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**EPIDEMIOLOGICAL ANALYSIS OF
TUBERCULOSIS IN CATTLE HERDS**

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ABSTRACT

Studies were made of the occurrence and distribution of bovine tuberculosis in the Taumarunui and Masterton veterinary districts, within which endemic wildlife tuberculosis is widespread. These districts were compared with the movement control herds of the surveillance areas of New Zealand, which are free of wildlife tuberculosis. The study covers the period from 1985-1990. The frequencies of tuberculosis infection for different categories of herds and different veterinary districts were examined and comparisons made of rates and risk of disease between the herd categories and districts under consideration.

A comparison of incidence measures showed a strong positive correlation between cumulative, corrected and true incidence values. Cumulative and corrected cumulative incidence values calculated for calendar, financial and test years were compared. Some disparity was found between annual cumulative incidences and annual corrected cumulative incidences calculated on the basis of calendar and test years, with measures calculated on the basis of test year having the highest values.

Regional comparisons showed that movement control herds in surveillance areas had higher incidences of tuberculosis than did herds in the Taumarunui and Masterton veterinary districts. Beef dry stock herds had higher incidences of tuberculosis than did dairy or beef breeding enterprises. Endemic areas had the highest incidence of all tuberculosis area classes.

Simple regression analyses indicated that the risk of tuberculosis for any animal in a herd was more closely related to the level of infection in adult cows than any other age or sex group. Cumulative incidence in yearlings was a poor predictor of risk for individual animals in a herd but there was a stronger relationship for the level of infection in 2 year-old animals.

Stepwise logistic regression was used to explore and quantify associations between cumulative incidence and putative risk factors. The odds of cattle testing positive in herds

in endemic areas was about five times as high as in herds in surveillance and fringe areas, where the risks of tuberculosis were about the same. The likelihood of reacting to the tuberculin test was considerably lower for animals in the Masterton and Taumarunui districts than for animals from movement control herds in surveillance areas outside those districts. The overall risk of infection increased slightly from 1985 to 1990.

Poisson regression was used to examine the relationships between incidence density and the same independent variables which were examined using logistic regression. The relative risks for infection were higher in beef breeding, beef dry stock and other herd types than in dairy herds. Herds in endemic areas had rates of infection about seven times those in Fringe and Surveillance area herds, where the rates were about the same. The incidence of infection in herds increased with increased herd size and was considerably less in the Masterton and Taumarunui districts than in movement control herds in the Surveillance areas. There was a good general agreement between the logistic and poisson regression models in the overall relationships between the predictor variables common to both models and their respective dependent variables.

Survival analysis showed that after going on to movement control for the first time, about 75% of herds could be expected to be still on movement control after 12 months and about 50% after 2 years. Herds in the Masterton veterinary district tended to stay on movement control longer than herds in the Taumarunui veterinary district and Surveillance areas (Risk ratio = 0.69). After 2 years of testing, about 60% of infected herds in the Taumarunui veterinary district and Surveillance areas had come off movement control, compared to 40% of infected herds in the Masterton veterinary district.

The survivorship probability of infected herds in Fringe, Surveillance and non-endemic zones for coming off movement control was lower than that for infected herds in endemic zones (Risk ratio = 0.61). The estimated median time on movement control was 3 years for herds from endemic areas and 2 years for herds in Fringe, Surveillance and non-endemic zones. The risk of coming off movement control decreased with increasing herd size. Herds with high levels of cumulative incidence were more likely to stay on movement control for longer periods than those with lower levels of incidence.

Simple linear regression indicated that distance from the nearest case herd with tuberculosis, distance from the Rangitoto buffer, distance from the nearest case in year one after breakdown and total number of cattle purchased were poor predictors of cumulative incidence.

A multivariate logistic regression analysis of the association between cumulative incidence and putative risk factors during the first year after a breakdown indicated that risk was lower on farms where the main activity was dairying (MAINOP) and in herds in which the proportion of adult cattle (ADULTCAT) was high. Over the whole period for which herds were under movement control, risk levels remained lower for dairy farms and increased as the proportion of beef cattle was increased.

Increased rates of infection were associated with the practice of buying replacements (BUYREPLA) in the first year after breakdown and rates were higher for the whole period in herds which ran cattle on agistment (OTHERCAT). Rates were lower on farms where cattle had access to bush (BUSHACCE), but despite the higher rates, survival analysis clearly showed that herds with access to bush could be expected to stay on movement control for longer periods than farms with no bush access. The median time on movement control was 1 year for farms without access to the bush and slightly more than 2 years for farms with access to the bush. Movement control farms without bush access were free from infection by 3 years, whereas farms with bush access took more than 5 years.

Both the risk and incidence of infection tended to be lower at higher stocking rates in the short and long term after first going onto movement control. The association between personal qualities of farmers and the risk and rate of infection were also examined using multivariable regression analyses.

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CHAPTER 1

INTRODUCTION

Despite the great and continued efforts made in terms of control and treatment, tuberculosis remains one of the world's most prevalent and devastating diseases of livestock and man. Tuberculosis caused by *Mycobacterium bovis* has been endemic in virtually all of the cattle populations of the world, although over the past 50 years, control efforts have been successful in reducing prevalences to very low levels in most developed countries.

Bovine tuberculosis was introduced into New Zealand by tuberculous cattle at the time of European settlement but the first national scheme for control did not start until 1945. This scheme was prompted by public health concern about the sale of milk from tuberculous cattle in town supply herds and was accompanied by legislation which directed that test positive cattle be slaughtered. The scheme was voluntary at its start but progressed to compulsory participation with all town supply herds under test by the end of the 1950s. In 1961, the scheme was extended to the manufacturing dairy industry which supplies milk for the export trade in dairy products. Beef cattle testing on a national basis started in 1967, and by 1977, all breeding cattle in New Zealand were under test and a surveillance system was in place at slaughter houses for all beef animals.

The dairy factory supply and beef cattle control schemes were undertaken primarily with the aim of ensuring continued access to competitive international markets. According to international standards set by the Office International des Épizooties (OIE), a country is considered free of tuberculosis when 99.8% of herds have been "officially free" for 3 years. Official freedom requires two clear herd tests 6 months apart, the first test being 6 months after the slaughter of the last infected animal. Annual testing, combined with either testing of purchased animals or purchasing from known free sources, are stipulated conditions for maintenance of official freedom. The prevalence of infected herds in New Zealand herds was 2.6% in 1994, but although some European Union countries had higher prevalences, viz. Spain 10.8%, Ireland, 8.8% and Italy 3.71% (O'Neil and Pharo, 1995), most European countries which trade

with New Zealand have disease free status and expect their trading partners to achieve a similar status.

Initial progress in controlling the disease was rapid, although in some areas prevalence was very high, with up to 60% of herds infected. By 1979-80, the percentage of reactors had reduced from an initial 8.6% to 0.05% in dairy cattle and from 1.5% to 0.1% in beef cattle. Since that time, there has been little positive progress in the reduction of incidence of reactors. The continued effort has only held reactor incidence steady and has had an undetermined effect of reducing the rate of dissemination and expansion of endemic areas. The reason for the continued lack of progress is the widespread established Australian brushtail tuberculous possum (*Trichosurus vulpecula*) populations and the reservoir status of possums for tuberculosis caused by *M. bovis*. The New Zealand population size was estimated to be approximately 70,000,000 by Batcheler and Cowan (1988).

For reasons of control, Ministry of Agriculture and Fisheries (MAF) authorities have categorised New Zealand into either Special Tuberculosis Control Areas, (STCAs), in which tuberculosis is endemic, or Surveillance Areas, where the disease is not endemic. An STCA has a central endemic zone, surrounded by a "fringe" zone. (An early classification also included a non-endemic zone, but this has now been amalgamated with the surveillance area). Each zone is defined, with due reference to possible possum movement and migration routes. The central endemic zone comprises a region containing known tuberculous possums, the fringe zone covers an area considered wide enough to contain tuberculous possums which might migrate into it. An area is declared endemic when tuberculosis is found in wild-life within that area. The surveillance areas also contain minor endemic areas which are well defined geographically and these areas are termed Special Tuberculosis Investigation Areas (STIAs) (Livingstone, 1992) where the whole possum population is very actively controlled. It is conceded that the disease cannot be eliminated from STCAs at this stage but eradication of disease from STIAs is considered technically feasible and is currently underway in some of these areas.

The number of endemic areas increased from 9 in 1980 to 23 in 1994, (6 STCAs and 17 STIAs), but more importantly, the area of New Zealand which is classified as

endemic increased from 10% in 1980 to about 24% at present and contains approximately 12% of all herds.

It is generally accepted that the New Zealand bovine tuberculosis problem is due to endemic infection in wildlife. Of the non-farmed species, the introduced Australian brushtail possum (*Trichosurus vulpecula*) is currently considered to be by far the most important.

A fairly traditional tuberculosis control scheme is operated by the Ministry of Agriculture and Fisheries. There is active surveillance via a regular caudal fold skin testing and passive surveillance via abattoir surveillance. This is supplemented by regular inspections of cattle and deer presented for sale. In surveillance areas ancillary comparative cervical testing is generally permitted, otherwise all caudal fold test-positive animals are slaughtered and subjected to detailed post mortem inspection (as are any comparative cervical positive animals). If tuberculosis is diagnosed in a herded, it is immediately placed in quarantine (i.e. movement control). The herd is then subject to further caudal fold testing with all positive animals being slaughtered. This animal tuberculosis 'test and slaughter' campaign is supplemented in STCA and STIA with possum control.

This thesis investigates the tuberculosis test history, patterns for a range of epidemiological measures of disease occurrence for the various types and categories of herds in two veterinary districts. Incidence measures are compared between herds and districts and the relative importance of putative risk factors to disease occurrence is investigated.

BOVINE TUBERCULOSIS

History of the disease

Tuberculosis is a disease of great antiquity. Bovine, human, avian, murine and cold blooded species of mycobacteria are recognized (Buxton and Fraser, 1977) and while they all arose from a common stock, the era in which they became differentiated cannot be determined. Tuberculous lesions have been found in the bones of Egyptian mummies (Smith 1909) and phthisis has been recognised in the Mediterranean littoral at least since classical times (Webb 1936). There is some evidence that human tuberculosis caused by *Mycobacterium tuberculosis* spread through Europe from the Mediterranean region and that the Americas and many other parts of the world were subsequently infected from Europe (Flick 1925).

