurs at a random time, animals from a large line are more likely to be sampled than animals from a small line. The sampling could be based on allocating numbers to the various lines during a day and then randomly selecting a number. This would give each line an equal chance on a daily basis to be selected.
However if during a day only large lines or only small lines were slaughtered, any of the large lines would have a greater probability of being selected on that day than any of the small lines during a day when small lines are slaughtered. Large lines are likely to be related to large farms. Small lines are not only related to farm size but may also be related to the health status of animals. During certain times of the year small lines may be more likely to contain animals that are (subclinically) diseased and the farmer may have had to keep them longer than other animals to gain the target weight.

The third option is to sample farms. Animals from the same farm are likely to have been subjected to similar farming practices, although to a lesser degree than animals from the same line. The sampling of farms would have the advantage that the chemical itself becomes less important. Rather compliance of a farm with the total set of relevant requirements becomes the issue. The above illustrates that if random sampling is intended, it needs to be clarified what the level of interest is (animal, line or farm) and what the limitations are.

Temporal analysis

A number of factors which influence the levels of residues in slaughter populations are dependent on time. These are the age at which animals are exposed to certain chemicals, the length of exposure and the period between exposure and slaughter. The time of the year of slaughter is relevant with regard to husbandry practices and the age of the animal at slaughter. These factors determine whether or not a chemical can be detected and if so at what level.

The trends in temporal distribution of residue levels can be divided into three groups:

- Short term.
- Cyclical (including seasonal) trends.
- Long term (secular) trend.

Temporal analysis of chemical residues can be performed by plotting data and by performing statistical analyses on these data. The techniques that are used to evaluate temporal patterns can be placed in several categories. They include:

- ANOVA and chi-squared tests.
- Quality control statistics.
- Sequential sampling.
- Regression models including Median Polish, ARIMA time series analysis and spectral analysis.

Plotting

Plotting of data is a quick way to acquire an appreciation of events. However plots may be misleading or difficult to interpret. Residue data are usually not collected at equally spaced points in time. For instance, slaughter occurs less frequently on Sundays than on other days of the week, and at certain times of the year premises may not operate. Often a number of samples for a chemical will be taken on one day, while no samples are taken on other days. This frequently results in an uneven spread of the days over which samples were taken. Aggregation of data in time can overcome this problem to some degree. Box-and-whisker plots can be used to display variation of levels over periods of time.

ANOVA and chi-squared tests

ANOVA and chi-squared tests can be used in a similar manner as has been described for classes of livestock. The data can be aggregated (eg on a monthly or three-monthly basis) and then analyses can be performed. The intention of these tests is to determine whether or not the values of various time periods are significantly different.

Quality control charts and statistics

Quality control charts and statistics are commonly used in a variety of industries. Their intention is to determine whether or not a process is under statistical control. These statistics can also be used for residue data if these data are considered to reflect the outcome of a process. This process consists of many components including approving new chemicals, the direct or indirect application of these chemicals to animals (eg anthelmintics and soil fertilisers), and the observation of withholding periods.

When variation occurs it should be ascertained whether it is the result of common cause or assignable cause variation (Mittag and Rinne, 1993). Common cause variation is the random variation that occurs in a process and it cannot be influenced without changing the process. Assignable cause variation occurs when the variation that occurs can be explained by a specific event. There are various guidelines which are used to establish that a process is out of statistical control. The Upper Control Limits (UCL) or Lower Control Limits (LCL) which show that the graph exceeds any of these two control limits are commonly used and they can be combined with other criteria. Tests to determine whether the process is out of control have an inherent trade-off between detecting nonconforming samples as soon as possible and false alarms.

Quality control charts which are used to monitor whether or not samples are conforming to a standard are called attribute control charts (Statistix®, 1992). One can define samples as non-conforming when they have violative or detectable levels or when a certain level is exceeded. Therefore the definition non-conforming with regard to residues may be somewhat arbitrary.

There are three charts for quantitative quality characteristics which are called charts with a memory. These are the MA (Moving Average), EWMA (Exponentially Weighted Moving Average) and CUSUM (Cumulative Sum) charts. Sampling can be performed by looking at each unit or groups of units at a given point in time in isolation. However at times it may be preferable that results from earlier tests are considered when evaluating a process, as could be the case for chemical residues. This is achieved by using such charts. The MA chart and the CUSUM chart have a limited memory. The MA chart will evaluate the result of the last predetermined number of samples only. On the other hand the EWMA chart has an unlimited memory in principle. Its memory is also non-uniform while the CUSUM and the MA chart have a uniform memory.

MA Chart — The test statistic of the MA chart is the mean value over a predetermined period which is sometimes called the lag. A value which occurred early in the lag period has the same weight as the last sample value.

EWMA Chart — The test statistic of the EWMA-mean chart is defined by the equation:
$Y_t = (1\text{-}d) \cdot Y_{t-1} + d \cdot \bar{x}_{tn}, \quad 0<d\leq 1$ (Mittag and Rinne, 1993)
The centre line of the graph will often consist of the mean value. The factor 'd' determines the weights of the distance in time. The larger the factor 'd' is, the smaller is the influence of the current sample value. The d-value should be chosen in such a way that the period covered becomes meaningful.

CUSUM Chart — The CUSUM chart consists of the sum of deviations of successive samples. It shows cumulative sums on the high side and on the low side. The CUSUM chart is used when small shifts in process averages should be detected quickly.
The Average Run Length (ARL) is associated with CUSUM charts. This shows the number of samples taken before an out-of-control point occurs under normal conditions

The MA chart will be evaluated in a later section with regard to its use for detecting clustering in time.

Sequential sampling

At times it is clear that knowledge of a residue level in a population will only be marginally improved by more samples. There may already be such a number of non-complying samples that it is obvious a problem exists. Alternatively it may also be clear that there is such a large degree of compliance that any non-complying product in the future would not alter this conclusion. In both situations allocation of further resources to testing is unnecessary. Sequential sampling systems enable the termination of sampling as soon as a conclusion has been reached. In the case

of chemical residues sequential testing could be seen as forward testing while the other tests that have been described previously are evaluating past events.

Regression techniques

There are a number of statistical techniques which can be used to model residue levels. Simple linear regression may not always be suitable. It may be of use when levels at a point in time are related to distances between points in time.

A convenient manner of comparing levels is by evaluating auto-correlation coefficients. The closer two samples have been taken in time, the more similar their values may be since they have been subjected to similar influences. Statistical software packages will often be able to calculate auto-correlation coefficients for time series. However, a major problem exists since the residue values are seldom equally spaced in time. In order not to lose data by aggregating it may be more convenient to use semi-variograms. This is an approach that is commonly used in spatial statistics. The differences in time are compared with the corresponding differences in values.

The median polish can be used to compare values over time if a number of years are available. This technique consists of creating a matrix with columns for the 12 months of each year and rows for the individual years (eg 1990, 1991, etc.). After a number of iterations the common effect, the row effect (eg year) and the column effect (eg month) are calculated.

The group of Box-Jenkins techniques have been developed to model time patterns. They include the ARIMA (Auto Regressive Integrated Moving Average) and SARIMA (Seasonal Auto Regressive Integrated Moving Average) regressions. Currently these techniques cannot be used for chemical residues since a considerable amount of data is required. In addition a philosophical issue arises. As time passes conditions under which animals are farmed will change. It becomes questionable whether data that were collected in the distant past are still relevant to current farming practices. One would be reluctant to use old data for setting baselines for residue testing. A decision needs to be made regarding the period of which the data can still be considered relevant to the present period.

Spectral analysis can be used for estimating spectral density functions of time series (Chatfield, 1989). It is a group of techniques which is suitable for modelling seasonal variations. One such technique, the Fourier analysis, is based on the sum of sine and cosine terms.

Spatial Analysis

Geography can play an important role in the distribution of chemical residues. Animals from a certain geographic area have often been exposed to the same climate. Although not as important as in the past the animals may have a relatively high degree of genetic homogeneity, for example because most farmers in the area keep a particular breed because of its local suitability. Soil

types and terrain are also geographically determined. Finally some farming practices may be more common in certain geographic areas than in other ones.

Spatial analysis is required to determine whether animals from some geographic areas are more likely to display violative levels of residues or higher levels of residues than animals from other areas. If this occurs an evaluation of the underlying causes and the extent of the problem can take place and ways of correcting the situation can be considered. Goodchild (1986) describes a taxonomy of spatial features as consisting of points, lines, areas and lattices. In the case of spatial analysis of chemical residues, points and areas are used for analysis.

A number of techniques regarding spatial analysis are relevant to residues:

- Display of geographical patterns.
- ANOVA and chi-squared analysis.
- Auto-correlation of data.
- Point pattern analysis of data.

Display of geographical patterns

Spatial patterns of data can be displayed on maps in Geographical Information Systems (GIS). The software programmes that have been developed for this purpose are either vector (points, lines and polygons) or raster (cells of a grid) based systems. Categorical data such as positive and negative results can be displayed by point locations. The same data can also be displayed on a regional basis. The ratio of positive animals to the total number of animals in a region that have been sampled can be displayed. Colour shading can indicate the ratio. The ratio of the number of tests in an area to the total number of animals in a certain category will indicate whether the testing has been applied equally over the country.

The display and interpretation of data is a subjective matter. The scale of the regions that are used can have an influence on the resulting data that are displayed. In addition boundaries of regions are often based on administrative criteria which do not necessarily bear any relationship to the distribution of animal diseases or chemical residues in livestock.

ANOVA and chi-squared tests

ANOVA and chi-squared tests can be used in a manner as has been described for livestock classes and temporal patterns.

Spatial statistics

Spatial data sets cannot be treated as containing independent observations, because of the

existence of auto-correlation in spatial data. Auto-correlation is defined as the relationship among values of some variable that is attributable to some underlying ordering of these values (Griffith, 1987). Therefore spatial auto-correlation is a concept which evaluates the degree to which data are influenced in space by distance between them. It compares the value of one point or area with the value of another point or area, having regard to the distance they are apart.

A number of techniques have been used for identifying spatial aspects of data. They include Moran's I test, Geary's c test, the G-statistics, the correlogram, nonparametric measures such as the rank adjacency statistic D, join count measures and regression with spatially correlated errors (Cliff and Ord, 1981; Walter, 1992a; Walter, 1992b).

Moran's I and Geary's c statistic: Moran's I test measures the I statistic which is the covariation between neighbouring regions. Geary's c statistic on the other hand looks at paired comparisons of data values (Walter, 1992a). There is no impediment however to looking at the relationship between all regions. Instead of using a 1/0 code indicating whether or not regions are neighbours, the weight matrix could consist of for instance the inverted difference in distance between points.

The formulas for Moran's I and Geary's c are respectively (Goodchild, 1986)

$$I = \sum_i \sum_j w_{ij} c_{ij} / s^2 \sum_i \sum_j w_{ij}$$

where w_{ij} is the weight matrix, $c_{ij} = (z_i - \bar{z})(z_j - \bar{z})$ and $s^2 = \Sigma_i (z_i - \bar{z})^2 / n$

$$c = \sum_i \sum_j w_{ij} c_{ij} / 2 \sum_i \sum_j w_{ij} \sigma^2$$

where w_{ij} is the weight matrix, $c_{ij} = (z_i - \bar{z}_j)^2$ and $\sigma^2 = \Sigma_i (z_i - \bar{z})^2 / (n-1)$

The common method used to evaluate spatial autocorrelation is to have a null hypothesis that spatial autocorrelation does not exist. The indices are complementary to some degree (Goodchild, 1986). However they may lead to conflicting inferences (Griffith, 1987). Upton and Fingleton (1985) suggest that while the I statistic is more influenced by extreme values, the c statistic is more influenced by absolute differences between adjacent pairs of data values.

G Statistics: The G statistics are a family of statistics which are of particular use to detect localised areas where spatial autocorelation exists. Getis and Ord (1992) argue that the G statistics are complementary to the I statistic, enabling the identification of patterns that would otherwise not be identified.

The general G(d) statistic is defined as:
$G(d) = (\sum_i \sum_j w_{ij} (d) x_i x_j) / (\sum_i \sum_j x_i x_j)$, $j \neq i$

The $G_i(d)$ statistic is defined as:
$G_i(d) = (\sum_j w_{ij} (d) x_j) / (\sum_j x_j)$, $j \neq i$

In the case of both statistics distances are defined for which the existence of spatial autocorrelation is evaluated (eg < 20 km or < 65 km). The difference between the two statistics is that whereas the G(d) statistic evaluates all pairs that are within this certain distance of each other, the $G_i(d)$ statistic evaluates each location individually.

Join count statistics: Join count statistics can be used for evaluating spatial autocorrelation for nominal values. In the case of chemical residues, differentiation can be made between regions with non-compliances and regions without non-compliances resulting in two classes. The joins between regions with non-compliances, without non-compliances, and with and without non-compliances can be counted (Cliff and Ord, 1991). It is also possible to rank the values of residues in the various regions and then to create nominal classes of levels of residues. Then again spatial autocorrelation can be measured.

The join count technique has several drawbacks. No account is taken of the degree of adjacency of the regions such as the length of their border. In addition only first nearest neighbours are evaluated although this can be extended by including higher order nearest neighbours. Weights can be introduced, for instance to give first nearest neighbours greater weights than second nearest neighbours etc.

From a residue perspective this technique may not be satisfactory. The number of samples per region, which are to represent each region, are small. Furthermore there is a large variation in the size of the various regions with regard to geographical size, the number of slaughtered animals and the number of samples taken. As an example 2 samples taken from the Southland area will at times be further away from each other than 2 samples taken from non-contiguous regions in the wider Auckland area. The use of the data would not be optimal.

Rank adjacency (D) statistic: The rank adjacency statistic is a nonparametric statistic. It is defined as the average absolute difference in ranks over adjacent pairs of regions.
$D = \sum_i\sum_j w_{ij} |y_i - y_j| / \sum_i\sum_j w_{ij}$ The statistical properties of this statistic are not well known and the sampling distribution is determined by Monte Carlo simulation (Walther, 1992a).

Nearest-neighbour analysis: A number of points are randomly selected from a pattern. The distance to the nearest neighbour is determined. If clustering occurred, then the average distance between nearest neighbours would be smaller than the average distance which could be expected under the assumption of Complete Spatial Randomness (Boots and Getis, 1988)

Quadrat analysis: Quadrat analysis measures the number of points within a quadrat and compares the findings with the number of points in the quadrat which would be expected under random conditions.

Spatial correlograms and variograms: A spatial correlogram is a graph which shows the spatial autocorrelation against spatial steps called lags. A variogram can be used to show spatial autocovariance between points when a process is continuous over space. Both process provide an insight how values of chemical residues change in space.

Time-space analysis

The above statistics have addressed statistics which address either temporal or spatial aspects of chemical residues. A combination of these two aspects, time-space clustering has been investigated by various workers (Knox, 1964; Mantel, 1967).

Evaluation of Current Sampling Plans

Introduction

Current procedures for assurances regarding chemical residues are based on sampling. Since not all product is inspected, product with violative levels of residues may enter the food chain under the present system. Commonly a sample of 300 animals of a slaughter population is taken to show that at the 95% confidence level not more than 1% of the population has violative levels. This implies a sensitivity of 100% and no clustering of cases in the population. At times this assurance may not be appropriate because violations can be expected to occur. Violative levels are based on toxicological studies. If the level that is deemed to be violative is relatively close to the mean of the chemical in the population and if the standard deviation is large, then a large number of violations can be expected.

Tissues of slaughter animals are sampled to acquire an understanding of the status of the population with regard to residues. At times the sensitivity and specificity of the chemical analysis procedures may be known. However knowledge of the performance characteristics of sampling procedures are required as well as the chemical analysis aspect to fully understand the implications of the results. These sampling characteristics are frequently unknown.

If a non-compliance is detected the question remains what conclusions to draw with regard to the levels of this chemical in the population. The objective of this section is to evaluate what information can be gleaned from monitoring systems. Trends in residue data can be defined as movements towards a different mean residue level in a population or a different level of non-compliances. In this section unacceptable levels will be considered of critical importance for taking corrective action.

The procedures described and evaluated in this section are based on current practices and include some minor amendments. The objective of this section is evaluate ways of determining whether or not trends have occurred with regard to levels of chemical residues which are deemed unacceptable. The assurance to consumers would be that no unacceptable trends have taken place rather than the assurance that no non-compliances have occurred or that not more than 1% of the population had violative levels. The next section evaluates a Decision Support System for chemical residues which addresses sampling and assurances in a substantially changed manner.

In general, the more samples which are taken from a population, the more accurately the parameters of these populations can be estimated. Therefore the sample size is of critical importance and this section focuses heavily on these aspects. However the literature provided no clear guidelines as to the required sample sizes. The system below describes ways to determine appropriate sample sizes.

This section will also show simulations in time and in space with the objective to evaluate how well trends in time and patterns in space can be detected. To some degree sampling for residues

over a period of time is similar to sampling as is commonly practised in industrial processes. Quality control charts and statistics such as Moving Average (MA), Exponentially Weighted Moving Average (EWMA) and Cumulative Sum (CUSUM) procedures can be used to monitor the process by monitoring the final quality of the product. In this section the MA approach has been chosen to simulate temporal clustering. The performance characteristics of sampling in space have been evaluated by using Moran's I, Geary's c, and the G statistics. Chemical residue data as provided by the New Zealand Ministry of Agriculture were used to create baselines. Levamisole data were used for simulations in time while cadmium data were used for simulations in space.

Methods and materials of sampling plans

Comparison between levels

The historical level which can be considered acceptable since no action was taken to reduce it, is the designated baseline. A new level, the Upper Action Point (UAP), is defined as the lowest unacceptable level and it is set as the trigger point where mean values become unacceptable. The UAP can be based on toxicological considerations where certain levels are deemed to be unacceptable. Alternatively it can be based on the historical baseline when a certain change is considered too large.

A historical sample which consisted of 163 sheep had a mean = 75.189 and standard deviation = 60.758. Statistica (StatSoft, 1993) was used to determine sample sizes which were able to distinguish between the historical mean and a new mean which was deemed to be unacceptable. It used one sided (right) tests with an alpha error = 0.0500 and a beta error = 0.1000. A normal distribution with the above mean and standard deviation was used. This may not reflect the actual situation, but the assumption was required to illustrate this example.

At times the number of violations of the MRL may be of more interest than a shift of the mean. In addition, the assumption of a normal distribution may not be appropriate. The binomial distribution was used for such a situation. Over a two year period 11 (2.76%) bulls were positive and 387 (97.24%) bulls were negative for ivermectin/milbemycin. The total number of bulls tested was 398. The UAP was based on the multiplication of the historical p value of the binomial distribution or on a new set value.

Random and stratified sampling

A random sample of sufficient size from a homogeneous population is deemed to be representative of that population within certain confidence levels. Therefore if for instance 40 samples are randomly taken from a population of 20,000 animals, the samples are deemed to be representative of these 20,000 animals.

In the proposed case a period will be set for which a guarantee will be provided that the process was under statistical control. For instance, this could be 2 months. A sample could be randomly taken from the population. The random aspect of sampling would apply to the animals. The timing of the sampling would be based on stratified sampling. For example each week during the 2 months a sample consisting of 5 animals (ie 40 animals / 8 weeks) would be taken. If the mean value over the 2 months were not significantly different from the historical baseline, then it would be concluded that the process was operating under the same conditions as previously.

The sample size is not dependent on the population under investigation. The critical point is the time over which the guarantee is given. The guarantee is that if a problem exists it will be detected within a certain period after which corrective action will be taken.

In the following week another 5 animals will be sampled. If the mean for the previous two months, as determined by this sample, is not significantly different from the historical baseline, then a guarantee could be provided regarding the slaughter population of these previous 2 months. The difference between this guarantee and the one before is that the last guarantee includes the most recent week but not the very first week while the one before included a week at the beginning of the period which was not included in the latter guarantee. The means that are created in this manner are moving averages which are not independent.

Sequential sampling

Fixed sampling plans in the case of chemical residues provide retrospective assurances. An alternative approach is the use of a sequential sampling programme. The guarantee could be re-evaluated for pre-determined periods such as for instance 2 monthly periods. First the maximum number of samples is determined to establish that the product was produced according to specification. Next the number of samples are spread proportionally over this period. As soon as it has been verified that the product complies, sampling will cease.

Results of sampling plans

Sample sizes for normal distribution with copper

Table 3.1 shows how many samples are required to detect whether or not the historical mean has increased to a certain level. For instance to detect whether the current mean is 0.10 higher than the historical mean, 560 samples are required.

The establishment of what constitutes an unacceptable degree of change of the mean is arbitrary. For the purposes of this example two types of shifts of the mean were considered. The mean could be increased by a certain percentage of the mean itself or by a percentage increase based on the standard deviation. If the UAP is only slightly larger than the historical mean, a large sample size is required. In this particular example (copper) the standard deviation was large if

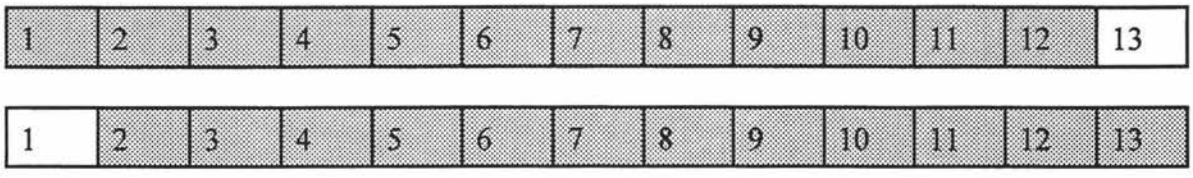
one considers the size of the mean.