The earliest known report about tuberculosis in animals comes from India. Cattle (*Bos brachyceros*) were first domesticated in Asia and later brought to Europe where they were probably crossed with the wild European cattle (*Bos primigenius*). It would therefore be of great historical interest if the existence of tuberculosis in the ancient cattle of Asia could be established. Ancient Hindu literature (2,000 B.C. or earlier) contains compelling evidence that tuberculosis was common in elephant in those times (Iyer 1937). Iyer gives an account of the recorded clinical signs and translates the old names as "slow wasting fever" and "pulmonary tuberculosis", but pathological evidence which would provide unequivocal evidence that the disease described was *M. bovis* tuberculosis is lacking. The issue is further clouded since even pathological evidence of tuberculosis in elephants would be inconclusive of *M. bovis* infection, because according to Dammann and Stedefeder, (1909), *M. tuberculosis* may also produce extensive disease in elephants.

Mosaic laws and the Talmud prohibited the consumption of tuberculous flesh (Friedberger and Frohner 1908). Nocard (1895) considered that tuberculosis of cattle was described by Columella (circa A.D. 0-50) who mentioned ulceration of the lungs as the last stage of the disease. Smith (1909), however, doubted whether tuberculosis could be inferred with certainty from Columella's description, but was confident that the descriptions by Vegetius (A.D. 450 - 500) of lung ulceration as a destructive and grievous distemper referred to tuberculosis. Taken as a whole, the available evidence

strongly indicates that bovine tuberculosis was established in Northern Italy at the beginning of the Christian era and probably existed there long before that time.

Switzerland and parts of Western Europe were probably infected with cattle introduced from Italy during the Middle Ages as trade in cattle gradually increased. Prentice (1942) attributes the origins of most of our modern dairy cows to a breed of high producing dairy cows in the Po valley. There are records of the introduction of those Lombardy cattle into the Netherlands in the thirteenth century, and Holland had become famous for its milk and cheese at least by and possibly before the sixteenth century. There were laws prohibiting the consumption of tuberculous flesh in Germany from the ninth century onward (Friedberger and Frohner 1908) and Drummond and Wilbraham (1939) record that as far back as 1319, sworn wardens of the City London, appointed for overseeing the flesh markets, condemned two carcasses of beef seized from "William Sperlyng of West Hamme" as being "putrid and poisonous". He was duly convicted for attempting to sell "bodies that have died of disease".

The ancient Britons possessed numerous cattle and the flesh and milk of these animals were important sources of food for them (The Commentaries of Caesar, cited by Youatt 1870.) The Romans imported their larger breeds of cattle into Britain and crossed them with the small black Celtic ox, *Bos brachyceros* (Prentice 1942), and bovine tuberculosis may have been introduced into the British Isles at that time. The large Roman cattle did not persist as a breed however, and it is unlikely that tuberculosis would have become at all common under the prevailing husbandry conditions of those times. Early in the seventeenth century and at intervals thereafter, cattle from Holland were introduced into York and Durham (Holderness) and these, together with the Teeswater cattle, helped to found the famous Holderness or Durham shorthorns (Prentice 1942). It was largely the Teeswater cattle which were used for the town dairies (Skellett 1807), which were notoriously filthy and unhygienic (Drummond and Wilbraham 1939) and it is reasonable to assume that tuberculosis first became common in British cattle when town dairies were established to supply the manufacturing towns, which developed during the latter part of the industrial revolution.

By this time, the local Shorthorns were infected with tuberculosis as were the introduced Dutch Friesian cattle, and it is easy to understand how the disease spread throughout Britain during the nineteenth century. The Shorthorn breed was used to improve local cattle and from the middle of the nineteenth century onwards, they were exported to France and throughout the developed world (Prentice 1942). When Bang commenced his work between 1880 and 1890, about 30 to 40% of dairy cows in Denmark were infected, and he held the view (Bang 1899) that Switzerland and parts of Western Europe had long been infected with tuberculosis, but the disease was only introduced into Denmark during the first half of the nineteenth century by Swiss, Dutch and English cattle. The literature of this period indicates that tuberculosis was probably introduced to most parts of the world as attempts were made to improve local breeds with British cattle.

Aetiology

The primary aetiological agent of bovine tuberculosis, *M. bovis*, was first clearly differentiated from other types of tubercle bacilli by Theobald Smith in 1898. It has a much wider range of animal hosts than other species of the genus. Other tubercle bacilli with some degree of pathogenicity for cattle are the "*Mycobacterium avium*" complex and *M. tuberculosis*. Pestana de Castro and Nemoto (1972) reported isolating *M. scrofulaceum*, *M. intracellulare* and *M. terrae* from lymph nodes of apparently healthy cattle slaughtered in Sao Paulo, Brazil. These species have little pathogenic importance but infections may cause nonspecific reactions to the intradermal tuberculin test.

Resistance of tubercle bacilli to environmental factors

Mycobacteria are enveloped in a thick waxy material and this structure makes them resistant to external influences, and the obligate intracellular parasites among the mycobacteria can survive for a considerable time outside the host. The original work of Maddock (1933) on survival of pathogenic mycobacteria outside the host is still valid. He showed that the bacilli present in pus and morbid discharges may remain viable for several weeks, while direct sunlight killed bacilli in cultures within a few hours. Bacilli in faeces remained viable for over five months and in artificially contaminated water for more than ten weeks. Further, he found that *M. bovis* survived on pasture for at least 49 days in summer in southern England. Williams and

Hoy (1928) found that tubercle bacilli exposed on pasture land retained their viability for at least five months in winter. The bacilli also withstood putrefaction by souring milk, salting or pickling. The saprophytic mycobacteria have been found to survive and multiply under a wide range of environmental conditions, including pH, temperature and metal content (Chapman 1971). Investigations both in France (Tacquet *et al.* 1966) and the United States of America (Chapman *et al.* 1965) recorded the presence of numerous saprophytic mycobacteria types in considerable numbers in raw milk. Harrington and Karlson (1965) and Chapman and Speight (1968) further demonstrated that these bacilli could persist through the pasteurisation process to packaged milk.

Effective chemicals for destroying tubercle bacilli include phenolic compounds (5% phenol), formaldehyde and alcohols. Susceptibility of the bacilli to ultraviolet light was reviewed by Hollender (1942) and since then, several workers (Collins 1971; David *et al.* 1971) have shown that ultraviolet irradiation from a germicidal lamp, when properly used, was very effective in killing tubercle bacilli, though *M. tuberculosis* and *M. marinum* were shown to be capable of photo- reactivation (David *et al.* 1971).

Characteristics of *M. bovis*

Details of the cell wall composition of the bovine tubercle bacillus were reviewed by Lederer (1971), lipid composition by Goren (1972), intermediary metabolism by Ramakrishnan *et al.* (1972), and biochemical properties by Bloch (1960). Mycobacterial cell walls have an unusually high lipid content (up to 60%) and this property is thought to account for their impermeability to stains, their acid-fast properties, resistance to some disinfectants the bactericidal enzymes of phagocytic cells. The wax D of *M. bovis* differs from that of *M. tuberculosis* in lacking peptide, and it is thought that this is the reason for its unsuitability as an adjuvant.

Mycobacterium bovis grows more slowly on solid or liquid media than *M. tuberculosis*, especially on primary isolation, and in contrast to *M. tuberculosis*, its growth is inhibited by glycerol. An egg medium with pyruvate replacing glycerol is favourable for growth, but the bacilli can be grown either on egg or agar based medium.

The colonial morphology of *M. bovis* varies with the medium. Primary colonies on Middlebrook's 7H-10 medium are colourless, flat, irregular, rough and dull, while those grown on Stonebrink's medium are white, moist, and convex and resemble those of *M. avium*.

The host range of *M. bovis* is exceptionally wide and includes other ruminants, man, dogs, cats, pigs, horses, badgers and parrots (Schmedial 1968; Huitema 1970; Little *et al.* 1975). It is also pathogenic for guineapigs, rabbits, rats, mice and hamsters. It is the least reactive of the mycobacteria to the biochemical tests used to identify the various species.

Modes of infection in cattle

Although mediate contagion can occur, infected animals are the main source of new infections. Organisms are excreted in the exhaled air, in sputum, faeces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges and discharges from open peripheral lymph nodes. The summary provided by Francis (1947) is still regarded (Morris *et al.* 1994) as the most appropriate synthesis of knowledge "The results of experimental aerogenous and alimentary infection together with the distribution of lesions found in naturally acquired tuberculosis show that about 80 to 90 percent of all cattle are infected by inhalation. Even in calves this is usually the most important route, although occasionally a whole group may be infected by tuberculous milk. Cattle are much more easily infected by the respiratory route than the alimentary route and although relatively large numbers of bacilli are passed in the faeces, pastures are probably not an important source of infection. The evidence suggests that even when heifers are pastured with heavily infected cows, the incidence remains low until they enter the cowshed. The primary lung lesion is a bronchopneumonic focus which may progress rapidly or may remain quiescent for many years. Despite this, however, it would appear that the complete healing of lung lesions which often occurs in man seldom takes place in cattle. This is a fundamental difference and in practice all tuberculous cattle are regarded as infectious to other cattle. The most important route of spread in the lung is along the bronchi; sputum passing up the trachea and being swallowed may also infect the intestine and mesenteric nodes, but other organs such as the spleen and kidney are infected by bacilli entering the blood stream from lesions in the lung."

Opportunities for infection by the oral route occurs at pasture from infected faecal contamination of feed and communal drinking water and feed troughs. Under natural conditions, stagnant drinking water may remain infective for up to 18 days after its last use by a tuberculous animal, but running stream would present negligible risk to cattle in downstream fields. It is difficult to give precise estimates of the persistence of infectivity of pasture and other inanimate objects because of the varying conditions under which organism survival experiments have been carried out. Viable *M. bovis* organisms can be recovered from the faeces of some infected cattle and have been isolated from soil contaminated by infected faeces for 6 to 8 weeks, but the duration of the potential infectivity of pasture to cattle shows wide variation. The period may be as short as one week if the weather is dry, or following harrowing, but may be much longer in wet weather. Experimental infections of cattle have been produced following contamination of pasture with emulsions of *M. bovis* organisms, produced from macerations of tuberculous organs of cattle (Maddock 1934, Schellner 1956) but the contamination rates used were very high. Schellner (1956) employed a suspension containing 10^5 – 10^7 organisms per ml applied to pasture at a rate of 1 litre per m². More recent studies (MAFF 1979; Duffield and Young 1985; Jackson *et al.* 1995) indicate that *M. bovis* disappears much more quickly from the environment than early studies suggested. The survival of *M. bovis* under Australian conditions was determined following artificial inoculation of animal faeces, dry and moist soil with *M. bovis* (Duffield and Young 1985). *M. bovis* survived for 4 weeks in non-sterile dry and moist soils held under 80% shade, in darkness and in the laboratory. No recovery of *M. bovis* was made at 4 weeks from dry or moist soils exposed to sunlight or from faeces held under any condition.