The sample size can be spread evenly over a period. For instance, based on 12 weeks of 2 samples each week, the assurance can be given at this point in time that over the last 12 weeks the mean did not shift by more than 0.5 mean from the historic mean. Next week 2 samples will be taken again and the same assurance will be given over the previous 12 weeks which equates to the previous assurance minus the first week of sampling plus the last week of sampling. Weeks covered by assurance at two consecutive points in time are shaded (Figure 3.1).

Table 3.1 Required sample size for testing copper in sheep based on historical data

Increase Historical Mean by	UAP	Required Sample Size
0.10 mean	82.7079	560
0.25 mean	93.9863	90
0.50 mean	112.7835	23
1.00 mean	150.378	6
0.10 SD	81.2648	857
0.25 SD	90.3785	138
0.50 SD	105.568	35
1 SD	135.947	9

UAP = Historical Mean + x mean, or
UAP = Historical Mean + x SD.



1,2 3 etc. denote sequential weeks

Figure 3.1 Illustration of the concept of providing assurances over a moving time frame

Sample sizes for binomial distribution with ivermectin/milbemycin

The required sample sizes to detect higher levels of non-compliance with regard to ivermectin/ milbemycin in bulls are shown in Table 3.2. The sample size depended heavily on the historical p-value, ie the historical level of non-compliance. For instance a sample size of only 22 is required to compare p = 0.284237 and p = 0.568475. These two p-values are ten times greater than the values described by Hist p (0.0284237) and Hist p *2 (0.0568475) in Table 3.2 which require a sample size of 293 to be compared.

Table 3.2 Required sample size for testing ivermectin/milbemycin in bulls based on historical data

Degree of change	UAP	Sample Size
Hist p * 2	0.0568475	293
Hist p * 3	0.0852711	74
	0.10	47
Hist p * 4	0.1136948	33

Sequential sampling with copper and ivermectin/milbemycin

Figure 3.1 shows a sequential sampling plan for copper in sheep. It shows a comparison between the historical mean (75.1890) and the UAP which is 0.5 SD higher than the historical mean. Once the cumulative deviations from the mean are above or below both dotted lines sampling can cease and the conclusion can be drawn whether change has occurred. Figure 3.2 is the sequential sampling plan for ivermectin/milbemycin in bulls. Tables 3.3 and 3.4 show the sample sizes which are expected to be required for drawing a conclusion whether or not the historical level has changed.

The expected number of samples under sequential sampling can be compared with the required number of samples under fixed sampling. A comparison between Tables 3.1 and 3.3, and between Tables 3.2 and 3.4 give an impression of the reduction of the number of samples which are required to give assurances regarding unchanged levels of non-compliances.

Discussion of sampling plans

The intention of the use of quality control charts is to take corrective action if the mean value of samples has reached an unacceptable level. Often quality control charts are intended to detect as

soon as possible when a process goes out of control. The great variability of the residue values (a large standard deviation) in meat and offal, and the ramifications of false alarms warrant caution in this regard. The low level of violations also makes it difficult to detect a relatively large change in terms of percentages which may be small in actual figures.

There is a need to screen out 'gross outliers'. These outliers are values that are far outside the range that can reasonably be expected. If such outliers are detected, an initial investigation should be carried out to determine whether the procedures which have been followed with regard to the livestock class, tissues, laboratory procedures and recording were correct. If any mistakes are detected which could no longer be corrected, the sample should be declared not valid and it should be replaced.

Table 3.3 Expected sample size for sequential sampling of copper in sheep

		Expected sample size under	
Increase of the mean by:	UAP	Hist. mean	UAP
0.10 mean	82.7079	261	311
0.25 mean	93.9863	42	50
0.50 mean	112.7835	11	13
1.00 mean	150.378	3	4
0.10 SD	81.2648	399	476
0.25 SD	90.3785	64	77
0.50 SD	105.568	16	20
1 SD	135.947	4	5

Table 3.4 Expected sample size for sequential sampling of Ivermectin/milbemycin in bulls

		Expected sample size under	
Change of probability of violations	UAP	Hist mean	UAP
Hist p * 2	0.0568475	137	163
Hist p * 3	0.0852711	35	41
	0.10	22	26
Hist p * 4	0.1136948	16	19

If the process is considered to be statistically out of control, the reasons for this need to be investigated and corrective action needs to be taken. Individual violations may still need to be investigated to ensure that the deterrent function of sampling remains in place. If farmers became less concerned about their violations being detected, the level of violations might very well change.

Information on residues should be collected and interpreted on an ongoing basis. This will make it possible at regular times to finetune the baseline and to check that it is still appropriate. Regular evaluations of the baselines should occur, for instance on a yearly basis.

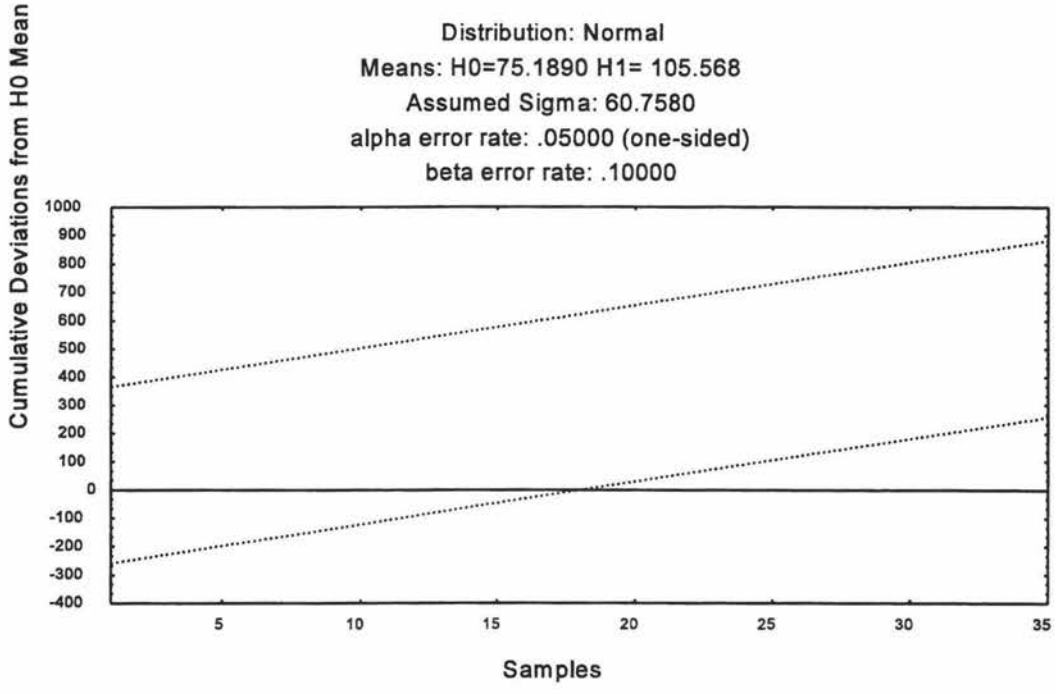


Figure 3.2 Sequential sampling plan for copper in sheep

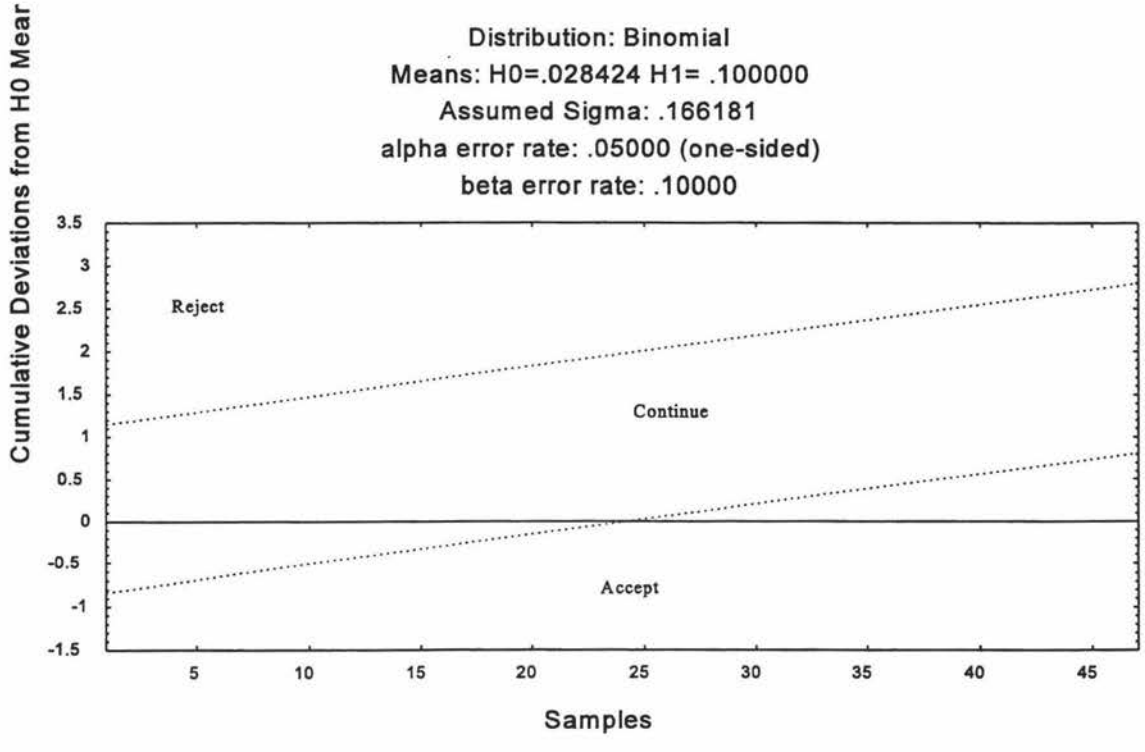


Figure 3.3 Sequential sampling plan for ivermectin/milbemycin

Materials and methods of temporal simulations

The prevalence of detectable levels of residues of levamisole of lambs was 66/483 = 0.137. A binomial distribution was used for the baseline and for predictive simulations of years with higher values. It was considered that clustering in time could be evaluated if the baseline over a period increased by the values listed below. The following binomial distributions were used for the distributions:

Baseline			p = 0.137	
Baseline	*	1.1	p = 0.151	(Baseline + 10%)
Baseline	*	1.25	p = 0.171	(Baseline + 25%)
Baseline	*	1.5	p = 0.206	(Baseline + 50%)

Comparisons of violations were made based on the number of animals that were sampled daily. The number of samples chosen was 1, 2, 5 10 and 50 animals per day. The mean, standard deviation (SD) and the Upper Control Limit (UCL) were calculated based on the first year of the simulations with the various number of animals per day (1, 2, 5, 10 and 50). A Moving Average (MA) approach was taken to evaluate the figures. The averaging period used consisted of 7 days.

The following formula for used for the UCL:

UCL = mean + 3*SD/√7

Table 3.5 Upper Control Limit used for temporal simulation of levamisole non-compliances in lambs

Animals/day	Mean	SD	UCL
1 animal/day	0.126027	0.332336	0.5028606
2 animals/day	0.297	0.511783	0.8703073
5 animals/day	0.663014	0.710113	1.4682065
10 animals/day	1.367123	1.100456	2.6149228
50 animals/day	6.846575	2.42662	9.5981034

In the analyses below a non-complying day is a day when the moving average level of non-compliances exceeded the UCL. As an example the two rows in Figure 3.4 denote two periods each of 13 days. The top row has five violative days which are denoted by the shaded cells. The bottom row has seven violative days. A violative run is a number of days in sequence which are all exceeding the UCL. The top row has three violative runs and the bottom row has four violative runs. It should be noted that a certain high level of violations on a particular day does not result in a violative day if the moving average does not exceed the UCL. Only when the mean over a period of seven days exceeded the UCL would a day be called a violative day.

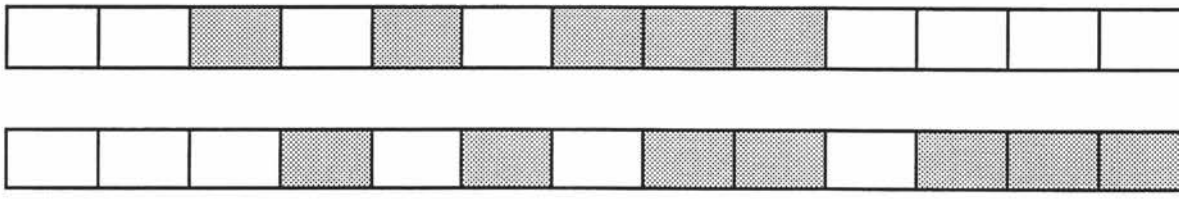


Figure 3.4 Explanation of difference of non-complying days and runs

Results of temporal simulations

The results of the simulations with regards to non-complying days and runs are shown in Table 3.6 and 3.7 respectively. If one animal was sampled per day, the MA was rather insensitive for determining whether the level of non-compliances was elevated to the level p=0.151 or p=0.171. In fact, under baseline conditions there were 17 violative days over the 5 year period. At p=0.151 a smaller number of days was violative than under normal conditions. At the level p=0.171 there were 21 violative days which was less than one extra violative day per year extra. The number of violative days increased to 85 days at the 50% higher level (p=0.206).
On a yearly basis there were variations from no violations to 8 violations when the level varied from p=0.137 to p=0.171. Even at the p=0.171 level there was still one year without violations. All yearly figures for the p=0.206 level were higher than for the other levels.
The number of violative runs displayed a similar pattern. There were again more violations at the baseline level than at the p=0.151 level over a five year period.

Sampling two animals per day resulted in lower numbers of violative days over five year periods compared with sampling one animal per day. Especially the number of violations at the p=0.151 and the p=0.171 level were low. This applied to both the number of days and the number of runs.

The number of violative days under normal conditions when five animals per day were sampled was higher than when one animal was sampled per day. However this was not reflected in the violative runs. The number of violative days and runs increased at the p=0.151 level compared with the sampling of one animal but the same did not occur for the p=0.171 level. A large increase of violations was detected at the p=0.206 level.

When ten animals per day were sampled there was only one violative day over a five year period under baseline conditions. The number of violative days remained relatively low, especially at the 0.151 level. As an example in the fifth year only one day was violative. Even at the p=0.171 level there was one year during which no violative days were detected.

When fifty animals were sampled per day there were no violative days at the baseline level. A comparison with the sampling of one animal per day showed that the number of violative days and runs at the p=0.151 level was respectively 35/14 and 15/7 times higher. Large numbers of violations were detected at p=0.171 and p=0.206 levels.

Table 3.6 Number of violative days per time period

animals sampled per day	p	year1	year2	year3	year4	year5	Total
1	.137		5	4	2	6	17
1	.151		1	4	7	2	14
1	.171	6	8	5	2		21
1	.206	10	19	23	11	22	85
2	.137	4		5		3	12
2	.151	1	5		1		7
2	.171		2	1	3	1	7
2	.206	5	12	13	6	6	42
5	.137	3	6	1	5	4	19
5	.151		6	2	13	5	26
5	.171	5		3	11	1	20
5	.206	35	43	37	33	36	184
10	.137	1					1
10	.151	4	2	8	4	1	19
10	.171		12	5	20	12	49
10	.206	22	51	36	59	25	193
50	.137						0
50	.151	7	8	8	3	9	35
50	.171	42	44	49	103	75	313
50	.206	Not determined on a yearly basis.					>1250

Table 3.7 Number of violative runs per time period

animals sampled per day	p	year1	year2	year3	year4	year5	Total
1	.137		2	2	1	3	8
1	.151		1	1	3	2	7
1	.171	5	3	2	1		11
1	.206	3	5	7	6	7	25
2	.137	2		2		1	5
2	.151	1	2		1		4
2	.171		1	1	2	1	5
2	.206	3	4	5	3	1	16
5	.137	1(>)	2 (<)	1	1	2	6
5	.151		4	2	6	2	14
5	.171	1		2	7	1	11
5	.206	8	15	13	9	12(>)	57
> and < Indicates a violative run which started in year 1 and finished in year 2							
10	.137	1					1
10	.151	3	2	5	2	1	13
10	.171		5	1	7	6	19
10	.206	11	14	13	13	9	60
50	.137						0
50	.151	4	4	2	2	3	15
50	.171	14	20	19	23	24	100
50	.206	Not determined.					

Discussion of temporal simulations

It should be noted that after a violative day there is more likely to be another violative day than after a day without a violation. This is because the value of the new day is added to previous values which are already high if the previous day was violative. Therefore the number of violative runs in addition to the number of violative days may place the findings in a broader perspective. However if a high level of violations starts to occur, long runs (consisting of many days) may occur resulting in a relatively smaller number of such violative runs.

In summary the MA approach seemed to have severe limitations in detecting trends of increased levels of violations. An increase from one to two or five animals sampled per day did not have much impact, if any, on reducing the number of false positive days. Violations at p=0.206 were detected relatively well but it should be considered that this is a very large increase under practical conditions. A low level increase of violations was usually not detectable on a yearly basis in a practical way ie by sampling a small number of animals.

If one animal of a livestock class per day is sampled, a total of 365 animals per year is sampled. This is a considerable number for a chemical residue programme under practical conditions. It could be argued that with the data set and simulations that were used ten animals per unit of time (ie day) were required to reduce the number of false positive values. This number of animals did not improve the sensitivity greatly at the p=0.151 level. In these simulations it performed even worse than taking five samples per day.

Thus increasing the sampling intensitiy from the typical very low value was ineffective (within the practical range) in reliably detecting a true increase in the occurrence of levamisole residues. It is clear that current monitoring systems used around the world for chemical residues have very poor sensitivity and specificity in detecting true changes in the occurrence of chemical residues, and merely increasing sampling intensity (even to totally unrealistic levels) offers little benefit in genuinely reducing whatever hazard consumers may face from such residues, Clearly a different approach is needed

The distribution of the values of cadmium in 73 kidneys of hoggets as supplied by the New Zealand Ministry of Agriculture (Table 3.8) was evaluated with a histogram. For the purpose of this simulation it was considered that this distribution could normally be expected in a hogget population. The mimimum value was 0 and the maximum value was 1.2 ppm. Steps of 0.15 ppm were used to create the histogram (Figure 3.5).

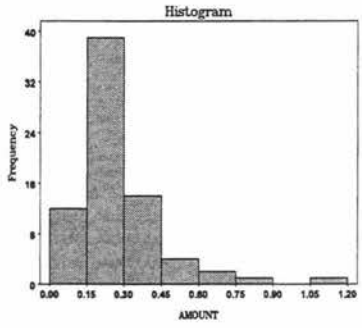


Figure 3.5 Histogram distribution of cadmium

Table 3.8 Frequency distribution of cadmium values in hoggets for a normal situation

Low value	High value	Number of animals	Percent
0.00	0.15	12	16.4
0.15	0.30	39	53.4
0.30	0.45	14	19.2
0.45	0.60	4	5.5
0.60	0.75	2	2.7
0.75	0.90	1	1.4
0.90	1.05	0	0.0
1.05	1.20	1	1.4

The actual cadmium values were multiplied by 1.10 (Table 3.9) and 1.25 (Table 3.10). The distributions are shown in Figures 3.6 and 3.7 respectively. The new distributions were to be used in the areas where clustering was going to be simulated.

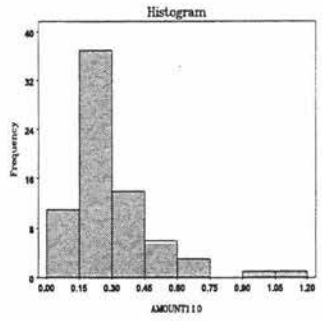


Figure 3.6 Histogram distribution if original cadmium values are multiplied by 1.10

Table 3.9 Frequency distribution of cadmium values which were 1.1 * normal situation

Low value	High value	Number of animals	Percent
0.00	0.15	11	15.1
0.15	0.30	37	50.7
0.30	0.45	14	19.2
0.45	0.60	6	8.2
0.60	0.75	3	4.1
0.75	0.90	0	0.0
0.90	1.05	1	1.4
1.05	1.20	1	1.4

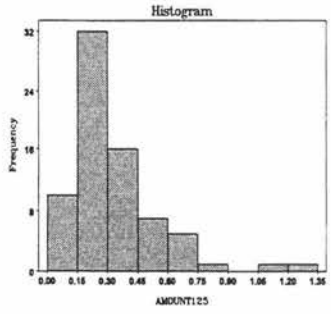


Figure 3.7 Histogram distribution if original cadmium values are multiplied by 1.25

Table 3.10 Frequency distribution of cadmium values which were 1.25 * normal situation

Low	High	Number of animals	Percent
0.00	0.15	10	13.7
0.15	0.30	32	43.8
0.30	0.45	16	21.9
0.45	0.60	7	9.6
0.60	0.75	5	6.8
0.75	0.90	1	1.4
0.90	1.05	0	0.0
1.05	1.20	1	1.4
1.20	1.35	1	1.4

The locations where the simulated samples were taken were established by determining the locations from which sheep had been submitted for slaughter. In some cases the precise coordinates of a location could not be determined because there are two or more locations in New Zealand with the same name. These locations were deleted from the group of locations which could potentially be sampled. Next random numbers were given to the remaining locations and the 64 locations with the lowest random numbers were used for the simulations. The location with the lowest random number (which happened to be Wanganui) was used as the area around which clustering was going be simulated.

Statistics to evaluate clustering were applied to the normal situation without clustering and to situations where clustering occurred. Clustering was considered to occur if the locations of the cluster had simulated residue levels which were 1.1 times higher than normal (coded as 'L') or 1.25 times higher than normal (coded as 'H'). The size of the clusters consisted of 2, 6 or 16 locations. Clustering was evaluated for Wanganui and its closest location (coded as 'L2' and 'H2'). It was also evaluated for Wanganui and its 5 nearest neighbours (coded as 'L6' and 'H6') and its 15 nearest neighbours (coded as 'L16' and 'H16').