In the summer months in England, *M. bovis* could not be recovered from grass contaminated with infected badger urine after 3 days or from naturally infected badger faeces after periods of 1 or 2 weeks (Anon 1979).

In a New Zealand study, (Jackson *et al.* 1995) *M. bovis* organisms absorbed on cotton ribbons were placed in different natural habitats on a farm in New Zealand. *M. bovis* was not re-isolated from ribbons placed on pasture after 4 days. Survival on ribbons was longest in brushtail possum dens, where the maximum period of survival in dens was less than 7 days in summer and greater than 14 days but less than 28 days in winter and spring. The maximum period of survival on a forest floor was intermediate

between pasture and dens - less than 4 days in summer and greater than 14 days but less than 28 days in winter. The overall probability of survival was influenced by season and was shortest in summer and longest in spring and winter. Survival time increased as minimum daily temperatures decreased. These recent studies show a relatively short period of survival of *M. bovis* outside hosts and support a conclusion that environmental contamination of pasture, particularly in summer months, may be relatively unimportant in the epidemiology of tuberculosis in cattle, deer and possums. Separation of infected and susceptible animals by a fence generally provides a sufficient and practical protection against spread of the disease

The central issue of epidemiological interest is not only how long *M. bovis* survives, but how long surviving organisms remain infective (Morris et al. 1994). Organisms may persist for very long periods in protected and sheltered locations such as in soil, but by virtue of their location be either inaccessible to a host or only available for infection by an inefficient route. Large doses of organisms are required to successfully infect cattle by the alimentary route (McFadyean, 1910.) and in that species environmental contamination other than by aerosol is relatively unimportant for initiating infection.

Our studies of survival showed there was a relatively short period of survival of organisms outside hosts and support a conclusion that environmental contamination of pasture, particularly in summer months, may be relatively unimportant in the epidemiology of tuberculosis in cattle, deer and possums.

Morris *et al.* (1994) provided a modern perspective on routes of transmission which apply to herds which are regularly tested; "Regular tuberculin testing of cattle herds has changed the relative frequency of different methods of transmission. Some which were important in the past have declined to insignificance in countries which have active bovine tuberculosis control programs, either because they required very large challenge doses or because they usually occurred only late in the course of the disease when clinical signs were beginning to develop. Francis (1947) summarised data which showed that in the absence of effective control measures about 5% of tuberculous cows had tuberculous metritis, originating from peritonitis, the external genitalia, or most commonly from haematogenous spread. Tuberculosis of the penis was fairly common

in bulls. Only 1% of calves from tuberculous cows were congenitally infected. One to 2% of tuberculous cows had tuberculosis of the udder due to haematogenous spread. Alimentary infection of calves was commonly secondary to those cases of udder tuberculosis. Thus, as these other routes have declined under the influence of control programs, airborne infection has become even more dominant”.

In herds in which no routine control programs are practised, as is the case in some developing countries, after respiratory transmission, the drinking of infected milk by young animals is the next most common method by which tuberculosis is spread. Less common routes of infection include intrauterine infection at coitus, by the use of infected semen or of infected insemination or uterine pipettes, and intramammary infection by the use of contaminated teat siphons or by way of infected cups of milking machine. The feeding of tuberculous cattle carcasses to pigs has also caused a severe outbreak of the disease in a piggery. Unusual sources of infection are infected cats, goats or even human care takers (Blood and Radostits 1989).

Bovine tuberculosis occurs in every country of the world, and in the developed world has been given priority status for control because of disease risk to humans, and especially children, through the ingestion of infected milk and milk products. In countries with extensive pastoral cattle rearing, the infection is often restricted to sporadic cases. Where intensive indoor cattle rearing is practised and where a lot of animal movement occurs, prevalence of disease can reach almost 100% (Blaha 1990). In spite of the low overall incidence in countries where cattle are at pasture all the year round, individual herds with 60-70% morbidity may be encountered and the Australian experience showed that beef herds grazed under very extensive conditions commonly had prevalences in the order of 10 - 20%.. Among beef cattle, the degree of infection is usually much lower because of the open range conditions under which they are kept. However, individual beef herds may suffer a high morbidity if infected animals are introduced and large numbers of animals congregate about water holes, especially during dry seasons. It is difficult to assess the economic importance of the disease in cattle. Apart from actual deaths, it is estimated that infected animals lose 10 -25% of their productive efficiency.

Spread of tuberculosis from animal to man makes this an important zoonosis. Infection in man occurs largely through consumption of infected milk by children but spread can also occur by inhalation.

Susceptibility

Of the domestic animals, cattle and pigs are the most susceptible to either natural or artificial infection with *M. bovis* (Francis 1947). Cattle are also susceptible to infection with some other species of mycobacteria, such as *M. avium*.

Several workers have described localised infection with the avian type bacillus in the udder, pleura and peritoneum, the mesenteric, supramammary or deep inguinal lymph nodes, and the uterus (Francis 1947; Henning 1956). However, in most cases a strong positive test with tuberculin from *M. avium* is often the only evidence of infection. Stuart and Marshall (1952) reported that, though extensive localised lesions may occur in natural infections of cattle with avian tuberculosis, there was usually no tendency for it to become generalised.

The frequency of avian tuberculosis in cattle is probably related in part to the prevalence of the infection in other species, particularly domestic poultry (Karlson 1962). Infection with organisms of this group may however also originate from environmental sources

It is generally agreed that the pathogenicity for cattle of *M. tuberculosis* is less than that of the avian type. However, several cases of infection of cattle with the human type bacillus have been reported (Karlson 1960; Black 1972; Kleeberg, 1975). Hillermark (1946) and Waddington (1965) isolated *M. tuberculosis* from cattle in tuberculosis free herds which had developed a positive tuberculin reaction. Waddington (1965), working in Kenya, demonstrated temporary sensitisation in cattle due to infection with the human tubercle bacillus, and also a significant relationship between the proportion of the human population reacting to the Mantoux test and the occurrence of this temporary tuberculin sensitisation of cattle in the same area. In Finland, when bovine tuberculosis had been virtually eradicated, some cattle that had been exposed to human beings with open pulmonary tuberculosis became positive tuberculin reactors (Huhatala 1953; Westermarck, 1954), but providing that the source

of infection was removed, such reactions became negative in about 6-8 months. Fourie (1952) also attributed some positive tuberculin reactions in South African cattle, which had been negative for years, to infection contracted from man. Some authors have considered it improbable that cows infected with human tuberculosis could transmit the disease to man (Jensen 1953; Paterson 1956). However, even though the infection in cattle does not result in progressive tuberculosis, human type tubercle bacilli may remain alive for some time in the organs and tissues, and bacilli could conceivably be excreted from the udder without signs of infection or inflammation (Kleeberg 1975).

The condition in cattle known variously as "skin lesions", *dermatitis nodose*, acid fast lymphangitis or skin tuberculosis has been reviewed by Wessels (1948) and Karlson (1962). The lesions are seen most commonly on the limbs and shoulders, and they consist of a string of small abscesses following the course of one of the lymphatic vessels. Grossly and microscopically, the abscess are similar to tuberculous lesions and acid fast bacilli can be demonstrated in sections or smears. Attempts to isolate mycobacteria from these lesions are usually unsuccessful. Although Yachida *et al.* (1973) reported isolation of atypical mycobacteria from one of five skin lesions after several attempts. Cattle sensitised by skin infection generally have smaller reactions to mammalian than to avian tuberculin, and the sensitivity wanes after the lesions become inactive and calcified.

Pathogenesis

Certain conditions need to prevail for successful infection via the respiratory route to occur. Key research, which established theories of airborne infection for man and animals, was reviewed by Langmuir (1961) and O'Grady and Riley (1963). Both reviews drew attention to the critical particle size needed for successful infection to occur in lung. In man, during speaking, coughing and sneezing, droplets are expelled into the immediate vicinity and either settle or dry out to become droplet nuclei which can remain suspended in air for long periods of time. From experimental infection studies in rabbits, Wells *et al.* (1948) were able to show that droplet nuclei were able to reach the alveolar surfaces of the lung and they finally concluded that a few bacteria in droplet nuclei could infect, while far larger numbers of organisms in coarse particles were strained out in the upper respiratory passages and swallowed. This conclusion was supported by Lurie *et al.* (1950) who estimated that about three bacilli must be

inhaled to produce a pulmonary tubercle. Particle size is critical for airborne infection. Evidence reviewed by Sonkin (1951) showed that particles above $5\ \mu\text{m}$ in diameter are trapped in the nose and that those smaller than $0.1\ \mu\text{m}$ stay suspended in the alveolar air and are ultimately again removed with the expired air. The size of tubercle bacilli is about $0.5 \times 2\ \mu\text{m}$ (Schlossberg 1988). Mucosal surfaces of the respiratory and gastrointestinal systems are difficult to infect with tubercle bacilli, and very large numbers are required to do so.

If not caught in the bronchial tree, an inhaled tubercle bacillus reaches the alveoli and is ingested by an alveolar macrophage. In such macrophages the bacillus can be destroyed or inhibited or can multiply intracellularly. If the bacillus multiplies, the alveolar macrophage dies and its bacillary load is ingested by other alveolar macrophages emigrating from the pool of circulating monocytes. Both types of macrophages are attracted to the site by released bacilli, cellular debris, and a variety of chemotactic factors of host origin. In time, macrophages from the circulation become completely responsible for the fate of the early lesion. The alveolar macrophages rarely participate in the established lesion, because they remain on the periphery rather far from the bacilli, which are almost always located more centrally (Dannenberg 1989).

The tubercle bacillus is an obligate intracellular parasite of mesenchymal cells, from which it can penetrate into perivascular connective tissue or reticular tissue. It has no effect on skin and mucous membrane as long as the epithelial layer is intact. However, when taken up by neutrophils or macrophages it can pass through these membranes into the corium or subepithelial tissue and become active. In cattle, a visible primary focus develops within eight days and calcification within three weeks (Jubb and Kennedy 1970). The exudative inflammatory focus is soon demarcated by granulation tissue consisting of epithelioid cells and Langhans giant cells which are again completely surrounded by lymphocytes. Tubercle bacilli which have multiplied in the focus are carried by monocytes to the regional lymph nodes at a very early stage, where they evoke a similar reaction. Usually, processes of the same kind occur simultaneously in the organ of entry (e.g. lungs) and in related lymph nodes. This "primary complex" is most frequently located in the respiratory tract (90 to 95% of infected cattle) or the digestive tract, or less often in the skin, genital tract and

mammary gland. In congenital tuberculosis of the calf, it is presumed that infection occurs in the majority of cases by the umbilical cord and thereby the foetal circulation to the liver and portal lymph nodes where lesions develop.

The characteristic lymph node lesion is well known, and consists of enlargement of the gland with diffuse cellular infiltration and early caseation. It is not a nodular type of lesion. A characteristic post mortem finding is early caseation of portal, caudal mediastinal, and bronchial lymph nodes with isolated nodules in lung, liver, spleen, etc (Stamp and Willson 1946).

The fate of this focus or complex varies considerably between species and is influenced by the condition of the animal. It may resolve into the organised connective tissue form, or become encapsulated by fibrous tissue, remaining unchanged for months or years so that the animal continues to harbour live bacilli. *M. bovis* infection in cattle is considered to be always progressive, while *M. tuberculosis* infection in cattle can be contained and eliminated (Cohrs 1967).

Pathology

Lesions develop most commonly in the lungs, pleura, and local lymphatic nodes (Henning 1956; Kleeberg 1966; Cohrs 1967; Blood and Radostits 1989). The lesions may be single or multiple and may occur within a lobe, or in subpleural locations in the dorso-caudal portions of the diaphragmatic lobes. There are almost always lesions in the regional lymph nodes, but they may be absent in some cases of chronic tuberculous pneumonia.

Lesions present a characteristic histological appearance. There may be more than one focus within a lung lobule (giving a clover-leaf appearance) and more than one lobule may be involved. The appearance of these lesions varies with their age and rate of progress. The earliest lesions are not encapsulated, but are small and surrounded by condensed alveolar tissue. The infection frequently extends from superficial pulmonary lesions to the pleura, resulting in the formation of characteristic, soft, greyish - red outgrowths of various shapes and sizes. Cauliflower-like masses are formed by the confluence of these outgrowths, producing the condition known as "pearl disease". Later there are extensive thickening of the serous membranes and even

adhesions. Chronic tuberculous pericarditis may occur in the same manner. Tuberculosis of the heart is relatively uncommon in cattle; it is characterised by calcification (McKay 1959).

Tuberculosis of the alimentary tract in adult cattle, as in man, is usually an extension of pulmonary infection. In a consecutive series of 100 cattle with tuberculosis, McKay (1959) found 21 cases of tuberculous enteritis, of which 12 had acute generalised infection and two chronic generalised infection. Calves which were suckled by cows with tuberculous mastitis all had extensive infection of the mesenteric lymph nodes. When present, tubercles were yellow or irregular ulcers extending deeply into the intestinal wall.

McKay (1959) observed a 35% prevalence of tuberculosis of the liver among the cattle he examined. Lesions occurred on the liver capsule whenever diffuse peritonitis or miliary tuberculosis was present. Occasionally, tuberculosis of either the liver or portal lymph nodes was found without abdominal lesions other than in the mesenteric lymph nodes. Tuberculosis of the spleen is rare, and is usually a consequence of congenital infection (Jubb and Kennedy 1970).

Immunity

Viable lymphocytes are capable of conferring protection against tubercle bacilli (Mackness 1969) and other facultative intracellular parasites such as certain protozoa (Frenkel 1967) and viruses, provided that humoral antibodies are absent.

Domestic animals develop resistance and delayed hypersensitivity after natural or artificial exposure to tubercle bacilli, but it is not known how this influences the course of the disease, because neither the degree of resistance nor the hypersensitivity has been subjected to reliable evaluation. Tuberculosis in animals, although not always continuously progressive, is probably rarely completely overcome.

The subject of acquired immunity in tuberculosis is still highly controversial despite the use of BCG vaccine. It is known that animals infected with tubercle bacilli become more resistant to reinfection, and that animals vaccinated with viable cells of attenuated mycobacterial strains, especially when young, acquire a resistance to infection later in life (Fenner 1951; Rich 1951; Rosenthal 1957; Youmans 1957).

Actively acquired resistance is characterised by an increased capacity of macrophages to kill tubercle bacilli, and also by a greater ability of the cells to inhibit intracellular multiplication of the parasite. Such intracellular bacteriostasis is the major manifestation of acquired immunity to tuberculosis. Tubercle bacilli may remain viable in the body for years without multiplying (Youmans and Youmans, 1969). The mechanism whereby phagocytic cells of immunised animals are able to exert such control over intracellular tubercle bacilli remains largely unknown.

The primary bacteriostatic function of the immune response in tuberculosis differs from the mechanism mediated by antibody against a bacterial cell component. The fact that immunity to tuberculosis cannot be passively transferred by antibody from tuberculous or vaccinated animals supports the minor role of antibody in the immune process (Raffle 1955). However, acquired immunity to certain other facultative intracellular parasites, such as *Salmonella typhimurium* and *S. enteritidis*, may involve a cytophilic antibody (Rowley *et al.* 1964; Mitsuhashi *et al.* 1965; Kurashige *et al.* 1967). A similar agent might be responsible for the increase in resistance following the transfer of macrophages from immunised animals to normal animals. This success in passively transferring immunity to tuberculosis by macrophages suggests that such cells may be solely responsible (Sever 1960; Suter 1961; Millman 1961; Mackaness 1969). Large numbers of lysosomes accumulate in the cytoplasm of such cells and they are more actively phagocytic (Berthrong 1970). Thus, acquired immunity to tuberculosis might result merely from more efficient functioning of these "activated macrophages". Nonactivated macrophages apparently cannot kill these facultative intracellular organisms. Activated macrophages are rich in lysosomal enzymes and produce relatively large amounts of reactive oxygen intermediates and other microbiocidins (Dannenberg 1989).

Clinical signs

Clinical signs depend upon the organs involved and the extent of the lesions. Pulmonary infection gives rise to a dry cough which increases in frequency as the disease develops and is accompanied by loss of weight. Infection of the udder sometimes produces no definite clinical signs although the tubercular mastitis may be extensive. Similarly, tuberculosis of other organs may not give rise to diagnostically reliable signs (Buxton and Fraser 1977).

Because of the universal dependence on the tuberculin test for diagnosis and the policy of slaughtering all positive reactors whether they are open cases or not, few clinico-pathological tests are now carried out. Sputum or discharges may be examined by inoculation into guinea pigs but improved cultural techniques have now generally superseded animal injection tests and made them unnecessary.

The basis of all tuberculosis eradication schemes is the tuberculin test and a knowledge of the various tests used; their deficiencies and advantages is essential. Clinical examination is still of value particularly in seeking out the occasional advanced cases which do not give a positive reaction to a tuberculin test. Much attention is being directed to devising tests to detect such animals but the eye of an observant clinician can still be the most important factor in problem herds where positive reactors keep recurring (Blood and Radostits 1989).

Diagnosis

Microscopic examination

Diagnosis by this method depends upon the identification of *M. bovis* in suspected materials including lesions, sputum, milk, uterine discharges, pleural and peritoneal fluids, urine or faeces. The usual method is to prepare smears stain by the Ziehl-Neelsen (ZN) method and examine microscopically for tubercle bacilli. Films prepared from sputa may need to be examined extensively before observing a single tubercle bacillus, and a negative result does not necessarily preclude the possibility of infection.

Conversely, it should also be remembered that saprophytic acid-fast bacilli may be present in sputa and milk. Samples of milk, urine, uterine discharges, pleural and peritoneal fluids should be centrifuged and films prepared from the deposits, stained and examined. Tubercle bacilli often occur in clumps and several microscopic fields may have to be searched before one or more organisms are identified (Buxton and Fraser 1977). The overall sensitivity of ZN histology was 37% and the specificity was 95% in a retrospective study of cattle slaughter house data in New Zealand by Ryan (pers comm), and these findings cast serious doubts on the cost effectiveness of the procedure.

Animal inoculation

Suspected materials can be inoculated into the thighs of guineapigs to diagnose the presence of infection. It is preferable to inoculate two animals with each sample. Animals are killed 4 - 6 weeks after inoculation and examined for typical lesions of tuberculosis in the liver, spleen and lymphatic nodes draining the site of inoculation.

Typing of bovine, human and avian strains can be carried out by animal inoculation methods although the procedure has generally been replaced by other purely laboratory procedures which do not depend on animal inoculation. Rabbits usually die from generalised infection with the bovine type 4-5 weeks after intravenous injection but survive similar inoculations with the human type. Guinea-pigs are highly susceptible to both the bovine and human types. The reliability of this test depends upon the use of weighed doses of culture. The avian type is less virulent for rabbits and guinea-pigs and produces a more chronic form of disease unless inoculated in unusually large doses by the intravenous route. It does not cause the production of macroscopically visible tubercles, although the spleen and liver become enlarged and contain many tubercle bacilli which can be readily seen in stained smears. Chickens are not affected by the human or bovine types but will die after inoculation of most strains of the avian type (Buxton and Fraser 1977).

Laboratory animal inoculation for the primary isolation of *M. bovis* is no longer recommended. For contaminated specimens, where this procedure has been most widely employed in the past, culture methods are at least as sensitive as animal inoculation (Corner 1994).

Serological tests

The tuberculin test fails to detect some infected humans and cattle in the advanced stages of the disease and it has been reported that the antibody responses in such cases of advanced disease were elevated (Grange, 1984, Plackett *et al*, 1989). Therefore a serological test coupled with a cellular test should give the greatest degree of diagnostic accuracy.

Three major avenues of serological investigations have been:

- (1) to study the immunochemical constituents of mycobacteria

- (2) to explore the possibilities of serological differentiation between various mycobacteria.
- (3) to investigate the presence and nature of antibodies against mycobacterial antigens in
- (4) serum from tuberculous humans or animals.