The chemical residue value which was considered to be representative for a location was derived from the mean residue value of 1, 2, 5, 10, 25 and 50 animals. One animal was modeled in @ Risk by one iteration, two animals by the mean value of two iterations etc. The simulations regarding different number of animals deemed to be representative for an area (1, 2, 5, 10, 25 or 50 animals) were performed independently of each other. This was initially done for the normal situation ie the situation for which no clustering was simulated. Subsequently these five simulations were saved for the different levels of clustering (L2, H2, L6, H6, L16 and H16)

where they continued to represent the locations which did not have clustering (ie simulated elevated levels of chemical residues). The areas with clustering (ie Wanganui and its 1, 5 or 15 nearest neighbours) were subjected to new simulations with the new low (L2, L6 or L16) or high (H2, H6, or H16) levels of clustering. These new simulated values replaced the original values for Wanganui and surrounding locations while the rest of the country where clustering did not occur retained its original values. A result of this approach is that chance clustering in the simulation of the normal situation would be copied to the other simulations (L2, L6, L16, H2, H6 and H16). For example if during the simulation with 10 sheep five neighbouring towns in the South Island displayed clustering, then this chance occurrence might show not only in the normal simulation but also in the L2, L6, L16, H2, H6 and H16 simulations. This could lead to the incorrect conclusion that the spatial statistics were able to discover the simulated clustering at L2, L6, L16, H2, H6 and H16, while in fact the clustering that was discovered occurred in a normal area. In the case of this particular example this situation would only apply to simulations with 10 sheep and not to simulations with 1, 2, 5, 25 or 50 sheep. Since this sitution can occur in reality it was considered a reflection of a potential problem.

The performance of a number of statistics were compared in Spacestat™ (geographical software). These were Moran's I, Geary's c, the G(d) and the $G_i(d)$ statistics. For all statistics the p-values or prob values as displayed by Spacestat were used to evaluate statistical significance. Both Moran's I and Geary's c use weight matrices based on inverted values of distances. During the simulations three weight matrices were used for these two statistics. They were based on the inverted distance, the inverted values of the squared distances and the inverted values of the distances to the power 3. The intention of using different weight matrices was establish the sensitivity of the I and the c statistic to the weight matrix. The calculations of the I and the c statistic in Spacestat were performed after row standardisation. This involves dividing all elements of a row by the sum of the corresponding row. Calculations by Spacestat were performed by permutation.

In order to detect local pockets of clustering the G(d) and the $G_i(d)$ statistic were used. In the case of these statistics, bands of distances need to be chosen. The distances chosen in these cases were 0-20 km, 0-65 km, 0-105 km and 0-205 km. It should be appreciated that although the first three bands were chosen so that they included the locations L2/H2, L6/ H6 and L16/H16 as described above they also included other locations which had distances between each other as described above. For instance 2 locations in the South Island could be less than 65 km distant from each other and therefore they would be included in the numerator for the $G_i(d)$ statistic for < 65 km.

Results of spatial simulations

Spacestat only shows ten entries for the largest and the lowest values. At times it may not show all statistically significant values. This is illustrated below by the code '+' which means there may have been (more) statistically significant values.

Simulation of a normal situation

In the simulation of the normal situation two relatively low p-values (p = 0.05) were detected by the I statistic (Table 3.11). However the c statistics were high (p= 0.95). In general the I and the c statistic were relatively similar but with regard to the 10 iterations they displayed a large difference.

The G(d) statistics did not show any evidence of clustering (Table 3.12).

Although no clustering was created the $G_i(d)$ showed a considerable number of locations with high values and others with low values (Table 3.13). It should be considered that if one location is statistically significant, other locations around it are more likely to be statistically significant as well. The distance (d) appeared to be of small importance in some cases. For instance when 2 simulations were performed locations in the area covered by numbers 62 and 63 were frequenly statistically significant. Generally, clustering of high values was more likely to occur than clustering of low values. Since no clustering was included, but statistically significant values were still detected, the values could be considered false positives.

Table 3.11 I and c, p-values for simulation of normal situation

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.12	0.23	0.21	0.30	0.27	0.30
2	0.53	0.59	0.51	0.78	0.47	0.81
5	0.24	0.24	0.13	0.31	0.13	0.35
10	0.06	0.93	0.05	0.95	0.05	0.95
25	0.18	0.15	0.42	0.39	0.43	0.60
50	0.14	0.15	0.09	0.09	0.09	0.11

Table 3.12 G (d), p-values for simulation of normal situation

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.10	0.17	0.11	0.50
2	0.40	0.34	0.44	0.43
5	0.33	0.34	0.15	0.21
10	0.42	0.17	0.45	0.34
25	0.31	0.34	0.25	0.08
50	0.48	0.31	0.41	0.41

Table 3.13 $G_i(d)$, clustered locations for normal situation

	Largest values		Lowest values	
Iterations	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1		2 7 11 22 27		
2	63 44			
5	36 35 61 27			
10	10	27 48		
25		63 46		
50				10 30
d < 65 km				
1	53 27 49	51 22		13 30
2	63 64 62			48 52 53
5	38 27 34	13 22 62 36 40		
10	1	21 2		
25	62	63 56 64 50		2
50		42 29 36	45	19 25 17 58
d < 105 km				
1	53 51	16 49 5 54 14 8	25	29 33 20
2	64 62	63 50 59 58		53 52 48 51
5		34		7
10				32 61
25	62 63 61 59	60 56 58 57 64	28	20 29
50		38	58	
d < 205 km				
1	53 51 49	48 19 52	32 35 30 36 34 28	38 61
2		62 59 60		48 55 53 52 51
5				26
10				

25	56 60 63 59 57 62 58 64 61		21	13 26 16 32 8 28 30 14 3 +
50	29	33 25		

Simulation of clustering at two locations at a low level (L2)

After 50 iterations of clustering at a low level, the I and the c statistic were able to detect a degree of autocorrelation (Table 3.14). The c-statistic was sensitive to the distance matrix used. It did not detect autocorrelation if the 1/d weight matrix was used.

The G(d) statistic did not discover any statistically significant findings for L2, even when 50 iterations were performed (Table 3.15).

Table 3.14 I and c, p-values for simulated clustering at L2 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.06	0.12	0.10	0.26	0.18	0.35
2	0.53	0.59	0.48	0.77	0.52	0.80
5	0.25	0.30	0.14	0.34	0.17	0.37
10	0.06	0.94	0.04	0.96	0.03	0.94
25	0.22	0.22	0.40	0.35	0.44	0.44
50	0.02	0.31	0.01	0.04	0.01	0.02

Table 3.15 G (d), p-values for simulated clustering at L2 level

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.13	0.15	0.21	0.27
2	0.37	0.37	0.39	0.50
5	0.30	0.35	0.17	0.25
10	0.39	0.18	0.46	0.35
25	0.39	0.45	0.40	0.17
50	0.17	0.46	0.37	0.24

Table 3.16 $G_i(d)$, clustered locations for simulation at L2 level

	Largest values		Lowest values	
Iteration	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1		7 11 22 27		
2	63 44			
5	36 35 61 27			
10	10	27 48		
25		2		
50	1 2	3		
d < 65 km				
1	53 27 49 51	22		13
2	63 64 62			48 52 53
5	38	27 34 13 22 62 36		
10		1 21 2		
25		62 63 56		30
50	6	2 3 8 4 5 1		45 19
d < 105 km				
1	53 51	49 54 22 52 48	25	13 29
2	64 62	63 50 59 58		53 52 48 51
5		13 34		
10				32 61
25	62 63 61	59 60 56 58	28	20 29 30 25
50	13 8 6			58
d < 205 km				
1	53 51 49	48 52	30 28 32 35	36 34 25 29 38 21 +
2		62 59 60		48 55 53 52 51
5				

10			
25	56 60 63 59 57	62 64 58 61	32 33 21 38 34 30
50	29	25 28 23 14 15 26 16 22 12 +	

Clustering of lower level values (original distribution * 1.1) was created at locations 1 and 2 which are < 20 km distant from each other. This pattern was distinguished by G_i(20 km) and not by the other statistics. However 25 or 50 iterations were required to detect the clustering(Table 3.16). When a lower number of iterations was used false positive values resulted. These values were often the same as the ones from the simulation of the normal situation.
At distance < 65 km locations 1 and 2 were also identified after 10 and 50 iterations. However a number of other locations were identified as well.

Simulation of clustering at two locations at a high level (H2)

At the H2 level, the I statistic detected autocorrelation after 25 and 50 iterations with five out of six matrices (Table 3.17). The c statistic was more conservative and only found spatial autocorrelation for H2 after 50 iterations. In addition it was very sensitive to the weight matrix used. A significant finding was detected with the $1/d^3$ weight matrix.Although both were statistically insignificant at $p < 0.05$, it should be noted that there was a large difference in p-values of the I and the c statistics after 25 iterations with the weight matrix 1/d.

The G(d) statistic detected a local pocket for clustering when the distance was less than 20 km (Table 3.18). This was consistent with the clustering at these two sites only. However this statistic required 50 iterations to detect the clustering.

Table 3.17 I and c, p- values for simulated clustering at H2 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.07	0.14	0.13	0.32	0.20	0.36
2	0.34	0.88	0.41	0.90	0.38	0.88
5	0.30	0.33	0.16	0.35	0.17	0.39
10	0.34	0.72	0.51	0.71	0.45	0.68
25	0.07	0.60	0.03	0.18	0.02	0.06
50	0.02	0.53	0.02	0.08	0.02	0.02

Table 3.18 G (d), p-values for simulated clustering at H2 level

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.051	0.111	0.112	0.422
2	0.126	0.467	0.375	0.257
5	0.170	0.426	0.245	0.354
10	0.215722	0.099721	0.233686	0.145255
25	0.062341	0.343223	0.273472	0.486322
50	0.028425	0.322814	0.141955	0.075958

The locations 1 and 2 were correctly identified by the $G_i(d)$ statistic after 10, 25 and 50 iterations and location 1 was identified after 2 iterations when d < 20km (Table 3.19). Other locations were identified as significantly higher after 1, 2 and 5 iterations when d < 20km. Locations 1 and 2 were also identified after 10 and 50 iterations and location 1 after 25 iterations when d < 65 km. A number of other locations were also positive when d < 65 km.

Table 3.19 $G_i(d)$, p-values for simulated clustering at H2 level

	Largest values		Lowest values	
	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1		7 11 22 27		
2	63 1 3 44			
5	36 61 35	27		
10	1 10	2 27		
25	1 2 3			
50	1 2 3			
d < 65 km				
1	53 27 49	51 22 11 7		13 30
2		63 64 62 8		52 48 53
5	38	27 34 13 22 62 36		

10	1 4	2 6 8 3 5		
25	4 8	1 6		
50	1 6 3 4 5 2	8		
d < 105 km				
1	53 51	49 16 54 5 22 14 8	25	29 33 20
2	64 62			53 52 48 51
5		13 34		
10		1 10 8 4 15		32 61
25	13	5 16		28 20
50	13	8 6 10 4 15 16 14 3 12 +		
d < 205 km				
1	53 51 49	48 52 19	30 32 28 35 36 34	38 25 61
2				48 55 53 52 51
5				
10		21 11 15 12 10 13 17 9		60
25				32 33 38 34
50		29 28 25 21 23 14 18 15 22 12 +		

Simulation of clustering at six locations at a low level (L6)

Both I and c statistics detected spatial autocorrelation after 50 iterations ($p < 0.05$) but not after 25 iterations in the case of simulations at the L6 level (Table 3.20).

The G(d) statistic was highly significant ($p < 0.01$) at $d < 65$ km but 50 iterations were required (Table 3.21). It did also detect the pocket of clustering at $d < 65$ km after 25 iterations, but the p-value was higher. Also significant findings were recorded after $d < 20$ km and $d < 105$ km.

Table 3.20 I and c, p- values for simulated clustering at L6 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.26	0.66	0.37	0.68	0.40	0.73
2	0.60	0.66	0.55	0.81	0.42	0.84
5	0.43	0.57	0.21	0.53	0.29	0.54
10	0.20	0.81	0.11	0.85	0.10	0.86
25	0.11	0.25	0.11	0.08	0.13	0.06
50	0.01	0.02	0.01	0.01	0.01	0.01

Table 3.21 G (d), p-values for simulated clustering at L6 level

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.069	0.142	0.112	0.496
2	0.348	0.282	0.337	0.449
5	0.261	0.361	0.373	0.436
10	0.473453	0.186250	0.394688	0.274516
25	0.350519	0.033573	0.117356	0.464908
50	0.040702	0.0088370	0.025468	0.052045

The cluster that was created included locations 1, 2, 3, 4, 5 and 6 and the relevant distance was d < 65 km. After 25 and 50 iterations these locations emerged according to the $G_i(d)$ statistic as sites where 'largest values' occurred (Table 3.22). Location 5 was not shown after 25 iterations but may have been part of the locations shown as "+". Also after 10 iterations 3 of the locations were detected. However it should be noted that many other locations were significant after 25 and 50 iterations as shown by the "+" in the table.

Table 3.22 $G_i(d)$, clustered locations for simulation at L6 level

	Largest values		Lowest values	
Iterations	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1	1 3	7 11 22		
2	63 44			
5	36 61 35	27		
10	10	27 48		
25	10	2		
50	3 2 10 4 1			
d < 65 km				
1	53 27	49 11 7 51 22		
2	63 62 64			48 52 53
5		38 27 13 34 22 62		
10	1	2 21 3		
25	1 7 4 9 11 12	8 3 2 6 +		30 38
50	1 2 3 5 6 4 8 7 9 11 +	+		45
d < 105 km				
1	53 51	16 49 5 14 54 8		25 29
2	64 62	63 59 50 58		53 52 48 51
5		13		
10				32 61
25	16	14 2 10 5 9 15 8 1 63 +	28	20 29 30 25
50	8 1 10 15 16 14 2 12 9 7+	+		58
d < 205 km				
1	53	51 49 19 48	30 32 35	36 34 28 38
2		62 59 60		48 55 53 52

5				
10		31		60
25		60 56 17 63	32 33 38	34 40 36 35 42 43
50	21 23 15 14 18 22 12 26 16 11 +	+		36 35 40 38 34

Simulation of clustering at six locations at a high level (H6)

Both the I and the c statistic detected spatial autocorrelation at the H6 level after 25 and 50 iterations (Table 3.23). The significant findings after one simulation were noted with interest since this pattern was not generally repeated after 2, 5 and 10 iterations. The I statistic also showed a significant finding after 5 iterations but the c statistic was not in agreement.

The G(d) statistic showed clustering for distance bands after 1, 25 and 50 iterations with the exception of $d < 205$ km after 1 iteration (Table 3.24). It was also significant in the $d < 20$ km band after 5 iterations and $d < 65$ km band after 10 iterations.

The $G_i(d)$ statistic performed relatively well after 1, 10, 25 and 50 iterations in the appropriate band ($d < 65$ km)(Table 3.25). After 2 iterations once again the values 63, 64 and 62 occurred, illustrating the difficulty separating out these values which were initially high by chance. Also when $d < 20$ km and $d < 105$ km the locations that were part of the cluster were often detected. At $d < 105$ km a large number of locations were detected which had statistically significant low p-values.

Table 3.23 I and c, p-values for for simulated clustering at H6 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.02	0.26	0.02	0.02	0.02	0.01
2	0.59	0.86	0.50	0.87	0.42	0.85
5	0.10	0.35	0.04	0.19	0.06	0.22
10	0.25	0.57	0.35	0.55	0.46	0.61
25	0.01	0.07	0.01	0.01	0.01	0.01
50	0.01	0.02	0.01	0.01	0.01	0.01

Table 3.24 G (d), p-values for simulated clustering at H6 level

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.000007	0.013	0.006	0.072
2	0.082	0.317	0.274	0.195
5	0.016	0.142	0.354	0.302
10	0.190233	0.032236	0.128847	0.104115
25	0.033875	0.000332	0.002497	0.034879
50	0.004856	0.000205	0.000923	0.003081

Table 2.25 $G_i(d)$, clustered locations for simulation at H6 level

	Largest values		Lowest values	
	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1	1 2 3			
2	63 1 3	44		
5	1 3 36 61 35	27		
10	10 2	3 1 4		
25	2 3 10 4	1		
50	2 3 1 4 10			
d < 65 km				
1	4 1 6 3 5	2 8 11 7		
2	8	1 63 64 62		52 48 53
5	1	8 38 27 13 34 22		
10	1 2 5 4 3 6	10 11 9		
25	1 2 4 3 7 9 5 11 6 8 +	+		

50	1 2 3 5 4 6 8 7 9 11 +	+		
d < 105 km				
1	16 8 14 5 11 3 10 7 13 6 +	+		25 29
2		64 62		53 52 48 51
5		13		29
10	1 10 8 15 2 9 16 4 14 11 +			32 61
25	10 16 2 1 14 15 8 5 9 7 +	+		28 30
50	8 10 1 15 16 2 14 9 12 7 +	+		
d < 205 km				
1	19 24 17	18 11 12 22 15 7 6 +	32 35 36 34	38
2				48 55 53 52 51
5		21 25		
10	21	11 15 12 13 10 17 9 18 14 +		60
25	24 17 19 18 11 12 7 21 22 15 +	+	32	33 38 34 40 36 35 42 43 41 +
50	21 24 18 19 17 11 15 22 12 23 +	+		35 36 32 40 38 34 42

Simulation of clustering at 16 locations at a low level (L16)

Both I and c statistics had significant findings after 25 and 50 iterations (Table 3.26). The I statistic also showed one significant finding after one iteration

The G(d) statistic was significant for all distance bands after 25 and 50 iterations (Table 3.27). Both the < 65 km and < 105 km distance bands were significant after 10 iterations. There were one significant finding after two iterations when d < 20km.

In the case of the $G_i(d)$ statistic, the category of most importance ($d < 105$ km) included the 16 clustered locations (and possibly other ones) performed well after 10, 25 and 50 iterations (Table 3.28). It should be noted that there are more locations with statistically significant findings as indicated by the '+'. However since the lowest p-values are in the left column ($p < 0.01$) the actual pattern can clearly be distinguished. A large proportion of the sites (16/64) constitute the cluster. This is also reflected in the columns with the lowest values on the right hand side of the page, which are starting to include more values than in previous simulations and they do not include the clustered locations.

Table 3.26 I and c, p- values for simulated clustering at L16 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.04	0.05	0.09	0.15	0.09	0.17
2	0.37	0.85	0.44	0.87	0.56	0.85
5	0.20	0.32	0.11	0.27	0.14	0.31
10	0.22	0.88	0.09	0.93	0.07	0.90
25	0.01	0.01	0.01	0.01	0.01	0.01
50	0.01	0.01	0.01	0.01	0.01	0.01

Table 3.27 G (d), p-values for simulated clustering at L16 level

Iterations	$d < 20$ km	$d < 65$ km	$d < 105$ km	$d < 205$ km
1	0.064	0.186	0.077	0.485
2	0.012	0.217	0.190	0.299
5	0.064	0.239	0.402	0.272
10	0.171073	0.008376	0.045888	0.125547
25	0.002163	0.000001	0.000013	0.009450
50	0.000158	0.000000	0.000001	0.000420

Table 2.28 $G_i(d)$, clustered locations for simulation at L16 level

	Largest values		Lowest values	
	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1	1 22 27			10
2	1 63 3 44			
5	2 36 61 35	4 27		
10	1 3 16	27 48		
25	12 9	14 2 3 11 16		
50	9 3 11 12	7 1 14 6 2		
d < 65 km				
1	53 22 27 49	51 16 8		17 30 13
2		63 64 62		52 48 53
5		38 27 13 34 62		
10	8 3 11 4 7	1 9 6 5 22		32 16
25	5 7 9 11 4 3 12 2 14 6 +	+		30 32 51 28 34 43 47
50	4 3 5 7 11 9 6 12 2 14 +	+		45 44 46
d < 105 km				
1	53 51 22	49 23 16 54 8 26 5 +	25 29	33 30 36 28
2		64 62		53 52 48 51
5	13			29
10	1 2 10 6	18 3 4 15 16 11 +	32	61
25	1 2 5 14 16 4 6 3 9 10 +	+	28 30 29 25	32 40 38 51 33 36 +
50	1 2 5 6 4 16 14 3 9 10 +	+		58 45 40 47 46 25 41 61 42 36 +
d < 205 km				

1	53 51 49	48 19 52 17	36 34 35 30	38 32 28 61 31
2				48 55 53 52
5		21		
10	18	13 19 11 12 24 17 10 22 7 +		32 60 61
25	17 24 19 11 12 15 6 7 10 18 +	+	38 34 40 36 42 43 41 33 35 32 +	+
50	17 24 11 18 19 12 15 14 22 23 +	+	36 40 34 38 42 35 41 43 44 45 +	+

Simulation of clustering at 16 locations at a high level (H16)

Both the I and the c statistic for H16 were significant after 10, 25 and 50 iterations (Table 3.29).

The G(d) statistic was more often statistically significant when d < 65 km than when d < 105 km (Table 3.30). In the lowest band (d < 20km), significant findings occurred if there were 5 or more iterations. In the highest band (d < 205 km) significant findings occurred at 2, 10, 25 and 50 iterations.

The $G_i(d)$ statistic performed well with the exception of five iterations. The relevant distance band (d < 105 km) discovered 5 out of 16 clustered sites after 1 iteration (Table 3.31).

Table 3.29 I and c, p- values for simulated clustering at H16 level

	weight matrix 1/d		weight matrix $1/d^2$		weight matrix $1/d^3$	
Iterations	I p-value	c p-value	I p-value	c p-value	I p-value	c p-value
1	0.14	0.34	0.23	0.43	0.27	0.45
2	0.21	0.57	0.28	0.60	0.34	0.56
5	0.23	0.28	0.16	0.30	0.20	0.39
10	0.01	0.01	0.01	0.01	0.01	0.01
25	0.01	0.01	0.01	0.01	0.01	0.01
50	0.01	0.01	0.01	0.01	0.01	0.01

Table 3.30 G (d), p-values for simulated clustering at H16 level

Iterations	d < 20 km	d < 65 km	d< 105 km	d< 205 km
1	0.59	0.046	0.078	0.430
2	0.12	0.025	0.019	0.040
5	0.003	0.018	0.105	0.188
10	0.004	0.000001	0.000008	0.000556
25	0.000043	0.000000	0.000000	0.000022
50	0.000015	0.000000	0.000000	0.000001

Table 3.31 $G_i(d)$, clustered locations for simualtion at H16 level

	Largest values		Lowest values	
	< 0.01	<0.05	<0.01	<0.05
d < 20 km				
1	10	7 11 22 27		
2	63 2	44		
5	7 36 61 12 35	27 11		
10	9 6 14	11 16		
25	9 3 12	11 2 16 1 4		
50	9 2 11 12 3	16 7 4 1 14 +		
d < 65 km				
1	53 27 7 1	49 51 11 3		30 13
2	2 4	1 6 14 12 7 63 11 64 +		52 53 48
5		38 27 13 2 5 22 3 34 4 62 +		
10	3 11 5 4 9 7 12 6 18 15 +	+		37 61 38
25	3 5 9 7 4 11 12 2 6 14 +	+		30 32 34
50	5 4 3 11 9 7 2 6 12 14 +	+		

d < 105 km				
1	53 51	16 8 14 49 54 5 7	25	29 33 30
2	2	16 14 1 5 9 4 12 7 3+		53 52 48 51
5		14 22 1		29 46
10	1 2 4 6 3 5 18 9 10 14+	+	32	61 35 34 30 36
25	1 2 5 4 6 16 14 9 3 10 +	+	28 30	32 40 29 25 38 36 34 33 +
50	1 2 5 4 6 16 14 3 9 10 +	+		40 30 32 36 45 41 42 28 35 46 +
d < 205 km				
1	53	51 49 19 48 17 15	30 32 36 35 34 28	38 61
2	27 24 17 26 19 13 12 8 2 23 +	+	48	55 53 52 51 49 45
5		20		54
10	17 13 11 18 24 15 19 12 6 7 +	+	36 38 34	60 33 42 35 61 40 32 +
25	17 24 11 19 15 12 18 7 6 10 +	+	38 34 36 40 42 43 41 35 44 45 +	+
50	17 24 11 18 13 15 12 19 14 7 +	+	36 40 34 38 42 41 43 35 44 45 +	+

Discussion of spatial simulations

The sampling took place at 64 locations and the number of animals sampled per location varied between 1 and 50. The simulations were based on available data which was considered the normal situation. The data showed a large degree of variation and the high values may in fact have been part of actual clusters themselves. Lessons can be learned from these simulations, however it should be considered that a different data set with less variation might have provided

a different appreciation of the usefulness of spatial statistics to detect clustering.

The following are generalisations of the results, and exceptions to the general pattern. The I and the c statistic were more likely to detect clustering at high levels of chemical residue values (H) than at low levels of chemical residue values (L).The I and the c statistic were more likely to detect clustering if occurring at a high number of locations than at a low number of locations. As the number of samples per location increased any clustering was more likely to be detected. However there were situations where clustering was detected after a small number of samples and not after a larger number (although clustering would then usually still be found after 25 and 50 samples). As an example, the simulation at the H6 level resulted in significant findings after one sample but not after two, five or ten samples. It is hypothesised that the risk of false positives is greater at low levels of sampling where at one location high values due to natural variation are not compensated by other samples from that site. This has ramifications for spatial statistics of chemical residues in general. One has to be extremely careful in evaluating clustering of chemical residues if the sample size per location is small and the variation in the population is high.

Goodchild (1986) suggests that a degree of complimentarity exists between the I and c statistic but the degree of mathematical complimentarity is never perfiect. Although the p-values of the I and c statistic were often in accordance in this study, this was certainly not always the case. In general the p-value of the c statistic appeared more conservative than the p- value of the I statistic. At times the difference between the two values appeared very large.

The G(d) statistic was more likely to detect clustering at high levels of chemical residue values (H) than at low levels of chemical residues values (L).The G(d) statistic was more likely to detect clustering if occurring at a high number of locations than at a low number of locations. As the number of samples per location increased any clustering was more likely to be detected. Frequently G(d) statistics for different distances were significant. Generally one would pay most attention to the distance band with the lowest p-values. However, the simulation H16 is an example that this may not always be correct. This finding is probably not surprising since the band d<65km included six of the 16 clustered locations.

Getis and Ord (1992) suggest that the use of the G statistics in conjunction with Moran's I or another measure of spatial autocorrelation deepen the understanding. Once the G(d) statistic had identified the distance of relevance for clustering, the $G_i(d)$ statistic would often be able to identify the locations with the high values, providing useful information for possible further investigation. However, again a large sample size per location was required. At low sample sizes the number of false positive locations could be considered unacceptably high.

Based on the findings of the simulations with this particular data set, one would generally need to sample at least 25 animals per location to detect clustering but even this number was not always sufficient. This would equal 1,600 animals in total. The use of small numbers of samples per location carries a great risk of drawing incorrect conclusions with regard to spatial clustering. Smaller sample sizes may suffice in the case of chemical data sets with less variation. Also the step below 25 samples per location in these simulations was ten. There may have been sample sizes between 10 and 25 which could have resulted in a satisfactory identification of areas with high residue values.

Risk-based Control System of Chemical Residues

Introduction

A number of systems are in place to ensure that food is safe to eat with regard to chemical residues. Currently these systems work in relative isolation from each other. A structured system which incorporates all these systems as integrated components appears to be lacking. Such a comprehensive system is important for a number of reasons. Consumers should be protected against harm in a reasonable, transparent way. Furthermore, resources should be allocated in a more cost-effective way.

Under GATT, countries cannot impose importing requirements which exceed their own standards. As a result there is a need to establish mechanisms to evaluate procedures both from an exporting and importing country's point of view.

It has been shown in the previous section that intensified sampling does not provide a workable answer to either detecting or controlling chemical residues in meat. An alternative approach has therefore been developed, in which the focus is on reducing the risk of consuming contaminated meat, as seen from a consumer's perspective. The approach taken is that of risk analysis, to assess the impact of various potential risk reduction measures on the assessed risk faced by the consumers in eating meat. Each major chemical group would need to be considered separately, but this may well enable measures for many chemicals to be simplified, while measures for a small number may have to be intensified.

A scenario set is developed below which describes steps that affect the risk of violative levels of chemicals in meat. The outcome of this risk reduction programme is the probability of a person consuming more than a certain predetermined quantity of meat (including offal) which results in the person exceeding the MRL. The MRL is considered to be an arbitrary value by some. However since it is the established value to evaluate food safety, it will continue at least for some time to be used for the purpose of risk assessment. Adjustement to MRLs can be made, without affecting the principles of the proposed system.

The scenario set includes steps that reduce the risk of people consuming violative food. The values and distributions that have been used in the accompanying figures are purely fictitious and do not bear any relation to reality. They are to demonstrate how data points in the model are handled. Furthermore it is appreciated that it will be a challenge to determine the actual figures. It should also be considered that the values for some data points will vary according to the resources that are allocated, ie if all other factors are the same a programme will be more effective if more money is spend on it.

Model

The evaluation of the model consists of establishing that the probability of a person who eats

- * edible portions per day (Cell D13)
- consisting of * kg of meat (Cell D12)
- over a period of * days (Cell D11)
- eats less than * kg of violative meat. (Cell D9)
- is less than * (Cell D8)

where * are values are entered by the evaluator in the model (Figure 3.8).

The number of violative portions ingested per person over the predetermined perios is denoted by $VPPP_{GPH}$ etc. after each step of the model. VPPP is a binomial distribution which includes the number of edible portions which were eaten during the year (cell X27) and the probability of a violation. This probability is initially set at stage 1, and is reduced in subsequent steps. The model has been developed in the risk analysis software '@Risk' which is an add-in to Excel spreadsheet software. Estimates in the model frequently consist of distributions.

Stage 1 *Good handling practice*

A national licensing authority can approve chemical'A' after receiving published information and information of trials carried out by pharmaceutical companies. Based on this information the withholding period (WHP) (Cells D25-D36) and the Maximum Residue Limit (MRL) (Cells E25-E36) are set for the various livestock classes (Cells B25-B36) (Figure 3.9). These measures are to prevent non-compliances.

Nevertheless, by fitting a curve to these values (example of distributions in Cells F25-F36) it might be possible to establish that a percentage of the animals is still likely to be non-complying despite good handling practices by farmers. Natural variation in metabolism and excretion rates in an animal population or possibly (subclinical) disease may cause this to occur. The probability of this category of violation occurring is shown in cells G25-G36. In addition, other factors which are the result of a lack of care by farmers can lead to violations. These factors include slaughter within the WHP (Cells H25-H36), application of a dose which is too high (Cells I25-I36), incorrect application of the chemical (Cells J25-J36) and finally the application of the chemical although not licensed for this livestock class (K25-K36) (Figure 3.10). Different distributions will apply to the respective livestock classes. The probabilities are summed for each livestock class resulting in the total probability of a violation per animal (Cells L25-L36). It should be noted that the probability of two reasons for violations occurring simultaneously for one animal (eg high dose and animal slaughtered during the withholding period) are not included in this model. Therefore the total probability of violation (Cells L25-L36) for each of the livestock classes is conservative.

Not all animals are treated with chemical 'A' and an estimate of the proportion of treated animals

is listed in Cells N25-N36. The number of slaughtered animals per livestock class (Cells O25-O36) multiplied by the average weight in kg of a boned out animal including edible offal (Cells P25-P36) equals the kilogrammes of meat and offal produced and subsequently consumed (Cells Q25-Q36). The weight of an edible portion can be entered in Cell D12 and is copied to Cell S25 (Figure 3.11). The number of portions produced per year per livestock class (Cells T25-T36) equals Cells Q25-36 divided by Cell S25. The number of violative portions which are produced on a yearly basis per livestock class (Cells U25-U36) equals the product of the probability of animals being treated (Cells N25-N36) times the probability of violation (Cells L25-L36) and the number of edible portions (Cells T25-T36). The total number of portions produced and consumed per year is calculated in Cell T37. The probability of an edible portion being violative P_{GHP} (Cell V37) equals U37/T37.

The number of edible portions the human population eats per day can be entered in Cell D13 and this is copied to Cell X25. For instance if this figure is 0.25, then the population eats 1 edible portion every four days. The period of interest can be entered in Cell D11 and this is copied to Cell X31, and the resulting number of edible portions over this period is calculated in Cell X27. The condition of acceptance of the chemical residue programme states that the estimate the probability of occurrence (Cell D8 and copied to cell X 35) of a quantity of violative meat (Cell D9) should not be exceeded. The quantity of violative meat (Cell D11) is converted into the number of violative portions (Cell X37). The number of violative portions consumed by one person over the predetermined period, $VPPP_{GHP}$, is calculated in Cell X42. The formula RiskBinomial (X27, V37) gives the number of violative portions eaten per iteration. The distribution of $VPPP_{GHP}$ after a large number of iterations in a simulation is the critical number for performance assessment of the chemical residue programme. It provides a base-line from which improvement can be monitored.

Comments on stage 1

The risk of a chemical to human beings is partly defined by the difference between therapeutic levels in animals and the level which can be safely ingested by humans. In addition the likelihood of abuse (ie violations) and the severity of the violations are relevant to evaluate the health risk. The model as described above applied to violations without regard to the severity of the violation. An alternative method is described below.

MRLs are considered levels at which chemicals become hazardous to human health, although they include safety margins. For the purposes of the risk reduction model, the risk evaluation could depend on the number violations which would occur if the residue levels in the population increased by a certain percent rather than on the current level of violations. Therefore the risk evaluation could be based on the historic results of residue testing plus a certain percentage. This percentage is arbitrary and 10% has been used in the examples below.

Some chemicals are obviously harmful at lower dose rates than other ones and consequently MRLs differ. Currently the underlying assumption is that if chemical 'a' just exceeds its MRL and chemical 'b' also just exceeds its MRL, then they are equally harmful. However if both chemicals exceed their MRL by 5 ppm, then they are not necessarily equally harmful. The lower

the MRL, the more harmful a chemical is if it exceeds its MRL by for instance 5ppm. Weighting factors can be created based on the extent to which the MRL has been exceeded.

Example
The 'level' described below is the level which was established on chemical analysis plus 10% (arbitrary percentage, see above).

chemical a lambs	sample 1	MRL 7ppm	level 9 ppm	excess 2/7 = 0.29	
chemical a lambs	sample 2		level 10 ppm	excess 3/7=0.43	
chemical a lambs	sample 3		level 6 ppm	excess N/A	
chemical a lambs	sample x				
chemical a lambs combined		excess (0.29 + 0.43) / 3 = 0.24			
chemical b ewes	sample 1	MRL 70 ppm	level 72 ppm	excess 2/70 = 0.03	
chemical b ewes	sample 2		level 75 ppm	excess 5/70 = 0.07	
chemical b ewes combined		excess (0.03 + 0.07) / 2 = 0.05			
chemical c heifers		MRL 8ppm	level 8ppm	excess 0/8	N/A
chemical d cows		MRL 5 ppm	level 4 ppm	excess -1/5	N/A

Table 3.32 Fictitious weighted risk factors

Weighted risk of violation	chemical a	chemical b	chemical c	chemical d	Total
lambs	0.24				
ewes		0.05			
heifers			0		
cows				0	
Total					2.6 (not detailed in this example)

Relative risk for chemical a: a / chemical a+b+c+d = 0.24 / 2.6 = 0.09
Relative risk for chemical b: b / chemical a+b+c+d = 0.05 / 2.6 = 0.02

The example showed estimates of relative risks that based on the 'predicted' violations and on the seriousness of these predicted violations.

The above sampling plan can be based on historic data eg data of the last 3 years. It is possible that during this period no violations would be predicted after multiplication of the test results by 1.1. This means that the probability of a violation in the future is considered small, provided no significant changes in management practices occur. If this is a widespread phenomenon, then it could be considered to divide all MRLs by a certain factor, eg 2 in order to acquire more combinations of livestock-chemicals which can be considered a risk. Unless violations are impossible for biological reasons a sampling programme for these chemicals should continue to occur, albeit at a low level. Each year the distribution over the previous five years will be

calculated in order to determine whether the various livestock-chemicals have remained a low risk. At the same time the values of each year will be considered in isolation to determine whether a significant shift has occurred.

Stage 2 Targeted high risk animals

The livestock classes are repeated in Cells B53-B64 (Figure 3.12). The probability of a violation for each livestock class was quantified in Cells L25-L36, while the number of violative portions that had been produced were shown in column U25-U31. The distributions which show the effectiveness of reducing the risk for the various livestock classes while the money spend on each livestock class is kept the same are shown in Cells D53-D64. Column G53-G64 shows the number of violative portions which will no longer occur if the risk reducing programmes were used for each of the livestock classses while Cells H53-H64 shows how many violative portions will remain. Cells I53-I64 shows which strategy is most effective in reducing the number of violative portions and this is based on ranking Cells G53-G64. For the purpose of this example, the livestock class displaying the greatest reduction will be subjected to a reduction programme. The other livestock classes will not be subjected to a special programme. Cells J53-J64 contain the same values as Cells U26-U36 with the exception of the livestock class of which the risk was reduced, for which the respective value from Cells G53-G64 was used. All violative portions are summed in Cell J65 and the ratio of the resulting number of violative portions and the old number of violative portions (J65/U37) results in the proportion reduction of violations, R_{animal} (Cell M54). The probability of violations, P_{GHP} (Cell V37 and M56) is multiplied by this proportion, resulting in a new probability of violations P_{animal} (Cell M58). $VPPP_{animal}$ (Cell M60) consists of the binomial distribution =RiskBinomial (X27, M58) which includes the number of portions consumed by a person per year (Cell X27) and P_{animal} (Cell M58). $VPPP_{animal}$ (Cell M60) denotes the number of violative portions consumed by one person per year at the end of stage 2.

Stage 3 Targeted high risk farms

Several types of high risk farms can be distinguished. There are farms with at least one violation over a certain period of time. Also clustering of violations may occur in a spatial and temporal sense.

A non-complying farm could be defined as a property that has submitted at least one animal for slaughter with a chemical exceeding the MRL. The probability of detecting individual non-complying farms, P_{ncf} (Cell H74) needs to be estimated (Figure 3.15). Next an estimate , E_{ncf} , (Cell H78) should be made of the effectiveness of a programme to reduce the number of subsequent violations of non-complying farms after they have been identified. The product of these estimates is the reduction of non-compliance of individual high risk farms, R_{farm}, (Cell H82). The product of R_{farm} (Cell H82) and P_{animal} (Cell M58) is P_{farm} (Cell L82) which is the probability of a violation once all above risk reducing steps have been taken (Figure 3.16). $VPPP_{farml}$ (Cell L84) consists of the binomial distribution =RiskBinomial (X27, L82) which includes the number of portions consumed by a person per year (Cell X27) and P_{farm} (Cell L82). $VPPP_{farm}$ (Cell L82) denotes the number of violative portions consumed by one person per year

after individual violative farms have been targeted.

Clustering of non-compliance may occur in space and in time. Available data should be evaluated for such events. The effectiveness of programmes targeting clustering in space, E_{space}, (Cell H86) and in time, E_{time}, (Cell H90) are to be estimated. It should be noted that there are difficulties of detecting clustering as discussed in the previous section. The product of E_{space}, (Cell H86) and E_{time}, (Cell H90) is the factor that denotes the effectiveness of risk reducing programmes based on targeting clustering $R_{clustering}$ (Cell H93). The product of P_{farm} (Cell L82) and $R_{clustering}$ (Cell H93) is $P_{clustering}$ (Cell L93) which is the probability of a violation once all above risk reducing steps have been taken. $VPPP_{clustering}$ (Cell L96) consists of the binomial distribution =RiskBinomial (X27, L93) which includes the number of portions consumed by a person per year (Cell X27) and $P_{clustering}$ (Cell L93). $VPPP_{farm}$ (Cell L96) denotes the number of violative portions consumed by one person per year after clustering in time and space has been targeted.

Stage 4 Reduction by targeted sampling

Commonly used sampling strategies for chemical sampling include random sampling, targeted sampling (eg animals with Injection site lesions) and 'one-off' surveys. In 1991 (Surveillance, *1993*) 19,395 animals were randomly selected for residue testing. A total of 58,180 unit analyses were carried out involving 106 chemical compounds. A number of categories of drugs can be distinguished such as antibacterial drugs, endoparasitic drugs etc. If a sample is taken from an animal it can be analysed for a number of drugs which are used for the same purpose such as aminoglycosides or beta-lactams in the case of antibacterial compounds. If a sample is tested for aminoglycosides, tests will be performed for dihydrostreptomycin, streptomycin, etc. Therefore the above shows that the 19,395 samples taken were often used to analyse more than one chemical. The previous sections have shown that large samples are required in order to acquire statistically significant results. Systems to improve the sensitivity of sampling programmes will improve the effectiveness of chemical residue testing.

Random sampling is often seen as the corner stone of chemical residue sampling systems. Sampling has two functions. It monitors the level of compliance and it functions as a deterrent. The more effective a residue sampling programme is in detecting non-compliance, the more care farmers will take to observe legal requirements. In general, numbers of slaughter animals are large and many chemicals are available as animal remedies. Subsequently the probability of detecting a non-complying farm by using a random sampling programme is slim. This is known to farmers. Sampling of farms from suspect lists is an example of a sampling system with an improved deterrent function. Targeted sampling systems will increase the probability of non-complying farms being detected and therefore they will have a stronger deterrent function than random sampling systems. In addition their results can still be used for statistical quality control charts to monitor the general degree of observance of requirements.

The livestock classes and the number of violative portions of chemical a are repeated in Cells B106-B117 and Cells C106-C117 respectively (Figure 3.17). Violative portions are also shown for chemicals 'b', 'c' and 'd' in Cells D106-D117, E106-D117 and F106-F117 respectively. The sum of all violative portions is calculated in Cell G118. The proportion of violative portions for

each livestock-chemical combination is shown in Cells J106-M117. The number of samples taken for each livestock-chemical combination will be proportional to these proportions. The reduction in violations for each livestock-chemical combination, $R_{sample\text{-}species}$, (Cells Q106-T117) will be a function of the number of samples taken for each (Figure 3.19). The number of remaining violative portions with chemical a after a targeted sampling plan is displayed in Cells V106-V117. They consist of the products of Cells U25-U36 and Cells Q107-Q117 and these products are summed in Cell V118. The reduction in violations is symbolised by R_{sample_total} (Cell Y106) which is V118/U37. The product of R_{sample_total} (Cell Y106) and $P_{clustering}$ (Cell L93) results in $P_{sampling}$ (Cell Y110) which is the probability of a violation once all above risk reducing steps have been taken. $VPPP_{sampling}$ (Cell Y112) consists of the binomial distribution =RiskBinomial (X27, Y110) which includes the number of portions consumed by a person per year (Cell X27) and $P_{sampling}$ (Cell Y110). $VPPP_{sampling}$ (Cell Y110) denotes the number of violative portions consumed by one person per year after a risk based sampling plan has been used.