These serological investigations mainly used the techniques of agglutination (Kuttler and Baisden 1962), complement fixation (deWitt *et al.* 1948) and precipitation tests on plates and in tubes. Lind (1960) tried the gel diffusion precipitin technique as a possible diagnostic aid for tuberculosis in cattle. Bennedsen (1969) examined the suitability of circulating antibodies in rabbits infected with *M. bovis* and *M. tuberculosis* for the fluorescent antibody test. Yugi and Nozaki (1972) compared the passive haemagglutination, haemolytic modification, kaolin agglutination and complement fixation tests in bovine tuberculosis. Cole *et al.* (1972) developed and evaluated a simple latex agglutination test for tuberculosis.

These tests have not proved to be very promising, for none is more specific than intradermal tuberculin tests. There was a lack of efficiency in detecting individual tuberculous animals and some tuberculosis-free herds were falsely classified as positive. Yugi and Nozaki (1972) claimed that the kaolin agglutination test gave accurate and consistent results in comparison with the other tests they used, though it was still less than 90% as specific as the tuberculin test.

The agglutination test is used for identifying "avian-3" mycobacteria (*M. avium* and Runyon Group 3), since neither cultural nor biochemical techniques are adequate to separate this group. Schafer (1965) introduced a scheme for recognition and classification of the group by agglutination with specific rabbit antisera. Schaefer's scheme was modified by Thoen *et al.* (1972). Known as the USDA system, it designates the different serotypes by Arabic numerals and uses only one species name, e.g. "*M. avium* complex serotype 2". This system has received world-wide acceptance (Pestana de Castro 1972; Thoen *et al.* 1975; Kleeberg 1975). No serotypes are known for *M. bovis* and *M. tuberculosis*.

Tests which rely on detection of a humoral response have proved disappointing for diagnosis of tuberculosis. The immune response to tuberculosis is largely cell mediated (Thorns and Morris 1983) with a minor humoral component. Agglutination, complement fixation, precipitation and anti-globulin tests have been investigated but have given poor results. Because enzyme linked immunosorbent assay (ELISA) has the ability to detect very low levels of circulating antibody, this method of testing has received some recent attention (Auer 1987; Plackett *et al* 1989; Fifis *et al* 1989; Wood *et al* 1991). Poor operating characteristics of all of these tests, either in relation to sensitivity, specificity or both preclude their use as primary tests, but they do have the ability to detect some anergic cattle and may have some future as a complement to a primary test. Circulating antibody may be more detectable in advanced cases of disease.

Lymphocyte transformation test (LTT)

The immunostimulation test is based on the ability of immunologically competent lymphocytes to recognise and interact with the specific antigen to which they are immune. This interaction causes characteristic change in lymphocyte form and function. The antigen used is specific PPD for the species of mycobacteria to be detected. The lymphocyte transformation is measured by the degree of uptake of tritiated thymidine. The test makes it possible to differentiate *M. bovis* from *M. paratuberculosis* and *M. avium* infections in cattle (Alhaji *et al.* 1974; Muscoplatt *et al.* 1975). The test has never become widely used, despite attempts to improve it since its inception in the early 1970s. The lymphocyte transformation test (LTT) for deer (Griffin and Cross 1986) uses an alternative method of assessing T-lymphocyte reactivity and has been accepted by MAF as a valid test for use in deer. A sensitivity of greater than 95% and a specificity of greater than 95% is claimed for the LTT (Griffin *et al* 1990). Improved values are claimed when an ELISA and inflammatory parameters are included with the LTT data. The combination of the 3 assays is termed the Blood Test for Tuberculosis (BTB).

Technical details of the assay system are described by Griffin and Cross (1989) and Griffin *et al* (1991) and the conduct of the test and indications for its use are explained by Griffin *et al* (1990). The BTB is claimed to be relatively insensitive to any

suppressive effects from prior injection of tuberculin and may be applied 14 days after an ID test.

The test was widely used after its introduction at a time when capital deer stock values were very high but its use has declined since then as deer prices have fallen. The BTB is labour intensive, technically demanding and expensive. The test takes about 6 days to complete

Gamma-interferon assay

In a tuberculous animal, T-lymphocytes are stimulated by contact with macrophages carrying *M.bovis* organisms to produce message carrying hormones which activate more macrophages. One of these hormones, gamma-interferon (IFN-g) was selected as worthy of further study for the development of a test because its basal level in the circulation is virtually nil, it is stable and its level rises rapidly as part of the CMI response.

The test involves collection of a heparinised blood sample which is incubated overnight with a small quantity of Tb antigen. Plasma from this sample is then assayed against an enzyme labelled monoclonal antibody to test for the presence of IFN-g and quantify its level.

Following evaluation in artificially infected cattle (Rothel *et al* 1990) and a limited trial involving cattle from infected herds (Wood *et al* 1992), the test was evaluated for final use in Australia in extensive trials (Wood *et al* 1991). The specificity was determined using more than 6000 cattle from tuberculosis free herds and depending on the cut-off point chosen, gave a specificity of 96.2% to 98.1%. Sensitivity varied from 76.8% to 93.6% depending on the method of interpretation. A maximum overall sensitivity of 95.2% was obtained by interpreting the IFN-g test and the CFT in parallel. In parallel test interpretation, a reaction to either test is interpreted as a positive test.

In further trials, Rothel *et al* (1992) determined that the heparinised blood samples should be stored at room temperature after collection and inoculated with antigen within 8 hours of collection. The ID test had no effect on the production of IFN-g in blood from uninfected animals but *M. bovis* infected animals showed a decline in IFN-g production. The authors recommended that animals should not be tested with IFN-g

assay for at least 59 days following an ID test. Blood was collected post-mortem to assess the value of the test application at slaughter. Blood collected 5 minutes after death from infected animals showed a reduction of greater than 50% of IFN-g production when compared with samples taken prior to slaughter.

The test has had some preliminary evaluation in New Zealand (Ryan *et al* 1991). This work indicated that the test may be a useful alternative to the CCT and a recommendation was made for further evaluation of it against the CCT. Test results can be available within 24 to 48 hours following blood sampling but the test is relatively expensive in terms of cost of reagents and laboratory use.

Tuberculin and the tuberculin test

Tuberculin tests have been used for the diagnosis of tuberculosis in cattle for more than 100 years and the test relies on a delayed-type hypersensitivity response which is maximal at about 72 hours post injection. The active principle of tuberculin is known to be a protein, with special properties, which is produced in culture medium supporting the growth of tubercle bacilli. Koch's "old tuberculin" (OT) was produced by growing tubercle bacilli on glycerol broth, removing the bacterial bodies, and reducing the volume to one-tenth by evaporation. The tuberculin was finally passed through a sterilising filter. However OT was a complex mixture which showed considerable variation between batches. Heat concentrated synthetic-medium tuberculin is produced in the same way but is about 22 times more potent (Dorset 1934). Asparagine is the source of nitrogen in the synthetic media used, consequently the tuberculin-protein is unmixed with other proteins, as in glycerol broth, and it can be precipitated and purified in various ways. It is then known as P.P.D. - purified protein derivative. Koch demonstrated the action of tuberculin in 1890 in the subcutaneous tuberculin test, in which 0.2-0.5 ml of OT was injected and the temperature reaction noted (Francis 1947).

The question of the relative specificity of tuberculin prepared from human or bovine strains of tubercle bacilli has not been settled. In 1962, Karlson observed that cattle were being tested with tuberculin made from the human tubercle bacilli in the United States, Great Britain and Australia, whereas in Canada, the Netherlands, Sweden, Denmark, and South Africa, tuberculin of *M. bovis* origin was being used. PPD tuberculins are the most widely used today but the use of heat concentrated synthetic

medium (HCSM) tuberculin is allowed in the European Community. Evidence based on field trials has increasingly favoured the use of *M. bovis* PPD for tuberculin testing in cattle. Francis *et al.* (1973) concluded that PPD tuberculin made from *M. bovis* gave considerably more specific results in cattle than human PPD tuberculin. This view was supported by Lesslie and Herbert (1975) in the Republic of Ireland, where tests were carried out on 510 cattle, 395 of which were shown by post-mortem examination to be tuberculous and 115 to be non-tuberculous. They concluded that, in the environment of the trial, bovine PPD was more specific for bovine tuberculosis than human PPD, and particularly so for differentiating "skin tuberculosis".

The use of bovine PPD for the tuberculin test in cattle has now become official in most countries.

The nature of the tuberculin reaction is still the subject of controversy. For many years the tuberculin reaction was looked upon as a unique immunological event, but it is now clear that several other types of immune reactions have the same basic mechanism. Thus it is possible to demonstrate "tuberculin-type" sensitivity to various other bacteria, fungi and viruses and even to purified protein antigen. A great number of organisms contain sensitising fractions similar to those contained by *M. bovis*, which can sensitise animals to mammalian tuberculin. These reactions are always smaller than the reaction to a similar dose of their own homologous tuberculin would be, but they can cause problems of interpretation when testing is carried out in the field using a single tuberculin. Although some other organisms cause tuberculin sensitivity, they usually fail to produce any lesions in the affected animal and at slaughter give rise to the no-visible lesion (N.V.L) reactor problem. Many organisms from outside the genus *Mycobacterium* have also been described as being able to sensitise animals or as possible sensitisers e.g. organism of the genera *Nocardia*, *Aspergillus*, *Trichopyton*, *Brucella* and *Actinomyces*. Liver fluke, hormonal influences and non specific infections, such as peritonitis and pleuritis, have also been suggested as possible causes. The most convincing evidence is however confined to the mycobacteria. (Worthington and Kleeberg 1965). In addition, contact sensitivity, rejection of solid vascularised tissue homeografts, the immune surveillance phenomenon and certain auto-allergic diseases result from immune responses of a similar type. The general terms currently used to describe this class of reactions are "cellular sensitivity" and

"delayed hypersensitivity", "cellular" because it is believed that the reactions are mediated by viable sensitized cells of the thymus derived or "T-cell" group of lymphocytes and "delayed because there is a lag period between contact of the sensitised host with an eliciting dose of antigen and the onset of the reaction. While the tuberculin reaction shares all the experimental properties of delayed hypersensitivity, it is complicated by additional factors. Antigen-antibody reactions elicited by protein antigens and attributable to endotoxin present in tuberculin can influence the basic mechanism under certain circumstances, but precise delineation of these components is difficult (Amason and Waksman 1964).