Stage 5 Farm certification programme

Farm certification programmes are currently designed by various livestock industries(eg pigs and deer). Such programmes include codes for superior farm management practices. As a result the number of violations per livestock class is likely to decline. The proportion of farms that are participating per livestock class are listed in cells F132-F143 and the effectiveness of the various programmes for the participating farms are listed in cells H132-H143 (Figure 3.20). The product of the number of violative portions per livestock class (Cells U25-U36), the proportion of participating farmers (Cells F132-F143) and the effectiveness of the programme (Cells H132-H143) provides the number of certified violative portions. The proportion of non-certified product (1-{Cells F132-F143}) multiplied by the number of violative portions per livestock class (Cells U25-U36) results in the number of non-certified violative portions of meat. The sum of these two numbers of violative portions provides the total number of violative portions. $R_{certified}$ (Cell N130) is the reduction in the number of violative portions after the implementation of farm certification programmes and is calculated as (J144+K144)/U37. The product of this factor with $P_{sampling}$ (Cell N132) results in $P_{certified}$ (N134). $P_{certified}$ (N134) is the probability of a violation once all above risk reducing steps have been taken. $VPPP_{certified}$ (Cell N137) consists of the binomial distribution =RiskBinomial (X27, N134) which includes the number of portions consumed by a person per year (Cell X27) and $P_{certified}$ (Cell N134). $VPPP_{certified}$ (Cell N137) denotes the number of violative portions consumed by one person per year after the effect of farm certification systems have been taken into account and it is the final stage of this model.

Discussion

Conventially, a number of tests for chemical residues in food are carried out and based on these tests, and if nothing untowards happens, customers (including importing countries) will trust that the product is safe to eat. However, sometimes chemical residues are detected and a consumer boycot may eventuate or borders from importing countries may be closed. Previous sections have

demonstrated that under certain circumstances the detection of non-complying levels of residues might be expected. The detection would not reflect that the system of handling chemicals is out of control, but would merely reflect the probability of detection given knowledge of an existing baseline.

It is to the benefit of both consumers and producers that a more structured approach is developed towards evaluating the safety of food with regard to chemical residues. The approach in this section was to determine, through a number of steps, what level of exposure to chemical residues was likely to be encountered by a person. The acknowledgement that low levels of chemical residues on a yearly basis are likely to exist and harmless will enable a rational discussion between all parties. In contrast the current situation where no violations are expected by some is unrealistic and prone to lead to extreme reactions.At the same time the assurance that the level is in fact low will be seen as reassuring by consumers.

The steps which were used in the DSS were often characterised by being estimates. Frequently it will not be known how effective the various steps precisely are. However by explaining how these estimates were made (transparency) and by showing how much influence they have on the intermediate results and the end result (sensitivity testing) consumers can still have confidence the the assurance was valid.

The DSS which was developed in this section has a large degree of flexibility. A number of 'what-if' scenarios can be run to determine the effectiveness of the various steps to reduce the probabilities and combined with a cost-benefit analysis optimum combinations can be established.

A number of issues need to be considered to make this DSS a practical option. The risk based sampling approach can only be used if extensive surveillance in the past has established the baselines. In many countries residue sampling has been in place for a number of years and baseline data will be available. Special surveillance projects may be required for some livestock-chemical combinations.

The possibility exists that extremely high (and potentially dangerous) levels which might occur in a relatively minor livestock class would still result in a small number of tests only if the number of exposed animals in this livestock class was small. Special programmes may need to be developed for these situations. Testing could be based on an initial allocation of a number of tests to the various livestock classes before any risk calculations are made.

Ongoing monitoring of farm management is required to ensure that sudden changes are detected in time. This can be done by surveys and expert opinion as discussed. Also the sales of chemicals in relation to livestock numbers can be monitored.

The cost of the DSS in relation to the benefits is an important consideration. At times one sample will be applied to one test which will provide information on several chemicals. The additional cost for more than one chemical may be small (eg reading various peaks in a graph). In such a situation it would be appropriate to combine the risks of violations for all the chemicals which are analysed in this test and consider it as one. Where in the past several different tests were

performed on one physical sample (eg split sample and use different extraction techniques), the chemicals could be considered separate.

The intention of the programme is to see whether the chemical residue programme in its entirety is under control. The need remains to verify that the underlying assumptions regarding the historical baselines continue to be correct. In some cases sampling at a low level may be required without the necessity to do so, if one considered historical data only as outlined in the model.

The targeted sampling could provide figures on a regular basis (eg weekly or monthly). Such figures would be the result of a number of values from different distributions (livestock-chemical combinations). Out-of-control situations would usually be identified by a number of tests (eg the graph exceeding the Upper Control Limit). However it is not known what distribution can be applied to this 'final figure' which is based on a variety of distribution. The solution may be to take results which have been acceptable in the past. After running a number of simulations the behaviour of the 'final figure' may become clear and decision rules may be made.

In summary the proposed program provides an alternative to currently commonly used procedures. There is a need initially to acquire new data to support this program but more importantly there is a need to change the approach to the evaluation of the risk of chemicals to human health and to assurances regarding safety of product.

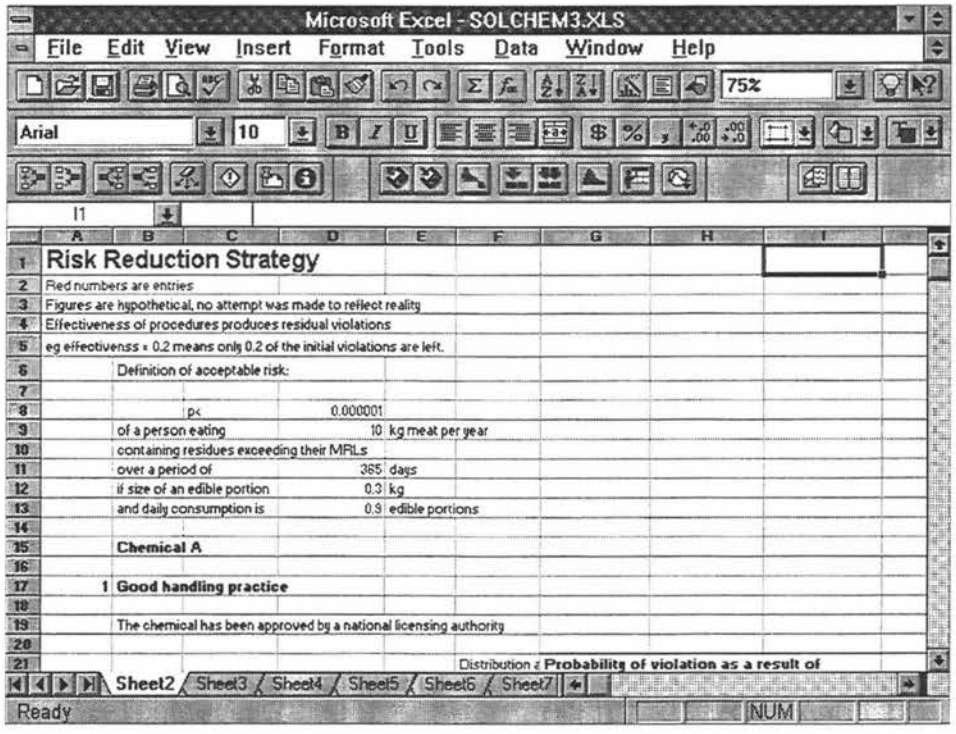


Figure 3.8 Screen - Risk reduction strategy

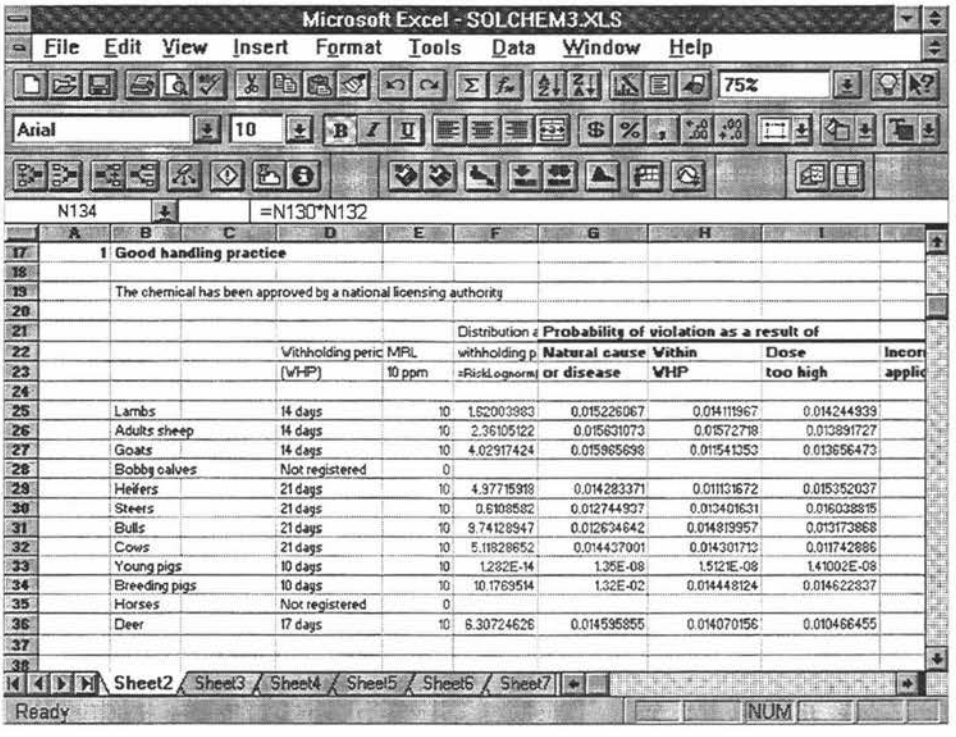
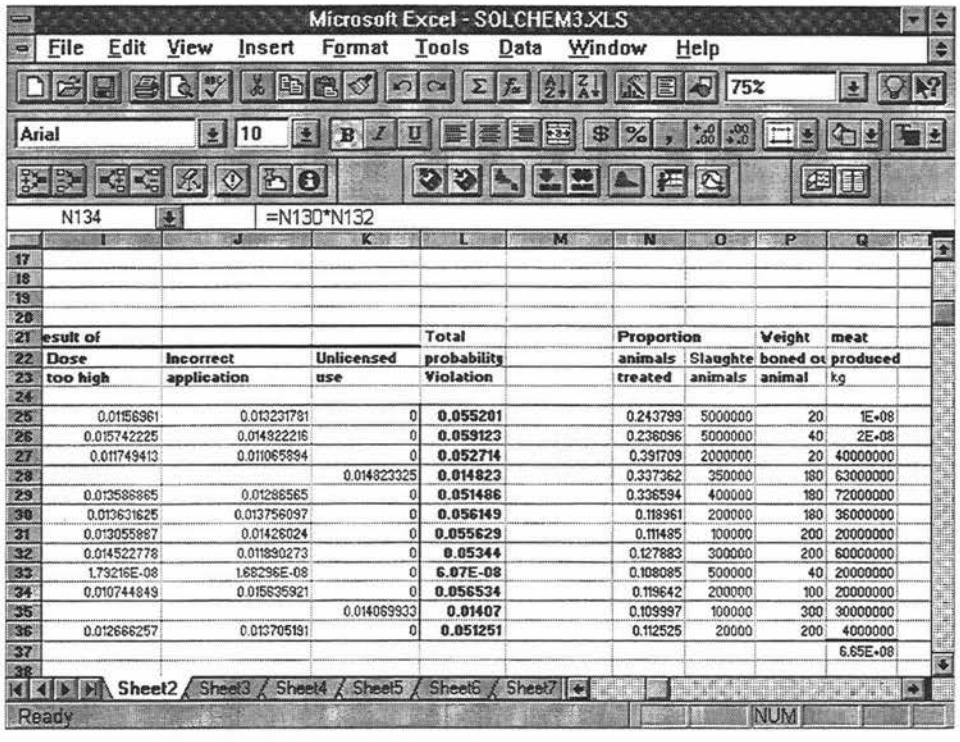
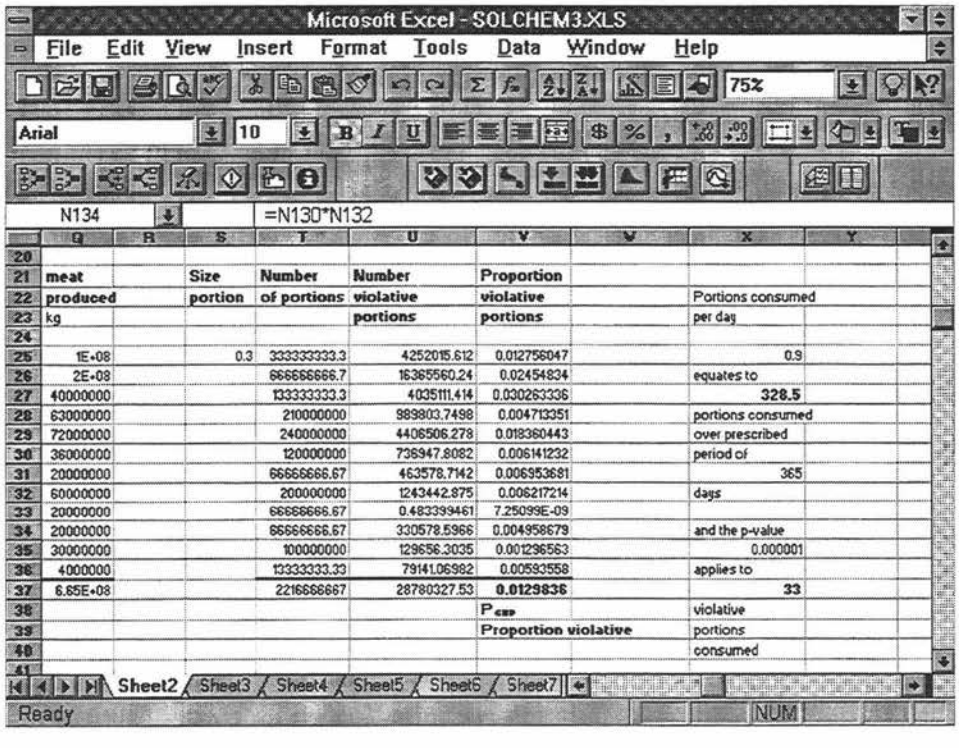


Figure 3.9 Screen - Good handling practice (1)



	I	J	K	L	M	N	O	P	Q
17									
18									
19									
20									
21	esult of			Total		Proportion		Weight	meat
22	Dose	Incorrect	Unlicensed	probability		animals	Slaughte	boned ou	produced
23	too high	application	use	Violation		treated	animals	animal	kg
24									
25	0.01156961	0.013231781	0	**0.055201**		0.243799	5000000	20	1E+08
26	0.015742225	0.014322216	0	**0.059123**		0.238096	5000000	40	2E+08
27	0.011749413	0.011065894	0	**0.052714**		0.391709	2000000	20	40000000
28			0.014823325	**0.014823**		0.337362	350000	180	63000000
29	0.013588865	0.01286565	0	**0.051486**		0.336594	400000	180	72000000
30	0.013631625	0.013756097	0	**0.056149**		0.118961	200000	180	36000000
31	0.013055887	0.01426024	0	**0.055629**		0.111485	100000	200	20000000
32	0.014522778	0.011830273	0	**0.05344**		0.127883	300000	200	60000000
33	1.79216E-08	1.68296E-08	0	**6.07E-08**		0.108085	500000	40	20000000
34	0.010744849	0.015835921	0	**0.056534**		0.119642	200000	100	20000000
35			0.014069933	**0.01407**		0.109997	100000	300	30000000
36	0.012666257	0.013705191	0	**0.051251**		0.112525	20000	200	4000000
37									6.65E+08
38									

Figure 3.10 Screen - Good handling practice (2)



	Q	R	S	T	U	V	W	X	Y
20									
21	meat		Size	Number	Number	Proportion			
22	produced		portion	of portions	violative	violative		Portions consumed	
23	kg				portions	portions		per day	
24									
25	1E+08		0.3	333333333.3	4252015.612	0.012756047		0.9	
26	2E+08			666666666.7	16365560.24	0.02454834		equates to	
27	40000000			133333333.3	4035111.414	0.030263336		**328.5**	
28	63000000			210000000	989803.7498	0.004713351		portions consumed	
29	72000000			240000000	4406506.278	0.018360443		over prescribed	
30	36000000			120000000	736947.8082	0.006141232		period of	
31	20000000			66666666.67	463578.7142	0.006953681		365	
32	60000000			200000000	1243442.875	0.006217214		days	
33	20000000			66666666.67	0.483399461	7.25099E-09			
34	20000000			66666666.67	330578.5966	0.004958679		and the p-value	
35	30000000			100000000	129656.3035	0.001296563		0.000001	
36	4000000			13333333.33	79141.06982	0.00593558		applies to	
37	6.65E+08			2216666667	28780327.53	**0.0129836**		**33**	
38						P_{exp}		violative	
39						Proportion violative		portions	
40								consumed	
41									

Figure 3.11 Screen - Good handling practice (3)

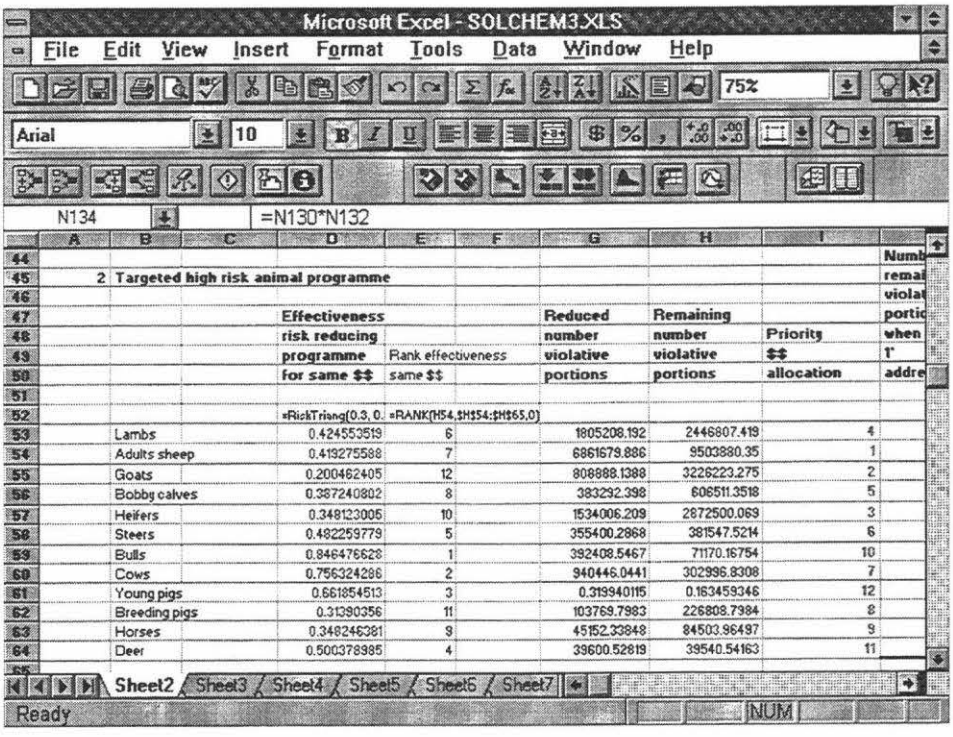


Figure 3.12 Screen - Targeted high risk animal programme (1)

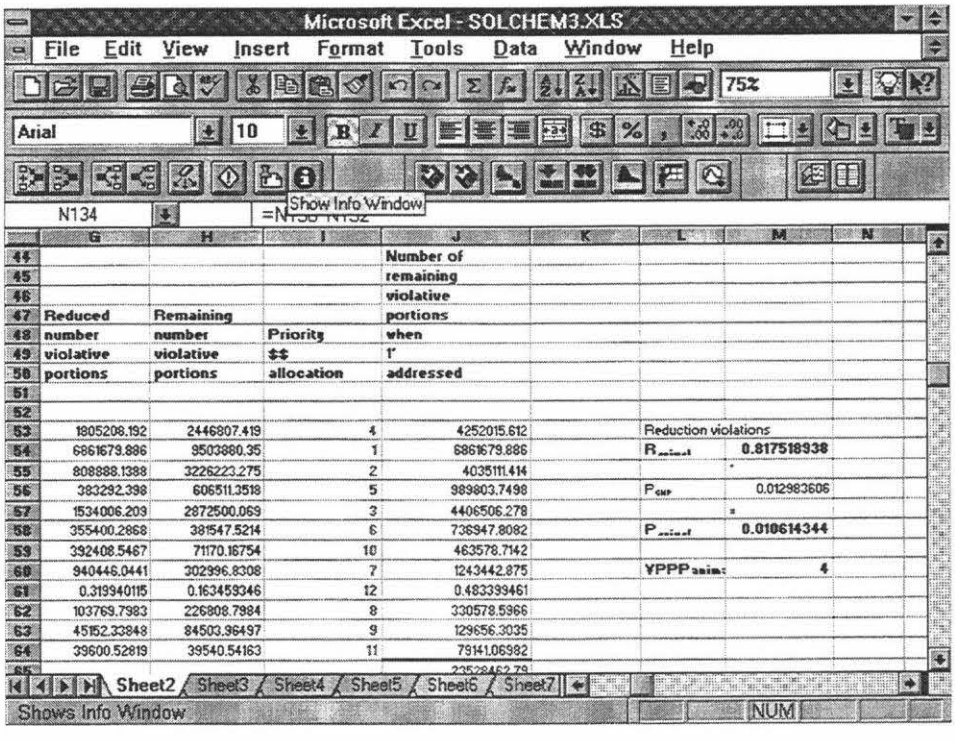


Figure 3.13 Screen - Targeted high risk animal programme (2)

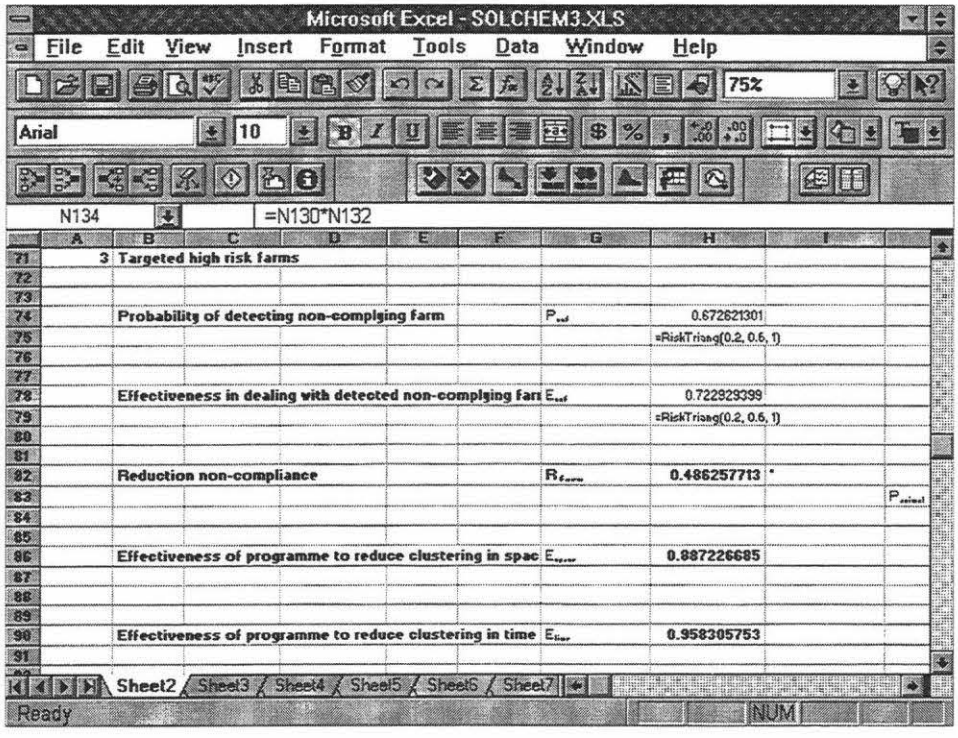


Figure 3.14 Screen - Targeted high risk farms (1)

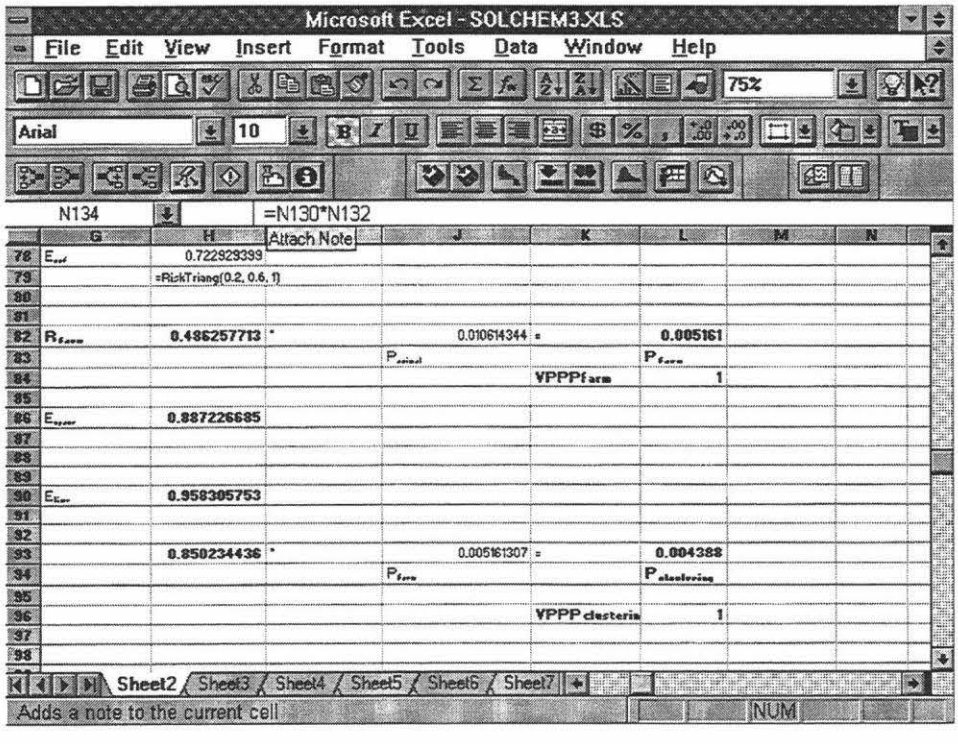


Figure 3.15 Screen - Targeted high risk farms (2)

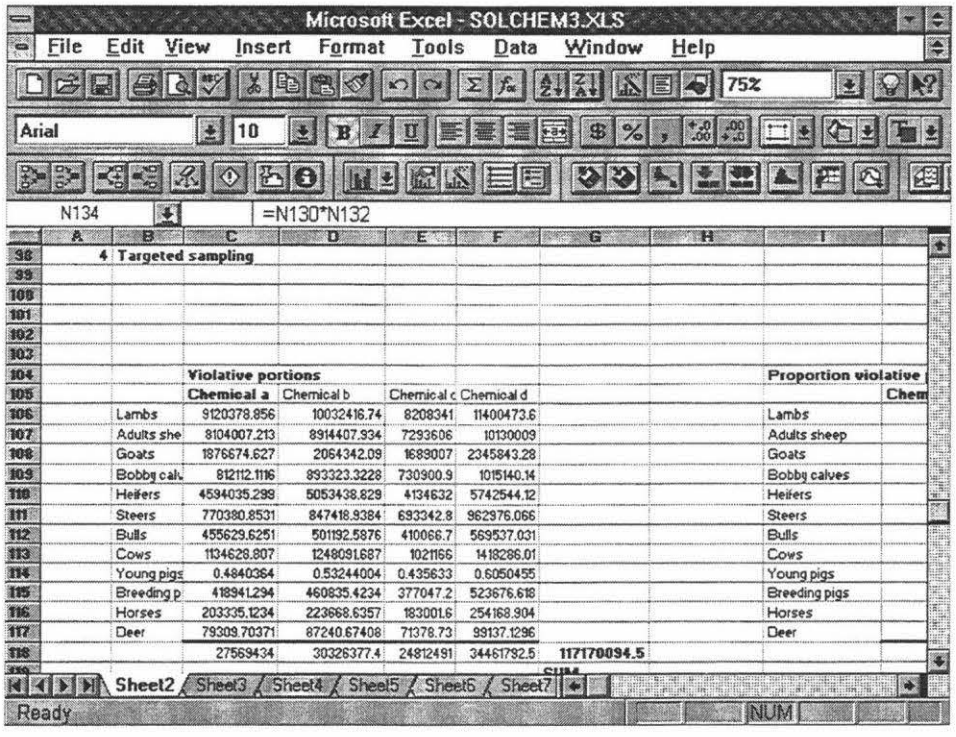


Figure 3.16 Screen - Targeted sampling (1)

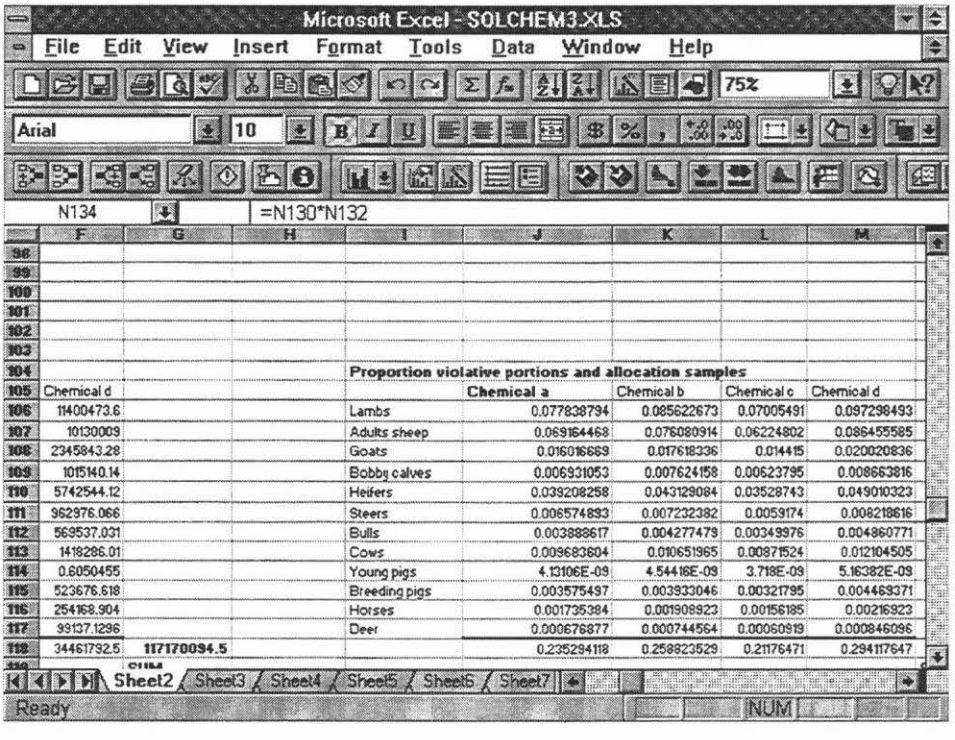


Figure 3.17 Screen - Targeted sampling (2)

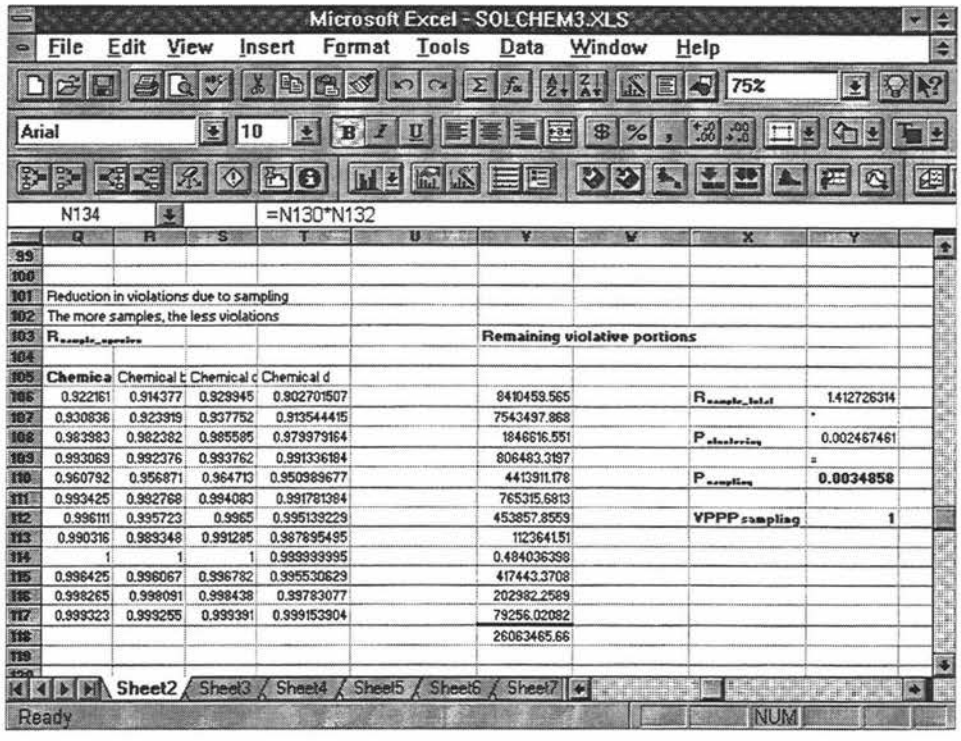


Figure 3.18 Screen - Targeted sampling (3)

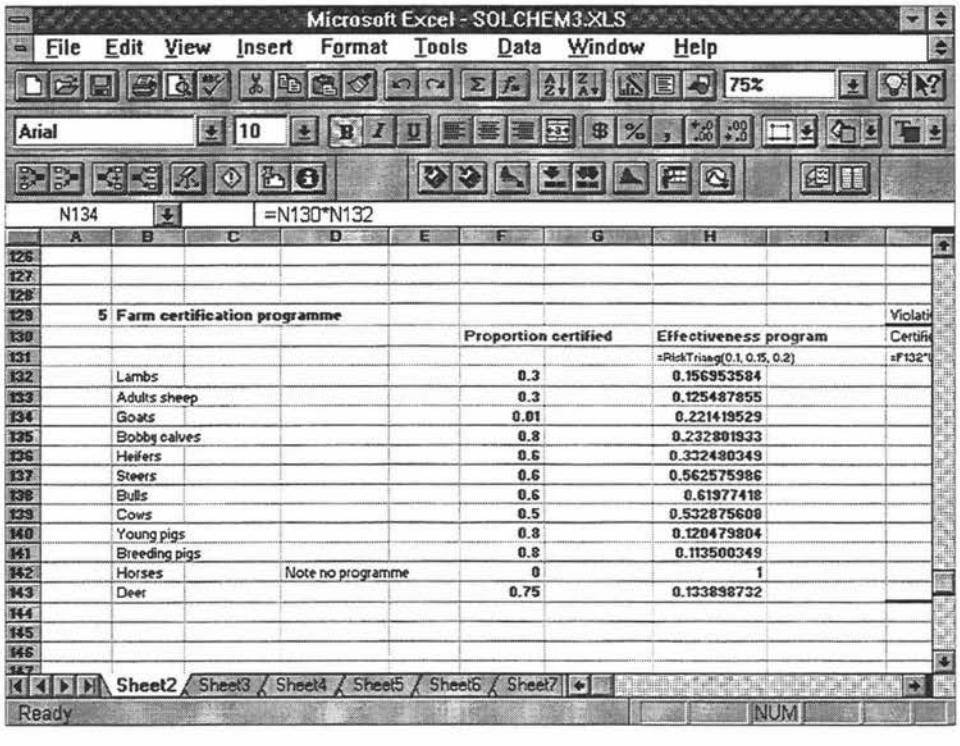


Figure 3.19 Screen - Farm certification programme (1)

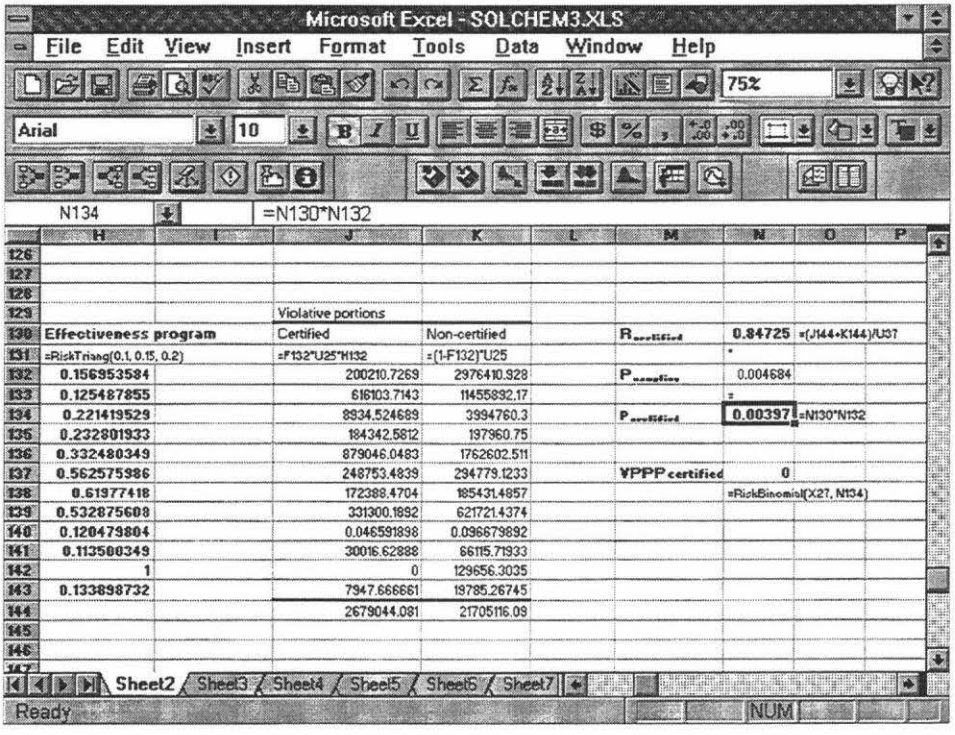


Figure 3.20 Screen - Farm certification programme (2)

CHAPTER 4

GENERAL DISCUSSION

This thesis explores a systematic approach to meat safety with measurable outcomes. Over the years meat inspection, a major component of meat hygiene, has developed into a set of procedures which purport to protect the public health. It is mainly carried out as a carcass by carcass evaluation of gross pathology, often without consideration being given to other factors (Snijders *et al.*, 1989). Its effectiveness needs to be evaluated critically and alternatives which can achieve the same outcomes need to be considered.

A number of issues related to public health were examined in this thesis. The hazard analysis of pleurisy in lambs demonstrated that its public health significance was in general very limited although there could be some exceptions. The sensitivity for detecting this disease at times left much to be desired. The setting of Maximum Residue Levels is constantly challenged. However with regard to chemical residues, the sensitivity for detecting clustering appeared to be less than desirable as well. The above factors show that the relevance of meat safety systems to public health protection need to be improved. There is a need to associate food safety procedures with defined public health outcomes. The resource which can be allocated to food safety is finite. Allocation should be based on risks and it should replace the current system which is based on historic decisions which may no longer be appropriate with the current knowledge of human and animal disease (Hathaway and McKenzie, 1991 (a); Bell, 1993).

In recent years the New Zealand Ministry of Agriculture has actively carried out applied research with a view to developing appropriate meat hygiene programs (Hathaway and McKenzie, 1991 (b)). Internationally MAF has developed a leadership role with re-evaluation of meat inspection procedures, and the development of HACCP as the foremost system to control processes and improve public health. This study continues the development of suitable methods for risk-based procedures in meat hygiene.

The study on pleurisy, especially the case-control study, showed procedures which can be used to investigate practices which are conducive to low or high levels of pathogens in animals. Studies which control as many factors as possible may not be appropriate if the levels of pathogens are determined by multifactorial interaction of these factors. Although necessary, this approach has a number of problems. There is a need to unambiguously identify case and control farms. In the pleurisy case-control study the definition suffered because only a fraction of all pleurisy cases will have resulted in downgrading of the carcass. Many pleurisy carcasses will therefore not have been identified. In addition the aetiology of the lesions is open to conjecture. The issues which were encountered in the pleurisy studies can be seen as contributing towards the building of models for similar studies for food borne pathogens. Similar uncertainties will also be found when sampling of carcasses for pathogens. In the case of food borne diseases in the future (eg *Salmonella* ssp., *Campylobacter* ssp., *Yersinia enterocolitica* and *Toxoplasma gondii*) identification by culturing or some other technique will be required. The cost of this will

be a major consideration when developing these trials while the pleurisy studies were relatively inexpensive. The positive outcome of the pleurisy studies gives confidence for future.

The potential benefits of meat inspection have not been fully developed yet (Martin *et al.* 1987). These benefits are not limited to public health, but animal health and production, and meat processing and marketing can also benefit from meat inspection. Although the public health risk of pleurisy may be debatable, there is certainly a great cost to the farming and the meat processing industries (Dysart, 1976; Alley, 1987b). Meat inspection in this regard can play an important role, not only for pleurisy but also for other diseases. Currently meat inspection is based on removing tissues with gross pathology from the human food chain. A preferable system would identify the outcomes, including the ones related to farming and processing practices, and design systems in such a way that maximum benefit is derived. In this case feedback based on meat inspection would provide advice to farmers to improve management of a farm to reduce the pneumonia prevalence.

The chapter on pleurisy demonstrated how a disease can be approached from a variety of angles for a better understanding. Studies with slaughterhouse data are valuable since large numbers of animals and farms can be included in a study at relatively low cost as shown in the case-control study. It is an additional advantage that most livestock will ultimately be slaughtered and inspected. The intervention studies demonstrated that problems at individual farms can be also investigated at slaughter by using a properly constructed study design. However, the disadvantages of slaughterhouse studies should not be overlooked. The sensitivity of inspection can be seen as a major problem. This is not surprising considering the speed with which animals are inspected. From a national disease perspective, slaughterhouse data will be biased towards certain animal categories. Young healthy animals predominate, but there is also a sizeable number of cull animals which have come to the end of their economic lives. As long as these limitations are appreciated, problems in interpreting the data can be overcome.

Automation of procedures was important on a number of occasions. The data which enabled the case-control study to proceed had been stored in a database. Initially another company has offered to provide data as well. Retrieval of the data appeared to be such a problem that the study proceeded without these. Furthermore, for a variety of reasons, the data that were finally used were not as detailed as initially intended. All these factors showed the importance of properly functioning data storage systems which are compatible with each other. The use of data capturing systems on slaughter boards appears to have progressed since these trials. Despite many efforts, one draft of lambs which participated in an intervention study was inspected without their details being recorded. This illustrates the importance of integrating company systems with systems of an inspection service. If these animals had been suspect for public health reasons they could equally have been missed for a more detailed inspection. The sensitivity of inspection was shown to be limited, especially for small lesions. Obviously their is a point where lack of sensitivity could lead to results which are not only useless to farmers but even misleading. Anecdotally, factors such as the time of the day may have a bearing on this. More work is required to identify and quantify factors which affect sensitivity of meat inspection. It supports the view that automation of inspection, ie the use of cameras and sensors, may ultimately be preferable for more reliable data. The same degree of sensitivity, but influenced by known factors would already be preferable to the current situation. Interpretation of data and feedback to farmers can

be further automated. Care should be taken to ensure that feedback is correct. Feedback systems should preferably take a number of factors and their interaction into account.

The production chain involves various groups of people. Farmers, meat processing industry and consumers are the main participants. Their prime objectives are basically different. They vary between making profit and consuming safe food. A better integration of the production chain which takes the different objectives into account will be to everybody's benefit.

The studies on chemical residues showed that even in a relatively new field procedures can quickly become inflexible, ie no major changes to the system are made and the underlying concepts are not questioned critically. The initial investigations showed the possible use of statistical quality control systems. However the simulations suggested that sensitivity to detect changing trends would not be adequate under practical circumstances. The DSS that was developed showed that especially in this area integration of procedures of the various participants in the meat production chain, could greatly assist in maintaining a high quality of product.