In the original test, tuberculin was injected subcutaneously and the temperature checked two hours later. A temperature rise indicated a positive reaction. Methods of testing local sensitivity include a number of skin tests, and ophthalmic, intrapalpebral, gingival and nasal tests. Of these, the intradermal test has been universally adopted for testing cattle.

The intradermal test was introduced by Moussu and Mantoux (1908), who found that the caudal folds (the folds of skin just below and on either side of the tail) were the most suitable site for injecting tuberculin and numerous trials were soon carried out to ascertain whether it was as reliable as the subcutaneous test. Moussu (1908) and Vallée *et al.*, (1909) found that the subcutaneous test caused some desensitisation to a subsequent intradermal test although this was not confirmed by Buxton and Glover (1939). In Vallée's trials the intradermal test was carried out just before the subcutaneous test. The tests agreed in 496 of the 521 animals but in 27 (5.2%) they differed, 12 of the animals being negative to the intradermal but positive to the subcutaneous test, and lesions were found in three of them. Fifteen were doubtful to the intradermal test and negative to the subcutaneous but a postmortem examination was carried out on only one of these animals and no lesions found. Vallée concluded that the intradermal test was much simpler to apply than the subcutaneous test and gave reliable results.

In a study in which the results were checked by post-mortem examination, Jowett (1914) found that the two tests agreed in 209 of 225 animals. In his hands the intradermal test was slightly more efficient than the subcutaneous test in detecting

tuberculosis. McFadyean and Sheather (1914) carried out simultaneous subcutaneous, ophthalmic and intradermal (caudal fold) tests on 50 animals, mostly calves, which had been artificially infected. All reacted to the subcutaneous test, whereas 16 failed to react to the ophthalmic and one to the intradermal test.

Ernest (1920) found lesions of tuberculosis in 6.75% of 965 animals which did not react to the subcutaneous test and 11.5% of 648 which did not react to the intradermal test. In another series, lesions were found in 96.12% of 9226 animals which reacted to the subcutaneous test and in 96.17% of 4171 animals which were positive to the intradermal test. Considering that at this period the various tuberculin were not strictly standardised, there is a remarkable similarity between the results obtained by the two tests. The intradermal test is simpler, takes less time to apply and uses less tuberculin than the subcutaneous test, and for these reasons it has been generally adopted.

Until 1947 the double intradermal test described by Buxton (1925) was widely used in some countries. In this test a second injection of tuberculin was made in the same site 48 hours after the first injection and the result read 24 hours later. A trial by the Ministry of Agriculture and Fisheries (1947) showed no advantage from the second injection. The single intradermal test then became the official method of tuberculin testing in Britain and elsewhere.

Single intradermal test (SID test)

This test is commonly applied by the intradermal injection of 0.1 ml of approved bovine tuberculin into centre of the caudal fold approximately 7 cm distal to the base of the tail. In New Zealand in cattle 1 mg/ml PPD (Wallaceville) is used for surveillance testing in tuberculosis free and accredited-free herds. In movement control herds, to increase the sensitivity of testing, 2 mg/ml tuberculin is used. The test site is assessed for the presence of induration or swelling, or the measurement of the reaction in millimetres, at 72 ± 6 hours following the injection. Animals are classified as positive or negative. In surveillance areas, in accredited-free herds, an increase in swelling of up to 4 mm (compared with the caudal fold on the other side) may be considered negative (this is called modified interpretation). Generally, however, standard interpretation is used; ie, any visible or palpable swelling is considered test-positive. In some countries an additional injection is made into the lip of the vulva at

the muco-cutaneous junction. The limitations of the test have been recognised for many years but the amount of published data to validate its performance is limited. This is primarily because experiments to establish the operating characteristics of the test are expensive and labour intensive, due to the requirement for slaughter and detailed postmortem examination of the sample of animals tested to confirm their true disease status. Despite the limitations of the test, it has enabled eradication of the disease or reduction to very low levels in countries where it has been used in national disease control programmes.

The skin of the neck area (the junction of the anterior and middle thirds of the neck) is more sensitive than that of the tail area (Francis *et al.* 1978; de Kantor *et al.* 1984) but the test suffers from the necessity to restrain each animal and measure all reactions carefully. Varying dose rates of tuberculin have been recommended and, with increasing demands for standardisation of the test to increase its accuracy, the exact dose for particular tuberculin should be strictly adhered to when the cervical skin test is used. In the U.S.A., 0.1 ml is recommended for herds of unknown status and 0.2 ml in known infected herds when cases with low sensitivity are to be carefully sought. A negative test result is recorded when there is an increase in skin thickness of less than 2 mm at the injection site with no clinical signs such as oedema, exudation, necrosis or pain. An inconclusive result is recorded if the increase in skin thickness is greater than 2 mm and less than 4 mm without any clinical signs. A positive reaction is recorded if there is an increase of 4mm or more in skin thickness with or without clinical signs. The method of injection of tuberculin also has more importance when the cervical site is used. A careful intradermal injection produces the largest swelling and a quick thrust the least. Variations in technique appear to have little effect on the size of reaction when the caudal fold is used.

The main disadvantage of the single intradermal test is its lack of specificity and the number of no-visible-lesion reactors (NVLs) which occur. Mammalian tuberculin is not sufficiently specific to differentiate between reactions due to infection with *M. bovis* and infection with *M. avium*, *M. tuberculosis* or *Nocardia farcinica*. The maximum permissible rate of NVLs is generally set at about 10% and when this rate is exceeded, tests other than the single intradermal test should be used. Other disadvantages of the single intradermal test include failure to detect cases of minimal

sensitivity which tend to occur in the early or late stages of the disease and in cows which have recently calved. This failure to detect tuberculous animals can be of considerable importance and must receive close attention when reactors are detected at an initial test. Serological tests to detect these cases of minimal sensitivity have been devised but are not sufficiently accurate for use in individual animals. The available tuberculin tests devised to overcome these deficiencies of the single intradermal test are short thermal, Stormont and comparative tests. The use of diluted tuberculin does not increase the specificity of the tuberculin test (Blood and Radostits 1989).

Short thermal test

Tuberculin (4 ml) is injected subcutaneously into the neck of cattle with a rectal temperature of not more than 39°C at the time of injection and for 2 hours later. If the temperature at 4, 6 and 8 hours after injection rises above 40°C, the animal is classed as a positive reactor. The temperature peak is usually at 6-8 hours. Preliminary evidence indicates a high efficiency of the test in detecting "spreader" cases giving negative intradermal tests. Occasional deaths due to anaphylaxis occur at the peak of the reaction, and there is one report of recumbency, which responded to intravenous therapy with calcium salts, in a large number of tuberculous cows submitted to this test. The test is seldom used nowadays.

Intravenous tuberculin test

The intravenous tuberculin test has been used experimentally (Kopecky *et al.* 1968).

In their experiment, the criteria for a positive reaction in tuberculous cattle were:

- a) a peak body temperature reached between 4 and 6 hours after injection
- b) a persistent elevated body temperature for 8 hours or longer exceeding 40°C
- c) a change in temperature exceeding 1.7°C

They concluded that the intravenous test (0.75 to 1.5 ml tuberculin) had two main uses:

- 1) to detect "anergic" cattle in a tuberculous dairy herd where frequent intradermal tuberculin skin testing had failed to detect all infected individuals
- 2) For a herd in which routine intradermal tuberculin testing had disclosed a large number of new reactors. Instead of sending all reactors to slaughter, the

intravenous tuberculin test may be conducted and only reactors to the test sent to slaughter

As with the short thermal test, the intravenous test is now seldom used.

Stormont test

This test has been devised to select those animals which are poorly sensitised for any reason and was used widely in Northern Ireland. The test is performed similarly to the single intradermal test in the neck with a further injection at the same site 7 days later.

An increase in skin thickness of 5 mm or more, 24 hours after this second injection is recorded as a positive result (Kerr *et al.* 1946). The increased sensitivity is thought to be due to the attraction of antibodies to the site by the first injection. The increased sensitivity begins at the fifth day, is at its peak at the seventh and ends by the twelfth day after the injection. Preliminary trials indicate very high efficiency in detecting the poorly sensitised animal but extensive field trials have not as yet been reported. Cattle infected with *M. avium* do not give a positive reaction but "skin tuberculosis" cases do.

A practical difficulty is the necessity for three visits to the farm. Special purified protein derivative (PPD) tuberculin of a specified potency must be used to fulfil the requirements of the test (Blood and Radostits 1989).

The comparative intradermal test

Where the presence of Johne's disease or avian tuberculosis is suspected or skin tuberculosis is apparent, non-specific sensitisation must be considered, and a comparative test used (Worthington and Kleeberg 1965). Transitory sensitisation may occur in cattle due to infection with *M. tuberculosis* but the comparative test will not distinguish that from bovine strain infection.

The comparative test depends on the greater sensitivity to homologous tuberculin. In Great Britain and Ireland, avian (0.1 ml, 2000 International Units) and mammalian (0.1 ml, 2000 Community Tuberculin Units) tuberculin are injected simultaneously into two separate sites on the same side of the neck between 12 cm and 12.5 cm apart, and the test is read 72 hours later. Care must be taken in placing the injection as sensitivity varies from place in the skin. A positive test result is recorded when the bovine reaction is more than 4 mm greater than the avian reaction or local clinical signs are present at the bovine site. An inconclusive result is recorded when the bovine

reaction is from 1-4 mm greater than the avian reaction and there are no clinical signs at the bovine site. A negative result is recorded when a negative bovine reaction, or a positive or inconclusive bovine reaction which is equal to or less than a positive or inconclusive avian reaction is recorded, in the absence of local clinical signs. The greater of the two reactions thus indicates the organism responsible for the sensitisation. The test is not generally intended for primary use in detecting reactors but is only to follow up known reactors to determine the infecting organism. However, the intradermal comparative test alone has been used successfully in the eradication programme in Great Britain. It was introduced in 1942 following validation in extensive trials by the British Ministry of Agriculture (Francis, 1947; Pritchard, 1988) and attestation was completed in Great Britain in 1960 (Pritchard, 1988). It was estimated that 6 to 12% of cattle in the United Kingdom would be classified as reactors to the SIT (Leslie *et al.*, 1975), so a comparative test is regarded as essential in those countries. Its use as a primary test is recommended when a high incidence of avian tuberculosis or Johne's disease is anticipated, or when vaccination against Johne's disease has been carried out. The comparative test is adequate to differentiate between vaccination against Johne's disease and tuberculosis and the distinction is easier the longer ago the vaccination was performed (Blood and Radostits 1989).