Risk assessment was a frequently occurring theme in this study. After designing initial qualitative assessments such as developing scenario sets, quantification is soon required to fully evaluate a risk. There are many problems in quantifying components of risk scenarios. Nevertheless, already the definition of these problems will be helpful in better understanding the problems. Resolution of problems requires resources. A logical corollary of the work in the future is the incorporation of cost-benefit analyses in some form. Since the health of a population is involved, moral dilemmas will arise inevitably. Economic studies have been carried out on the cost of food borne disease (Archer and Kvenberg; 1985; Todd, 1989) and on the values of reducing risks of death (Fisher *et al.*, 1989). The available resources are finite and it can be argued that it is preferable to make the resource allocation transparent within a budget with their outcomes, rather than continuing historically developed systems which may be vague, and not efficient in the allocation of resources. However, as mentioned previously, the current lack of understanding of a correlation between food safety procedures and their impact on public health makes this approach currently of academic value only.

There is a need to improve the flexibility of current systems. Not only should it be possible to reallocate resources within the meat inspection system. It should also be considered whether at times it might not be more cost-efficient to allocate resources from inspection to prevention. It is appreciated that opportunities to do so are currently limited. However the interest in certification of farms with superior farm management practices is an example of this approach.

A number of issues raised have the potential to reduce the extent of procedures of conventional meat inspection. However at the same time one should warn against hasty decisions that do not take the full potential of meat inspection into account with regard to animal health and animal production. It may be proper to view this as an opportunity to create flexibility at all aspects of the chain and to integrate public health objectives with other, commercial, objectives.

APPENDICES

APPENDIX 1

Questionnaire on factors relating to pleurisy and pneumonia in slaughter lambs.

Thank you for your participation in this project. Below is a short explanation of filling in the questionnaire and then the questions follow.

This questionnaire applies only to the farm to which it was addressed. It does not apply to other farms that may be owned or managed by the person who completes this form.

This questionnaire applies to events of the previous season (the 1992-93 season) only. Therefore any recent changes in management practices or to the farm itself are not included in this questionnaire. For the purposes of this questionnaire the word 'lamb' applies to the lambs that were born in the second half of 1992.

Shaded areas indicate where written answers are expected. Where choices are offered, please tick the correct answer. In the case of some questions more than one answer may apply. If so, please tick all correct answers.

In some cases a question may not be applicable to your farming practices. In such instances will you please answer the question with 'Not applicable' or 'NA'. If you feel that a question does not properly address the situation at your farm, please clarify your situation on the form or on a separate page.

It is appreciated that some questions are difficult to answer with complete accuracy. In these situations your best estimate is sufficient. Please answer 'Not known' or 'NK' if you feel that your estimate would be too imprecise to be worth giving. In the case of dates you may perhaps not know the exact date. The month would then be enough.

Name

Location of farm

Contact telephone

1 Details of 1992/93 lambs.

1.1 By what date were the rams put out and when were they removed for the 1992 mating period ?

/ /19 when rams were put out.
/ /19 when rams were removed.

1.2 What method(s) was (were) used for docking?

- ☐ Cutting with knife.
- ☐ Rubber rings.
- ☐ Heated docking iron.
- ☐ Tails were not cut.

1.3 What type of yards did you use for docking?

- ☐ Temporary
- ☐ Permanent

1.4 At what age (range) did you dock the lambs?

days or weeks

1.5 What method(s) was (were) used for castrating the ram lambs that went for slaughter?

- ☐ Cutting with knife.
- ☐ Rubber rings.
- ☐ Heated docking iron.
- ☐ Cryptorchid.
- ☐ No castration.

1.6 By what date or over what period (in case of separate groups) were the lambs weaned?

Date: / /19 or
Period: from / /19 to / /19

2 Treatment.

2.1 What **vaccinations** (if any) were given to the lambs since birth?

Product names	Full date or month
	/ /19
	/ /19
	/ /19

2.2 What **worm drenches** (if any) were given to the lambs?

Product names	Full date or month
	/ /19
	/ /19
	/ /19
	/ /19
	/ /19
	/ /19
	/ /19
	/ /19

2.3 Has drench resistance been diagnosed on the farm by a laboratory?

☐ Yes
☐ No

2.4 What products were used on lambs for the prevention of **fly strike**?

Product names	Full date or month
	/ /19
	/ /19
	/ /19

2.5 Which were the method(s) of application of the products listed in the previous question (2.4)?

☐ Plunge dip
☐ Shower dip
☐ Spray race (jetting race)
☐ Hand jetting (eg gorse gun)
☐ Spray-on or wand

2.6 Which other measures were taken to monitor or control fly strike?

☐ Fly traps
☐ Crutching
☐ Shift lambs to high ground
☐ Shift lambs to windy areas
☐ Bury dead animals quickly
☐ Other, please specify:

2.7 What **other products** have been given to lambs to improve health or performance?

Product names	Dates	Purpose?
	/ /19	
	/ /19	

3 Yarding.

3.1 On average, how long did it take to muster the lambs before and after weaning (excluding docking)?

Before weaning ____ hours

After weaning ____ hours

3.2 On average, how long did the lambs stay in the yards or holding paddocks before and after weaning (excluding docking)?

Before weaning ____ hours

After weaning ____ hours

3.3 How many times have the lambs been yarded **before** weaning (excluding docking)? ____ times

3.4 How many times have the lambs been yarded **after** weaning? ____ times

You may have had groups of lambs slaughtered at different times during the season. If so, answer for the last group of lambs slaughtered before 30 September 1993.

3.5 What kind of aids did you use to muster your slaughter lambs in the majority of cases after weaning?
Please tick the main methods.

- ☐ None
- ☐ Motor bike (2, 3 or 4 wheel)
- ☐ 4 Wheel drive vehicle
- ☐ Tractor
- ☐ Dog
- ☐ Horse
- ☐ Other, please specify:

3.6 After weaning have the lambs ever been kept indoors (within 4 walls) for more than two hours (For example in a shearing shed)?

- ☐ Yes
  - _____ hours on average
  - _____ maximum number of times
- ☐ No

3.7 How many sets of permanent yards do you have? _____ sets of permanent yards

3.8 What was the estimated surface of the yards that was covered with a roof? _____ percent

3.9 What was the type of flooring of the permanent yards?

Yard 1
Yard 2
Yard 3

3.10 When were the lambs shorn?

/ /19
/ /19

3.11 Do you use scales to record:

Lamb liveweights	☐ Yes	☐ No
Ewe liveweights	☐ Yes	☐ No
Ewe fleeceweights	☐ Yes	☐ No

4 Diseases

4.1 In your opinion, what were the diseases/health problems of your lambs that were most costly in their effects on your flock? Costs of preventative measures such as vaccinations, drenches or dips should be excluded.

Please rank them in order of cost to you.

(1 is the most serious)

1
2
3
4
5

(5 is the least serious of the above problems)

4.2 In your opinion, what were the diseases/health problems of your ewes that were most costly in their effects on your flock? Costs of preventative measures such as vaccinations, drenches or dips should be excluded.

Please rank them in order of cost to you.

(1 is the most serious)

1
2
3
4
5

(5 is the least serious of the above problems)

4.3 Did you see more severe nasal discharge in the lambs in the '92/'93 season than in other seasons?

☐ Yes
☐ No

4.4 Did you hear more coughing in the lambs in the '92/'93 season than in other seasons?

☐ Yes
☐ No

5 Details of farm.

5.1 What was the total farm size?

acres or hectares

5.2 What was the effective grazing area?

acres or hectares

5.3 Into how many paddocks (other than holding paddocks) was the farm subdivided?

paddocks

5.4 What was the altitude range of the grazing area of the farm?

From metres (lowest level) to metres (highest level).

5.5 How would you describe the predominant topography of your farm where your lambs were kept?

☐ Flat
☐ Rolling
☐ Steep

5.6 How would you describe the degree of exposure to strong winds of the grazing area where your lambs were kept?

☐ Mainly exposed
☐ Mixed
☐ Mainly sheltered

5.7 To what water sources did the lambs have access? Please tick all options which apply.

☐ Trough
☐ River/creek
☐ Lake/pond/dam
☐ Other, please specify:

5.8 What system of grazing management for lambs did you use after weaning?

☐ Daily shifts
☐ Shifts every 2-5 days
☐ Shifts every 6-10 days
☐ Shifts at intervals of longer than 10 days but not set stocking
☐ Set stocking

5.9 What was the approximate stocking density at which lambs were kept after weaning?

lambs per acre, or

lambs per hectare.

5.10 Has supplementary feed been provided to lambs?
- ☐ Yes
- ☐ No

5.11 Did you graze different species (cattle, goats, etc.) with lambs in the same paddock?
- ☐ Yes, please state which other stock:
- ☐ No

5.12 Did sheep from neighbouring farms and your lambs get mixed because of broken fences?
- ☐ Frequently
- ☐ Sometimes
- ☐ Never

6 Livestock numbers.

6.1 What breeds and cross-breeds of ewes (hoggets, two tooth ewes and older ewes) were mated in 1992. Please provide the number in each breed.

	Breeds	Number
Ewes		

6.2 What breeds and cross-breeds of rams were used for mating in 1992. Please provide the number in each breed.

	Breeds	Number
Rams		

6.3 What were the livestock numbers at your farm as at 1 July 1993?

Lambs/hoggets
Two tooth ewes
Older ewes
Rams
Cattle
Deer
Goats
Other, please specify:

6.4 What was your lambing percentage over the last three years?

percent in 1990
percent in 1991
percent in 1992

6.5 What percentage of your lambs died between weaning and slaughter?

percent

6.6 How many of your lambs were affected by pneumonia and how many lambs died of pneumonia?

lambs affected by pneumonia

lambs died of pneumonia

6.7 How many lambs were sent for slaughter?

lambs

6.8 What was the annual ewe death rate?

percent ewes

6.9 How many sheep of the following categories were brought onto the farm? This includes sheep that were purchased.

Lambs

Hoggets

Two tooth ewes

Older ewes

Rams

6.10 From how many different flocks did the sheep originate that were mentioned in the previous question (excluding rams)?

different flocks

6.11 From what sources have you bought your sheep (excluding rams)?

- ☐ Sale yards
- ☐ Through stock agent
- ☐ Private
- ☐ Clearing sales
- ☐ No sheep bought
- ☐ Other, please specify:

6.12 Did you keep the introduced sheep separate from your home-bred lambs.

- ☐ Yes
- ☐ No

7 Respondent's information.

7.1 What is your relation to the property?

- ☐ Owner
- ☐ Manager
- ☐ Lessee
- ☐ Other, please specify:

7.2 How many years have you worked in farming?

- ☐ Less than 5 years
- ☐ Between 5 and 10 years
- ☐ Between 10 and 20 years
- ☐ More than 20 years

THANK YOU FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.
Will you please mail the completed questionnaire in the stamped, addressed envelope that is enclosed.

APPENDIX II

Protocol for post mortem inspection of lambs for pleurisy and pneumonia.

1 Goal.

To evaluate some aspects of the feasibility of pre-harvest food safety by subjecting groups of trial lambs to various farm management practices.

2 Background.

This trial is part of the MAF programme to establish a scientific basis for meat inspection.Pleurisy has been chosen for this project because of its importance to the meat industry. It is the disease that results i more lamb carcasses being diverted to the detain rail than any other disease.

A case-control study of lambs in the North Island has been carried out to determine risk factors for pleurisy. A number of factors have been identified and the current intervention studies have been designed to further explore some of these risk factors and their interaction.

Compared to other trials only limited work is required. For instance there is no need for detailed inspection. However significant resources have been invested by the participating farmers and accurate recording is of paramount importance.

Two farms take place in the current trials, they are Farm A and Farm B. At Farm A 4 groups of lambs are exposed to combinations of 2 different risk factors. These are the use of oral or injectable drenches and the time lambs are kept in the yards. At Farm B the factors under investigation are the use of oral or injectable drenches and the use of a shower dip or wand for the prevention of flystrike.

The number of lambs from each farm is approximately 1,000. They will be submitted for slaughter in several drafts. The exposure groups will be identified by different colour eartags. It is important that all animals that are submitted for slaughter are inspected according to this protocol. There are no other lambs that can be used to compensate for lambs of which the inspection results have not been recorded. In addition incorrect recording of disease with regard to the eartag colour would lead to erroneous conclusions.

The lambs will receive routine inspection for all diseases. If pleurisy or pneumonia are detected additional classification and recording of these diseases will be necessary. The recorded data will show all diseases (including the classification) per individual animal. The weights and grades of the lambs by eartag colour will be recorded.

3 Carcass inspection including inspection for pleurisy.

The carcasses will be inspected in accordance with normal procedures. These are:

3.1 View the back of the carcass as it approaches, from the hocks to the neck.

3.2 View the rectal cavity.

3.3 Insert two fingers into the rectal cavity and pull the tail back. View the muscular groove on either side of the tail. Palpate the ischiatic lymph nodes.

3.4 View and palpate the hind leg joints, precrural and superficial inguinal lymph nodes. Palpate the popliteal lymph nodes.

3.5 Palpate the back of the carcass.

3.6 View the front of the hind legs and ventral surface of the abdomen.

3.7 View the pelvic cavity including the iliac lymph nodes.

3.8 View and palpate the abdominal and thoracic cavities including the diaphragm and kidneys if present.

3.9 Palpate the prescapular lymph nodes and view and palpate the foreleg joints.

3.10 View the brisket , forelegs, axillae and neck.

4 Viscera inspection including inspection for pneumonia.

The viscera and head will be inspected in accordance with normal procedures with the exception of the lungs. These procedures are:

4.1 Heart, observe and palpate.

4.2 Pericardium, observe in conjunction with heart inspection.

4.3 Liver, observe and palpate both sides.

4.4 Hepatic lymph nodes, observe in conjunction with a visual examination of the visceral surface of the liver.

4.5 Bile duct, observe and palpate in conjunction with liver inspection.

4.6 Kidneys, observe both kidneys lifted from the viscera table.

4.7 Gastro-intestinal tract, observe.

4.8 Mesenteric lymph nodes, no routine inspection.

4.9 Oesophagus, no routine inspection

4.10 Spleen, observe

The lung procedures are:

4.11 View the dorsal surface of the lungs, and the visible lymph nodes (bronchial and mediastinal) as the tissue lies on the viscera table. pick-up the lungs via the dorsal surface (most inspectors use only one, spread hand), invert, and hold so that the ventral surface of the lungs can be viewed. Palpate the lungs, the bronchial and mediastinal lymph nodes. This action is usually carried out while the lungs are being picked up and turned over for inspection of the ventral surface.

The current routine NZ procedures for inspection of the lungs which require observation of the lungs and no routine inspection of the bronchial and mediastinal lymph nodes are therefore replaced by 3.11.

The heads are inspected or not as required by commercial practices at the slaughterhouse. The results need not be recorded.

5 Classification of pleurisy.

During inspection of the thoracic cavity the pleurisy lesions shall be classified and recorded as detailed below. This classification is similar to the one that has been used by MAF on previous occasions when investigating pleurisy. The code used on the recording sheet is printed in bold letter type below.

5.1 Chronic Type I pleurisy (**Chronic large**).

(a) Fibrous adhesions with thickening of the pleura, extending over an area greater than 50 mm in any dimension. Grey to white in colour.

(b) Fibrous adhesions, as above, that include purulent or densely fibrous foci. Variable colour and extent.

5.2 Chronic Type II pleurisy (**Chronic flimsy**).

Flimsy fibrous adhesions and tags with very little thickening of the pleura. Less than 50 mm in the greatest dimension, and whitish opaque in colour. Difficult to distinguish upon drying of the pleural surfaces.

5.3 **Acute localised** pleurisy.

Hyperaemic, fibrinous adhesions, usually more than 50 mm in greatest dimensions. variable appearance, and often with a gelatinous consistency.

5.4 **Acute** pleurisy with **systemic** involvement.

Septicaemic pneumonia-pleurisy complex.

5.5 Any pleural lesions that are not covered by any of the above categories are to be separately described.

6 Classification of pneumonia.

6.1 **Mild** pneumonia; small discrete areas of consolidation in one or both apical lobes. Maximum 3 cm diameter on dorsal surface and/or 3 cm diameter on ventral surface.

6.2 **Moderate** pneumonia; consolidation of the apical lobes and/or ventral portion of the cardiac lobes and/or the adjacent antero-ventral aspect of the diaphragmatic lobes, with less than one third of the lung parenchyma involved.

6.3 **Severe** pneumonia; consolidation of the apical, cardiac and/or diaphragmatic lobe to such an extent that an estimated one third or more of the lung parenchyma is involved.

The main purpose of this trial is to record the different classification for pleurisy and pneumonia. Other lesions in the carcass and the lungs (as described in the Appendix) are to be recorded as supporting data. Pneumonic lesions which are consistent with parasitic infections as described in the Appendix are to be classified as parasitic lesions.

7 Recording.

Since there are not likely to be electronic recording systems in place and operational at premises that are likely to participate a dedicated recorder will be required who will be able to correlate the findings of the carcass inspectors and the viscera inspectors.

If **pleurisy and/or pneumonia** are detected they will be recorded **in accordance with the classification** described above, on an animal-by-animal basis.

All other diseases and defects that are found during inspection are to be recorded on an animal by animal basis. Defects in the carcass will be recorded according to the Disease and Defect recording system. In the case of OC (other causes), the actual defect is to be recorded. Since the disease and defect system usually does not extent to the viscera, standardised descriptions for these conditions are not available. In the case of viscera, the actual defect will be recorded if tissues of the viscera are downgraded or condemned. Defects in the head are not recorded.

A **separate recording sheet** is to be used **for each** group of lambs of one **eartag colour** slaughtered on one day.

The **weights and grades** of the lambs are to be recorded based on the colour of the eartags and the day of slaughter.
Example: 26 February 1995, red eartag, 5 lambs YL 10.0 kg
10 lambs YL 12.0 kg etc.

Contamination, wounds & bruises, and agonal haemorrhages are not recorded in this trial. Care is nevertheless to be taken that lungs with contamination will still receive a full inspection to detect other abnormalities.

8 Trial manager's responsibilities.

The trial manager should contact company management to explain the purpose of these trials and to ask for cooperation where required.

Before the actual trial lambs from Farm A and Farm B are inspected, a pilot run should be carried out at the premises to ensure that the trial manager at the premises is satisfied that the recording can be performed in accordance with this protocol.

In the past approximately 30,000 lambs, sheep or cows were inspected per post mortem inspection trial. Therefore compared with other trials the number of animals is small. However these trials are different in that much work has been carried out by the participating farmers to ensure that lambs were exposed to risk factors in accordance with the protocol. In addition numbers of lambs that are not inspected during these trials cannot be compensated by using other lambs at a later stage. Therefore the following are critical factors in this trial:

- **The inspection of all animals and the recording of all diseases according to the protocol.**
- **Recording of all diseases on sheets which correctly describe the colour of the eartag.**
- **Recording of correct correlation of diseases in carcass and offal.**

9 Appendix: lung abnormalities (Jub, Kennedy and Palmer, 1994).

Various types of pneumonias are described in this reference. During this trial no attempt will be made to differentiate between the different types of pneumonias with regard to aetiology and stage.

The following descriptions are provided to refresh memory and to differentiate pneumonia with other disease conditions in the lungs.

Pneumonia

The typical gross appearance of bronchopneumonia is of irregular consolidation in cranioventral regions. The cranial and middle lobes are most often affected in those species having well-defined lobation. Consolidated lung varies from dark red, through gray-pink to more grey, depending on age and nature of the process. Palpable firmness (consolidation) of the tissue is the single most important gross criterion of pneumonia. Lobar pneumonia is one in which entire pulmonary lobes, or major portions of lobes, are diffusely and uniformly consolidated.

Atelectasis

The atelectatic lobules are distinct because they are dark red, depressed below the surface of the surrounding aerated lung, and in contrast to pneumonic lung, have a flabby consistency.

Dictyocaulus filaria

Lesions caused by *Dictyocaulus filaria* are typically seen as large wedge-shaped areas of dark red or greyish consolidated lung at the posterior border of the caudal lobes.These consolidated areas have firm consistency and are slightly depressed below the surface of surrounding inflated or sometimes hyperinflated lung. Patchy consolidation may also occur in other dorsocaudal regions.

Muellerius capillaris

The nodules produced by *Muellerius capillaris* may occur anywhere in the lung, but most of them are located beneath the pleura of dorsal regions of the caudal lobes. The nodules range in size from 1 mm to several centimetres. They are soft and hemorrhagic early in an infection. Later they are greenish grey and project above the pleural surface of adjacent lung at necropsy. Some of them become calcified.