Special aspects of sensitivity to tuberculin

Site of injection

Sensitivity to tuberculin injected intradermally varies considerably from site to site on the body. In cattle the relative sensitivities of different areas to tuberculin and to johnin have been determined as follows: back 1, upper side 1.75 lower side 2.5, neck 2.75-3. The cervical area is also much more sensitive than the caudal fold, and has the advantages that reactions are more pronounced, animals can be retested immediately and the area is more sanitary. Its disadvantages are that restraint of each animal is necessary and the proportion of NVLs increases.

Potency of tuberculin

In the search for more specific and potent allergens, bovine and human types have been used to prepare tuberculin for comparison, the latter being more potent. However, for maximum specificity, tuberculin prepared from *M. bovis* is recommended, and for preference it should be a purified protein derivative (PPD). Purified protein derivatives

are now in general use because of their greater specificity and the greater ease with which they can be standardised. One of the important problems in tuberculin testing is deciding the optimum amount of tuberculin to be used to get maximum specificity. More accurate determination of the amount to be used could dispel many of the difficulties of non-specific reactions. A dose rate between 5000 and 10 000 tuberculin units (0.1 ml tuberculin containing 1 or 2 mg of bovine PPD) is considered to be most suitable.

Desensitisation during tuberculin testing

When a suspicious reaction is encountered, the question of when to retest is complicated by the phenomenon of desensitisation. When tuberculin is absorbed into the body, desensitisation occurs and its degree increase in general terms with the amount of tuberculin and the amount of other foreign proteins absorbed. Thus desensitisation is more marked and of longer duration after a subcutaneous than after an intradermal injection. A characteristic of the allergic reaction is the variation in differential white cell count of the blood which occurs. Polymorphonuclear cells increase and lymphocytes decrease and there is a suggestion that the greater the variation in cell count the longer the duration of desensitisation. After a single intradermal injection, the leucocytic reaction is of a minor degree and is not a very reliable guide to the diagnosis of tuberculosis. Moreover the period of desensitisation is short and the animal can be retested within a few days. However, after a Stormont test the leucocyte reaction is very marked in sensitive animals and is of diagnostic significance. Although the period of desensitisation after this test is not known definitely, it is of relatively long duration although not more than 6 months. The diagnostic value of this leucocyte reaction is vitiated to some extent by the variation in the time at which it occurs (6-24 hours) and by factors such as parturition, injection of adrenal cortical hormone and infective processes which produce a similar reaction.

A further aspect of the desensitisation phenomenon is that use can be made of it to obscure a positive reaction. If tuberculin is injected so that the test is made in the desensitised period, no reaction will occur in infected animals. Tuberculous cattle go through a period of desensitisation immediately before and after calving and as many as 30% give false negative reactions, returning to a positive status 4-6 weeks later. The loss of sensitivity is probably due to the removal of fixed cell antibodies from the

skin into the general circulation and subsequent drainage into the colostrum. Calves drinking this colostrum give positive reactions for up to 3 weeks after birth even though they are not infected (Blood and Radostits, 1989).

Non-specific sensitisation to tuberculin

The so-called no visible lesion reactor or NVL was recognised as soon as tuberculin testing was begun (Bang, 1892) and caused problems in Finland soon after 1910 (Stenius, 1938).

When an animal becomes infected with *M. bovis* the body becomes sensitised by certain fractions of the organism. The animal is then in an allergic state and an acute defensive reaction (Koch's reaction) will occur at the infection site if the animal is re-infected with *M. bovis*.

The genus *Mycobacterum* contains a large number of species including pathogens, free living saprophytes and potential pathogens. A great number of these organisms contain sensitising fractions similar to those contained by *M. bovis*, which can sensitise animals to mammalian tuberculin. They can cause problems of interpretation when testing is carried out in the field using a single tuberculin. Although these organisms cause tuberculin sensitivity they usually fail to produce any lesions in the affected animal (Karlson, 1962).

Judgement should not be made on each animal individually but only after the results of the whole herd have been considered. A few reactions in heifers can hardly be considered positive when there are no reactors amongst the older cows. Similarly if only a few reactions occur in a large herd, and in particular if the herd has been closed for some time, they should be considered with extreme caution. When any reactors are found unexpectedly in previously clean herds, a careful check should be made to find out whether there have been any new introductions or whether the cattle have been in contact with other cattle. While testing, a careful watch should be kept for the occurrence of "skin lesions" in the herd. It is a generally held belief that non-specific reactions are more common in heifers and steers than in other cattle.

The nature of the reaction as well as its size should be considered and recorded. The typical specific reaction is hot, painful and diffusely oedematous, whereas non-specific reactions are hard and circumscribed. It is unwise however to attach excessive value to the nature of the swelling as hard circumscribed reactions are sometimes found in tuberculous animals. If there is reason to suspect *M. tuberculosis* sensitisation then all persons having contact with the animals should be screened for active lung tuberculosis (Worthington and Kleeberg 1965).

Repeat testing is essential in both tuberculous herds and in problem herds. Tuberculous herds should be retested after 2-3 months as animals which were in a pre-allergic state at the first test will have converted to positive by this stage. Tuberculous animals having become positive will retain their sensitivity indefinitely, the only exception being the occasional animal which becomes de-sensitised when the disease is in an advanced state. In practice, it is considered that the completely anergic tuberculous animal is encountered only rarely.

In the case of non-specific sensitised animals, it has been said that they should be tested until they become negative. This statement, although somewhat cynical, contains a great deal of truth. At a retest of a non-specifically sensitised herd we generally find that some of the reactors have become negative, some have decreased reactions, some have similar or increased reactions and a number of new reactors may be found. The problem may continue in the herd for a long time, or the reactions may suddenly and inexplicably vanish. When dealing with problem herds, records should be kept of the injection site measurements of all reactor cows over all tests. Examination of these records may allow a non-specific sensitivity pattern to be more easily recognised (Worthington and Kleeberg, 1965).

Conclusions on tuberculosis tests drawn by 20 representatives of various countries and from the Food and Agricultural Organization and World Health Organisation who attended the second symposium on the eradication of bovine tuberculosis in Rome in 1960 were:

- 1) The intradermal technique continues to be the most satisfactory method for the application of the tuberculin test and should not be replaced.

- 2) A comparative test using mammalian and avian tuberculin is of great value in some countries for differentiating between specific and non-specific reactors.

However, there is still no universally accepted method for tuberculin testing, even for routine use (Lesslie and Hebert 1975). In the USA., the single caudal fold test is applied for routine testing and is followed by the single cervical comparative test on suspect reactors within seven days of the initial test (Roswurm and Konyha 1974). In the Netherlands, the single cervical test is routinely used, and suspicious reactors are subjected to the comparative test, using equal strengths of bovine and avian PPD (Huitema 1970). The Canadians also used the single caudal fold test for routine testing (Wells 1973).

The concentration of PPD tuberculin used is variable because of differences in production and standardisation from one country to another. A standard concentration for PPD tuberculin for use in cattle has been difficult to establish. Currently there is reasonable good agreement on the relative potencies of all PPD tuberculin in use. Most PPD tuberculin are used in the concentration of 0.5 mg/ml for avian PPD, 1 mg/ml for bovine PPD, and 2 mg/ml for human PPD. The dose for the intradermal test is 0.1 ml for each PPD (Lesslie and Herbert, 1975; Huitema, 1970; Kleeberg 1975). There are other types of tuberculin, but PPD is considered the best tuberculin because of its relative purity, specificity and ease of standardisation.

Immunity in tuberculosis

The waxy substance present in the wall of tubercle and other acid-fast bacilli serves to protect them against the antagonistic influences of the body. Even in naturally nonsusceptible species of animals, tubercle bacilli usually are destroyed only after a long time. In the sensitised animal a cellular immunity resides in the activated macrophages, enabling them to consume bacteria. Frequently the immune response in such species consists of a walling-off reaction in which the nidus of bacilli is surrounded by a dense wall of tissue that keeps the organisms more or less dormant (Dannenberg, 1989).

That a form of immunity does occur in the course of tubercle infection was first shown in the so-called phenomenon of Koch. It was observed by Koch that guinea pigs which were already infected and had developed low-grade tuberculosis reacted differently to a

second inoculation of a culture of high virulence to animals which had no primary infection. The animals that were already infected proved to be refractory to the second dose. Whereas the previously normal animal developed an acute progressively fatal disease, the previously infected developed only a swelling at the point of inoculation. This became a local abscess, which opened to the surface and sloughed away the necrotic tissue and the virulent bacilli without involvement of the neighbouring lymph nodes. Using a dose of virulent tubercle bacilli intravenously which produced acute, fatal, miliary tuberculosis in normal cattle, it was found that tuberculin-reacting cattle (infected) could not be killed (Calmette and Guérin, 1911). These animals showed an immediate reaction from which they rapidly recovered, and then they continued on their course of life as if nothing had happened. These studies showed that a new acute infection could be superimposed upon one already established and that a chronic state conferred protection from a more acute form.

These altered reactions are manifestations of a state of allergy. The relation of allergy to immunity is a question over which there has been great deal of controversy (Schlossberg, 1988). Some believe that allergy and immunity are identical while others consider that they are different properties with no relationship to one another. The allergic condition evidently has an important role to play in the course of disease. When small infective doses of tubercle bacilli reach individuals of the susceptible species that have not previously encountered infection (initial infection), there is a marked tendency of the tissue to wall off the infection and to reduce it to a latent state. In allergic individuals, on the other hand, there is a tendency for the tissues to react with an acute, inflammatory reaction when tubercle bacilli, escaping from a primary lesion, find themselves deposited in a new location. These lesions do not tend to heal but rather to spread and to destroy large amounts of tissue. Delayed hypersensitivity in tuberculosis is both beneficial and detrimental. In low concentrations, tuberculin stimulates the development of immunity in macrophages. Therefore, the presence of hypersensitivity is an asset in preventing pulmonary tuberculosis, for only small units of one to three bacilli reach the alveolar spaces where the infection begins. In high concentrations, tuberculin kills macrophages and thus is responsible for caseation and much of the tissue injury accompanying the disease (Dannenberg, 1989).