Abscess

APPENDIX III

TABLES WITH ANALYSIS RESULTS OF RESPIRATORY DISEASE AT FARM B

AppendixIII Table 1 Cumulative prevalence of various categories of pneumonia at Farm B

Date	Treatment Categories		p-value	No. cells < 5.0
mild pneumonia				
10/4/95	wand	shower	0.9716	
10/4/95	oral	injection	0.4513	
13/6/95	wand	shower	0.7862	
13/6/95	oral	injection	0.8334	
30/8/95	wand	shower	0.3904	
30/8/95	oral	injection	0.9474	
Moderate and severe pneumonia				
10/4/95	wand	shower	0.2620	
10/4/95	oral	injection	0.2322	
13/6/95	wand	shower	0.0432	*
13/6/95	oral	injection	0.6249	
30/8/95	wand	shower	0.0307	*
30/8/95	oral	injection	0.5961	
pneumonia of any severity				
10/4/95	wand	shower	0.4803	
10/4/95	oral	injection	0.9856	
13/6/95	wand	shower	0.1108	
13/6/95	oral	injection	0.9229	
30/8/95	wand	shower	0.0322	*
30/8/95	oral	injection	0.8062	

AppendixIII Table 2 Cumulative prevalence of various categories of pleurisy at Farm B

Date	Treatment Categories		p-value	No. cells < 5.0
Chronic flimsy pleurisy				
10/4/95	wand	shower	0.7655	
10/4/95	oral	injection	0.3065	
13/6/95	wand	shower	0.3922	
13/6/95	oral	injection	0.2525	
30/8/95	wand	shower	0.1699	
30/8/95	oral	injection	0.1393	
Chronic large pleurisy				
10/4/95	wand	shower	0.0545	
10/4/95	oral	injection	0.7942	
13/6/95	wand	shower	0.0332	*
13/6/95	oral	injection	0.7613	
30/8/95	wand	shower	0.0696	
30/8/95	oral	injection	0.3925	
All acute pleurisy				
30/8/95	wand	shower	0.8964	
30/8/95	oral	injection	0.2649	
Pleurisy of any degree				
10/4/95	wand	shower	0.2235	
10/4/95	oral	injection	0.4430	
13/6/95	wand	shower	0.4830	
13/6/95	oral	injection	0.1828	
30/8/95	wand	shower	0.7562	
30/8/95	oral	injection	0.5296	

Appendix III Table 3 Cumulative prevalence of pleurisy and/or pneumonia at Farm B

Date	Treatment Categories		p-value	No. cells < 5.0
10/4/95	wand	shower	0.2392	
10/4/95	oral	injection	0.7179	
13/6/95	wand	shower	0.0875	
13/6/95	oral	injection	0.9507	
30/8/95	wand	shower	0.0808	
30/8/95	oral	injection	0.7732	

Appendix III Table 4 Prevalence of chronic flimsy pleurisy

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	6	88	0.064	
10/4	20	293	0.064	0.064
13/6	27	178	0.132	0.087
30/8	9	77	0.105	0.089

Chi-squared test of Table 4 with Date and numbers of lambs as variables

overall chi-squared value	8.05
p-value	0.0449
df	3

Appendix III Table 5 Prevalence of chronic large pleurisy

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	5	89	0.053	
10/4	17	296	0.054	0.054
13/6	27	178	0.132	0.080
30/8	29	57	0.337	0.112

Chi-squared test of Table 5 with Date and numbers of lambs as variables

overall chi-squared value	58.51
p-value	0.0000
df	3

Appendix III Table 6 Prevalence of acute localised pleurisy

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	1	93	0.011	
10/4	3	310	0.010	0.010
13/6	8	197	0.039	0.020
30/8	0	86	0.000	0.017

Chi-squared test of Table 6 with Date and numbers of lambs as variables

overall chi-squared value	8.60
p-value	0.0351
df	3

Note: there are 3 cells with expected value <5.0

Appendix III Table 7 Prevalence of pleurisy of any degree

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	11	83	0.117	
10/4	40	273	0.128	0.125
13/6	62	143	0.302	0.185
30/8	38	48	0.442	0.216

Chi-squared test of Table 7 with Date and numbers of lambs as variables

overall chi-squared value	54.71
p-value	0.0000
df	3

Appendix III Table 8 Prevalence of mild pneumonia

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	44	50	0.468	
10/4	157	156	0.502	0.494
13/6	135	70	0.659	0.549
30/8	4	82	0.047	0.487

Chi-squared test of Table 8 with Date and numbers of lambs as variables

overall chi-squared value	91.34
p-value	0.0000
df	3

Appendix III Table 9 Prevalence of moderate and severe pneumonia

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	7	87	0.074	
10/4	41	272	0.131	0.118
13/6	17	188	0.083	0.106
30/8	0	86	0	0.093

Chi-squared test of Table 9 with Date and numbers of lambs as variables

overall chi-squared value	14.79
p-value	0.0000
df	3

Appendix III Table 10 Prevalence of pneumonia of any degree

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	51	43	0.543	
10/4	198	115	0.633	0.612
13/6	152	53	0.741	0.655
30/8	4	82	0.047	0.580

Chi-squared test of Table 10 with Date and numbers of lambs as variables

overall chi-squared value	126.53
p-value	0.0000
df	3

Appendix III Table 11 Prevalence of pleurisy and/or pneumonia of any degree

Date	Affected lambs	Unaffected lambs	Prevalence	Cumulative prevalence
7/3	53	41	0.564	
10/4	205	108	0.655	0.634
13/6	165	40	0.805	0.691
30/8	38	48	0.442	0.660

Chi-squared test of Table 11 with Date and numbers of lambs as variables

overall chi-squared value	41.35
p-value	0.0000
df	3

APPENDIX IV

CORRELATIONS (PEARSON) OF PLEURISY LESIONS AT VARIOUS PREMISES

Correlations (Pearson) of Major pleurisy on a half-monthly basis for all premises

	ME17	ME20	ME42
ME20	0.9508		
ME42	0.9059	0.9835	
ME47	0.8551	0.9379	0.9726

Cases included 12 missing cases 13

Correlations (Pearson) of Major pleurisy on a half-monthly basis excluding ME 20

	ME17	ME42
ME42	0.9387	
ME47	0.9049	0.9832

Cases included 15 missing cases 10

Correlations (Pearson) of Minor pleurisy on a half-monthly basis including all premises

	ME17	ME20	ME42
ME20	0.8003		
ME42	0.7205	0.7597	
ME47	0.8154	0.9048	0.8998

Cases included 12 missing cases 13

Correlations (Pearson) of Minor pleurisy on a half-monthly basis excluding ME20

	ME17	ME42
ME42	0.8044	
ME47	0.8550	0.9374

Cases included 15 missing cases 10

Correlations (Pearson) of Major and Minor pleurisy on a half-monthly basis for all premises.

	ME17	ME20	ME42
ME20	0.9235		
ME42	0.9101	0.9848	
ME47	0.8600	0.9595	0.9826

Cases included 12 missing cases 13

Correlations (Pearson) of Major and Minor pleurisy on a half-monthly basis excluding ME20

	ME17	ME42
ME42	0.9389	
ME47	0.8992	0.9877

Cases included 15 missing cases 10

Correlations (Pearson) of Major pleurisy/Minor pleurisy on a half-monthly basis for all premises

	ME17	ME20	ME42
ME20	0.9236		
ME42	0.3739	0.4769	
ME47	-0.6187	-0.5415	0.0071

Cases included 12 missing cases 13

Correlations (Pearson) of Major pleurisy/Minor pleurisy excluding ME20

	ME17	ME42
ME42	0.1921	
ME47	-0.6741	0.2217

Cases included 15 missing cases 10

Correlations (Pearson) of Acute pleurisy on a half-monthly basis for all premises

	ME17	ME20	ME42
ME20	-0.4012		
ME42	-0.1150	-0.3435	
ME47	0.3559	-0.5700	0.6713

Cases included 12 missing cases 13

Correlations (Pearson) of Acute pleurisy on a half-monthly basis excluding ME20

	ME17	ME42
ME42	0.0001	
ME47	0.4350	0.6875

Cases included 15 missing cases 10

Correlations (Pearson) of Septicaemia on a half-monthly basis for all premises

	ME17	ME20	ME42
ME20	M		
ME42	M	M	
ME47	0.1837	M	M

An "M" is displayed when a coefficient cannot be computed

Cases included 12 missing cases 13

Correlations (Pearson) of Septicaemia on a half-monthly basis excluding ME20

	ME17	ME42
ME42	M	
ME47	0.2192	M

An "M" is displayed when a coefficient cannot be computed
Cases included 15 missing cases 10

Correlations (Pearson) of Acute and Septicemic pleurisy on a half-monthly basis for all premises

	ME17	ME20	ME42
ME20	-0.4193		
ME42	-0.0734	-0.3414	
ME47	0.4024	-0.5710	0.6418

Cases included 12 missing cases 13

Correlations (Pearson) of Acute and Septicemic pleurisy on a half-monthly basis excluding ME20

	ME17	ME42
ME42	0.0456	
ME47	0.4792	0.6619

Cases included 15 missing cases 10

APPENDIX V

PREDICTIONS OF THE PREVALENCE OF PLEURISY BASED ON VALUES OF OTHER PREMISES

Pearson correlation coefficients for ME047, ME063 and ME035 (area M)

	ME047	ME063
ME063	0.28	
ME035	0.74	0.02

Regression coefficients and the p-values of a linear regression to predict ME047

	Coefficient	p-value
Constant	0.01	0.50
ME063	1.42	0.00
ME035	0.27	0.00
R-squared	0.62	

There were 56 cases included and 76 missing cases

Pearson correlation coefficients for ME042, ME008 and ME010 (area W)

	ME042	ME008
ME008	0.84	
ME010	0.84	0.76

Regression coefficients and the p-values of a linear regression to predict ME042

	Coefficient	p-value
Constant	0.00	0.27
ME008	0.62	0.00
ME010	0.63	0.00
R-squared	0.81	

There were 105 cases included and 27 missing cases

Pearson correlation coefficients for ME017, ME034 and ME016 (area T)

	ME017	ME034
ME034	0.86	
ME016	0.89	0.88

Regression coefficients and the p-values of a linear regression to predict ME042

	Coefficient	p-value
Constant	0.01	0.52
ME034	0.53	0.00
ME015	0.64	0.00
R-squared	0.82	

There were 84 cases included and 48 missing cases

Pearson correlation coefficients for ME020, ME050 and ME022 (area O)

	ME020	ME050
ME050	0.81	
ME022	0.75	0.76

Regression coefficients and the p-values of a linear regression to predict ME020

	Coefficient	p-value
Constant	0.01	0.51
ME050	0.54	0.00
ME022	0.39	0.00
R-squared	0.70	

There were 72 cases included and 60 missing cases

Simple linear regressions to evaluate the different levels of pleurisy prevalences.

Pr47	=	0.11 + 0.24	*	Pr63	(R- squared = 0.02)
Pr47	=	0.03 + 1.33	*	Pr35	(R- squared = 0.55)
Pr47	=	0.04 + 0.73	*	Pr42	(R- squared = 0.25)
Pr47	=	0.05 + 1.07	*	Pr17	(R- squared = 0.15)
Pr47	=	0.05 + 1.19	*	Pr20	(R- squared = 0.20)
Pr42	=	0.01 + 1.09	*	Pr8	(R- squared = 0.71)
Pr42	=	0.01 + 1.11	*	Pr10	(R- squared = 0.72)
Pr42	=	0.02 + 1.28	*	Pr17	(R- squared = 0.70)
Pr42	=	0.04 + 1.16	*	Pr20	(R- squared = 0.63)
Pr17	=	0.006 + 1.33	*	Pr34	(R- squared = 0.63)
Pr17	=	0.005 +0.97	*	Pr16	(R- squared = 0.74)
Pr17	=	0.01 + 0.73	*	Pr20	(R- squared = 0.74)
Pr20	=	0.006 + 0.97	*	Pr22	(R- squared = 0.57)
Pr20	=	0.008+0.78	*	Pr50	(R- squared = 0.68)

The Pr value shown (egPr16) relates to the export works with the same number (eg ME16).

APPENDIX VI

EXAMPLES OF TESTS USED IN EVALUATING DECISION SUPPORT SYSTEMS

Month 1- 1993	pleurisy prevalence	not pleurisy
All Farms	2	98
Farm A	6	94
Chi-squared value	2.08	
p-value	0.1489	
2 cells with expected value < 5.0		

Appendix VI Figure 1 Chi-squared test to evaluate pleurisy prevalence in Month 1

Month 2 - 1993	pleurisy prevalence	not pleurisy
All Farms	6	194
Farm A	14	186
Chi-squared value	3.37	
p-value	0.0665	

Appendix VI Figure 2 Chi-squared test to evaluate pleurisy prevalence in Month 2

Month 3 - 1993	pleurisy prevalence	not pleurisy
All Farms	15	285
Farm A	20	280
Chi-squared value	0.76	
p-value	0.3838	

Appendix VI Figure 3 Chi-squared test to evaluate pleurisy prevalence in Month 3

Month 4 - 1993	pleurisy prevalence	not pleurisy
All Farms	16	184
Farm A	20	180
Chi-squared value	0.49	
p-value	0.4846	

Appendix VI Figure 4 Chi-squared test to evaluate pleurisy prevalence in Month 4

Month 5 - 1993	pleurisy prevalence	not pleurisy
All Farms	19	181
Farm A	25	175
chi-squared	0.92	
p-value	0.3377	

Appendix VI Figure 5 Chi-squared test to evaluate pleurisy prevalence in Month 5

Appendix VI Table 1 Cumulative data of slaughtered lambs and lambs with pleurisy

	Farm A				Area X			
Month	Pos. 1992	Kill 1992	Pos. 1993	Kill 1993	Pos. 1992	Kill 1992	Pos 1993	Kill 1993
10								
11								
12								
1			6	100			5	300
2	11	300	20	300	20	600	14	600
3	31	600	40	600	41	900	29	900
4	56	800	60	800	41	900	29	900
5	67	900	85	1000	41	900	29	900
6	67	900	85	1000	41	900	29	900
7	91	1000	85	1000	41	900	29	900
8								
9								

Month 1 - 1993	cumulative pleurisy prevalence	not pleurisy
All Farms	2	98
Farm A	6	94
Chi-squared value	2.08	
p-value	0.1489	
2 cells with expected value < 5.0		

Appendix VI Figure 6 Chi-squared test to evaluate cumulative pleurisy prevalence in Month 1

Month 2 - 1993	cumulative pleurisy prevalence	not pleurisy
All Farms	8	292
Farm A	20	280
Chi-squared value	5.39	
p-value	0.0202	

Appendix VI Figure 7 Chi-squared test to evaluate pleurisy prevalence in Month 2

Month 3 -1993	cumulative pleurisy prevalence	not pleurisy
All farms	23	577
Farm A	40	560
Chi-squared value	4.84	
p-value	0.0278	

Appendix VI Figure 8 Chi-squared test to evaluate pleurisy prevalence in Month 3

Month 4 - 1993	cumulative pleurisy prevalence	not pleurisy
All farms	39	761
Farm A	60	740
Chi-squared value	4.75	
p-value	0.0293	

Appendix VI Figure 9 Chi-squared test to evaluate pleurisy prevalence in Month 4

Month 5 - 1993	cumulative pleurisy prevalence	not pleurisy
All farms	58	942
Farm A	85	915
Chi-squared value	5.49	
p-value	0.0191	

Appendix VI Figure 10 Chi-squared test to evaluate pleurisy prevalence in Month 5

Month 1 - 1993	cumulative pleurisy prevalence	not pleurisy
All Farms	5	295
Farm A	6	94
Chi-squared value	5.27	
p-value	0.0217	
1 cell with expected value < 5.0		

Appendix VI Figure 11 Chi-squared test to evaluate pleurisy prevalence in Month 1

Month 2	cumulative pleurisy prevalence	not pleurisy
All Farms	14	586
Farm A	20	280
Chi-squared value	10.33	
p-value	0.0013	

Appendix VI Figure 12 Chi-squared test to evaluate pleurisy prevalence in Month 2

Month 3	pleurisy	not pleurisy
All Farms	29	871
Farm A	40	560
Chi-squared value	9.73	
p-value	0.0018	

Appendix VI Figure 13 Chi-squared test to evaluate pleurisy prevalence in Month 3

Month 4	cumulative pleurisy prevalence	not pleurisy
All Farms	29	871
Farm A	70	730
Chi-squared value	23.60	
p-value	0.0000	

Appendix VI Figure 14 Chi-squared test to evaluate pleurisy prevalence in Month 4

Month 5	cumulative pleurisy prevalence	not pleurisy
All Farms	29	871
Farm A	85	915
Chi-squared value	23.39	
p-value	0.0000	

Appendix VI Figure 15 Chi-squared test to evaluate pleurisy prevalence in Month 5

Month 1	slaughtered	not slaughtered
All farms	300	600
Farm A	100	900
Chi-squared value	155.17	
p-value	0.0000	

Appendix VI Figure 16 Chi-squared test to evaluate slaughter pattern in Month 1

Month 2	slaughtered	not slaughtered
All Farms	600	300
Farm A	300	700
Chi-squared value	255.44	
p-value	0.0000	

Appendix VI Figure 17 Chi-squared test to evaluate slaughter pattern in Month 2

Month 3	slaughtered	not slaughtered
All Farms	900	0
Farm A	600	400
Chi-squared value	4.56	
p-value	0.0000	

Appendix VI Figure 18 Chi-squared test to evaluate slaughter pattern in Month 3

Month 4	slaughtered	not slaughtered
All Farms	900	0
Farm A	800	200
Chi-squared value	201.18	
p-value	0.0000	

Appendix VI Figure 19 Chi-squared test to evaluate slaughter pattern in Month 4

Month 5	slaughtered	not slaughtered
All Farms	900	6*
Farm A	1000	6*
Chi-squared value	0.03	
p-value	0.8556	

Appendix VI Figure 20 Chi-squared test to evaluate slaughter pattern in Month 5

*An arbitrary small number (6) has been chosen for 'not slaughtered' in month 5, otherwise a chi-squared test would not have been possible.

	A	B	C	D	E	F	G	H	I
1	**APPENDIX VII**								
2	**Model of cross-contamination**								
3									
4	**Prevalence**		**0.154698**						
5	**Cross-contaminatio**		**0.9**	**0.8**	**0.6**				
6									
7				**Cross-contamination**					
8				One		Two		Three	
9				lung set		lung sets		lung sets	
10			**Originally**	back		back		back	
11	Lungs	Prevalenc	**Infection**	0.9		0.8		0.6	
12	1	0.167236	0						
13	2	0.165777	1	0	0				
14	3	0.119985	0	0.9	1	0	0		
15	4	0.136457	0	0	0	0.8	1	0	0
16	5	0.119923	0	0	0	0	0	0.6	0
17	6	0.114256	1	0	0	0	0	0	0
18	7	0.171608	0	0.9	1	0	0	0	0
19	8	0.108156	0	0	0	0.8	1	0	0
20	9	0.172283	0	0	0	0	0	0.6	1
21	10	0.192869	0	0	0	0	0	0	0
22	11	0.184297	1	0	0	0	0	0	0
23	12	0.159018	0	0.9	1	0	0	0	0
24	13	0.138587	1	0	0	0.8	1	0	0
25	14	0.191037	0	0.9	1	0	0	0.6	0
26	15	0.14382	0	0	0	0.8	1	0	0
27	16	0.10666	0	0	0	0	0	0.6	1
28	17	0.133252	0	0	0	0	0	0	0
29	18	0.105605	0	0	0	0	0	0	0
30	19	0.18439	0	0	0	0	0	0	0
31	20	0.18357	0	0	0	0	0	0	0
32	21	0.158423	0	0	0	0	0	0	0
33	22	0.123683	0	0	0	0	0	0	0
34	23	0.103328	0	0	0	0	0	0	0
35	24	0.17806	0	0	0	0	0	0	0
36	25	0.191087	0	0	0	0	0	0	0
37		**SUM**	**4**						

	J	K	L	M	N	O	P	Q	R
1									
2									
3									
4									
5						If handwashing is			
6						partially effective			
7				Proportion		every	**5**	lung sets	
8	Cross-	**Positive**		positive lung sets		One	5	Two	4
9	Contamina	**lung set**		if handwashing		lung back		lungs back	3
10	Lungs			100% effective		0.5		0.3	
11			Lungs	every x lungs		0.9		0.8	
12	0	0	1	0		0	0	1	0
13	0	1	2	0.5		0	0	1	0
14	1	1	3	0.666667		0.9	1	0	0
15	1	1	4	0.75		0	0	0.8	0
16	0	0	5	0.6		0	0	0	0
17	0	1	6	0.666667		0	0	0	0
18	1	1	7	0.714286		0.9	1	0	0
19	1	1	8	0.75		0	0	0.8	1
20	1	1	9	0.777778		0	0	0	0
21	0	0	10	0.7		0	0	0	0
22	0	1	11	0.727273		0	0	0	0
23	1	1	12	0.75		0.9	1	0	0
24	1	1	13	0.769231		0	0	0.8	1
25	1	1	14	0.785714		0.9	1	0	0
26	1	1	15	0.8		0	0	0.8	1
27	1	1	16	0.8125		0	0	0	0
28	0	0	17	0.764706		0	0	0	0
29	0	0	18	0.722222		0	0	0	0
30	0	0	19	0.684211		0	0	0	0
31	0	0	20	0.65		0	0	0	0
32	0	0	21	0.619048		0	0	0	0
33	0	0	22	0.590909		0	0	0	0
34	0	0	23	0.565217		0	0	0	0
35	0	0	24	0.541667		0	0	0	0
36	0	0	25	0.52		0	0	0	0
37	**Sum**	**13**							

	S	T	U	V	W	X	Y
1							
2							
3							
4							
5							
6							
7			**Positive**		**Proportion**		
8	Three	3	**lung**		**of contaminated carcasses**		
9	lungs back	2	**including**		**if handwashing**		
10	0.2	1	**handwashing**				
11	0.6						
12	1	0	0		0		
13	1	1	1		0.5		
14	1	0	1		0.666667		
15	0	0	1		0.75		
16	0.6	1	1		0.8		
17	0	0	1		0.833333		
18	0	0	1		0.857143		
19	0	0	1		0.875		
20	0.6	0	1		0.888889		
21	0	0	0		0.8		
22	0	0	1		0.818182		
23	0	0	1		0.833333		
24	0	0	1		0.846154		
25	0.6	0	1		0.857143		
26	0	0	1		0.866667		
27	0.6	0	1		0.875		
28	0	0	0		0.823529		
29	0	0	0		0.777778		
30	0	0	0		0.736842		
31	0	0	0		0.7		
32	0	0	0		0.666667		
33	0	0	0		0.636364		
34	0	0	0		0.608696		
35	0	0	0		0.583333		
36	0	0	0		0.56		
37							

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