Artificial immunisation

Because of its great importance, it is probable that more attempts have been made to find immunisation methods for tuberculosis than for almost all other disease of man or animals. Unfortunately these methods have not met with any real measure of success. Ever since Koch claimed that tuberculin had curative or protective properties, workers have been interested in the possibility of immunising animals and man against tuberculosis. Virulent *M. tuberculosis* vaccines were first used to immunise cattle and they were employed on a fairly extensive scale. Eber (1907) concluded that they did not confer a degree of immunity on cattle sufficient to protect them against the natural risks of infection. It was soon shown by several workers, however, that human tubercle bacilli could be isolated from the internal organs and the milk of vaccinated cows, and the method fell into disuse.

McFadyean *et al.* (1913) confirmed the immunising value of human and avian tubercle bacilli injected intravenously. Immunity was tested by the subcutaneous inoculation of bovine bacilli and post mortem examinations were carried out at various intervals after immunisation. Both human and avian strains produced some immunity, but when the cattle were placed in order of ascending severity of the lesions and numbered from 1-29, the average number of those vaccinated with human tubercle bacilli was 7.9 and those with avian 17, indicating that the avian type was a considerably less potent immunising agent. These workers noted no ill effects following the intravenous inoculation of avian tubercle bacilli into calves but it is known that they may sometimes produce fatal disease (Bang, 1908).

BCG vaccine

The letters by which this vaccine is designated stand for the bacillus of Calmette and Guérin. The strain was originally isolated by Nocard from the milk of a cow (Griffith, 1932). The French workers cultured a bovine-type tubercle bacillus continuously on a bile-saturated medium for 13 years, during which the culture was renewed 70 times. Under these conditions it gradually changed in physical characteristics and lost virulence until, at the end of the period, it had completely lost its tuberculogenic properties. For many years this culture has been used as a vaccine on many kinds of animals and man. It has proved to be quite harmless, and it confers an appreciable,

but not absolute, resistance to tuberculosis on the part of individuals into which it has been injected.

To be effective, it must be given before virulent tubercle infection has occurred. This means that it must be given very early in life, in most instances. It can be given to very young calves in areas in which bovine tuberculosis is prevalent, to reduce their susceptibility to tuberculous infection.

The inoculation of BCG may give rise to positive reactions to the tuberculin test in cattle, and although it is a safe vaccine, the degree of immunity which develops subsequent to vaccination is variable and uncertain. For these reason it has not been generally accepted as a method for controlling tuberculosis in cattle (Buxton and Fraser, 1977).

The vole bacillus vaccine

The vole bacillus was isolated by Wells from voles or field mice in England in 1937. Wells and Brooke found that, although this organism had relatively low virulence for guinea pigs, it had appreciable immunising properties against both human and bovine types of tubercle bacilli. In comparison with BCG, they concluded that the vole bacillus gave the stronger protection. Griffith and Dalling (1940) found that the vole bacillus considerably enhanced the resistance of calves against inoculation with virulent bovine tubercle cultures, was manifested by the vaccinated calves living considerably longer than the control animals. Nine calves were vaccinated with the vole bacillus and there were two controls. Immunity was tested 71 to 175 days following vaccination. Both controls developed a moderate infection, five of the vaccinated had trivial lesions, and in four there were no macroscopic lesions. The authors concluded that the results were unexpectedly good and better than would be expected with BCG. All vaccinated guinea pigs, however, had living virulent tubercle bacilli in there tissues and their is little doubt that they would eventually have produced a progressive disease.

Chemotherapy

A large number of substances have been tried in the hope of finding a chemical agent that would destroy or restrain the development of tubercle bacilli in animal tissues and

that would at the same time be tolerated by the host. For many years there were no hopeful results but Rich and Follis (1951) reported that sulphanilamide inhibited the development of experimental tuberculosis in the guinea pig.

According to a report to the Council of Pharmacy and Chemistry of the American Medical Association, it appears that streptomycin, p-aminosalicylic acid (PAS), and isoniazid constitute the major chemotherapeutic agents used in the treatment of tuberculosis in man. To this may not be added the combination of rifampicin-isoniazid, which upon oral administration has proved effective and less toxic than the best regimen previously available. The use of terramycin is indicated when streptomycin resistance develops. Viomycin is limited in efficacy and is toxic. Amithiozone (tibione) is relatively ineffective. Pyrazinamide (aldinamide), although possessing immediate therapeutic effect, soon produces toxic manifestations in the patient. Synthesised modifications of isoniazid appear to be no more than secondary weapons-substitutes for streptomycin and isoniazid when tubercle bacilli become resistant to these drugs. Neomycin exerts too toxic an effect on the kidney and the auditory branch of the eighth cranial nerve. Mycomycin and erythromycin are ineffective in vivo. The report indicates that the treatment of tuberculosis has been advanced by the use of certain drugs, but much more effective ones must be found before we can rely on chemotherapy to cure tuberculosis. Multiple drug resistance to *M. tuberculosis* is increasing worldwide. Patients with records of prior treatment develop chronic disease or die more frequently than patients without prior therapy.

Much work has been done on the use of isoniazid in the prevention and treatment of bovine tuberculosis in South Africa (Kleeberg and Worthington 1963; Kleeberg 1966; Kleeberg *et al.* 1966). The results showed :

- 1) chemotherapy and chemoprophylaxis against bovine tuberculosis can replace vaccination or segregation in areas where the prevalence is high
- 2) the minimum dose rate of isoniazid was 10 mg/kg given in the feed once daily for at least seven months
- 3) farmers who co-operate in all respects can remove all reactors within three to five years, because cured cattle gradually lose tuberculin sensitivity
- 4) healthy cattle are capable of overcoming natural infection due to isoniazid-resistant, catalase-negative *M. bovis*

- 5) recognised advanced cases must be eliminated before treatment starts, but it is possible to retain 50-75% of infected cows for normal production of calves and milk
- 6) isoniazid is not toxic to ruminants at a dosage of 16-20 mg/kg. At higher dose rates, the toxic effects appear to be caused by its breakdown products
- 7) isoniazid is excreted in milk in small amounts, but it is broken down and rendered harmless by pasteurisation

Kleeberg and his colleagues concluded that a combination of chemoprophylaxis, chemotherapy and selective slaughter is feasible for eradication of bovine tuberculosis in certain economic and agricultural situations (Alhaji, 1976).

THE CONTROL OF BOVINE TUBERCULOSIS

The only rational approach for reducing and eliminating losses caused by the infection in herds and for preventing human cases due to *M. bovis* lies in the establishment of a control and eradication program of bovine tuberculosis. In man, prevention of *M. bovis* tuberculosis is based on pasteurisation of milk, vaccination with BCG, and, principally, control and eradication of bovine tuberculosis. In many countries, bovine tuberculosis has been virtually eradicated by reduction to insignificant levels, although complete eradication of *M. bovis* continues to be elusive. In the Western Hemisphere, Canada and the United States have reduced the infection rate to very low levels. In the United States, out of 4.5 million cattle examined in 1969, 0.06% reacted to tuberculin and no lesions were evident in the great majority of those reactors at post mortem inspection. The methods used have depended on a number of factors but ultimately the test and slaughter policy has been the only one by which effective eradication has been achieved.

Control on a herd basis

Effective control in a herd depends on removal of infected animals, prevention of spread of infection and avoidance of further introduction of the disease. All three points are equally important and neglect of one may result in a breakdown of the eradication programme.

Tuberculous cattle, even when affected with generalised or advanced disease are seldom clinically detectable with any degree of confidence, and identification of infected animals depends largely upon the use of a tuberculin test. The single intradermal test is the most widely used test although other tests should be employed when indicated. All animals over three months of age should be tested and positive reactors disposed of according to local legislation. At the initial test, a careful scrutiny for clinical cases which may be anergic is worthwhile while suspicious reactors and any animals likely to have reduced sensitivity, in particular old cows and those calved within the previous 6 weeks, may be tested by one of the special tests described above or retested subsequently. The comparative test should be used where widespread infection with *M. paratuberculosis* or *M. avium* is anticipated or where a high incidence of reactors occurs in a herd not showing clinical or post mortem evidence of the disease (Worthington, 1965).

If the prevalence of reactors is high at the first test, or if open lesions are found at necropsy in culled animals, emphasis must be placed on repeat testing at short intervals to give rapid containment of the disease. Tests may be conducted at 2-monthly intervals in such cases. Lower prevalence herds may be retested at 3-monthly intervals until a negative test is obtained. A further test is normally conducted 6 months later, and if the herd is again negative, it may be classed as free of the disease. Subsequent whole herd tests should be carried out annually.

Sanitary measures to prevent the spread of the infection should be instituted as soon as the first group of reactors is removed. Although the risk of infection from fomites is low, when high prevalence herds are identified feed troughs should be cleaned and thoroughly disinfected with hot 5% phenol or equivalent cresol disinfectant. Similarly, water troughs and drinking cups should be emptied and disinfected. Suspicious reactors being held for retesting should be isolated from the remainder of the herd. If a number of reactors are culled, attention must be given to the possibility of infection being reintroduced with replacements which should ideally only come from accredited herds. Failing this, newly introduced animals should be tested before or at entry, isolated and retested in 60 days (Blood and Radostits, 1989).

The following measures are recommended for the rearing of tuberculosis-free calves from an infected herd.

- 1) segregation of calves from cows
- 2) isolating new born calves into a separate calf shed immediately after their birth and avoiding close contact with the dam after birth. "Dry-licking" of new born calves by cows should be avoided. Heat treated milk may fed to the calf apart from the first offering of colostrum.
- 3) Calves should be tested at weaning and thereafter at intervals of 4 to 6 months

Replacement stock should be similarly kept separate from the infected main herd. Although it is sometimes recommended, a two herd system with separate operation of two cow-sheds of infected and tuberculosis-free cows is extremely difficult to maintain in practice. There is continued danger of a breakdown due to the possible transmission of disease into the tuberculosis free unit. For that reason, the period of operation of any two herd system should be as short as possible, or avoided by buying animals officially free from bovine tuberculosis. A cost benefit analysis is useful to test alternative strategies when it is unclear as to which method should be adopted.

Complete replacement of a heavily infected herd after careful sanitary measures has a high initial cost but is reliable and effective for small herds. Under some circumstances in developing countries where there are large herds with several subunits, the separate rearing of calves and young breeders and the separate management of tuberculosis-free and positive herds is an effective means of eradication. In such cases, for the protection of humans and the minimisation of the danger of infection in tuberculosis-free populations or subunits, clinically diseased animals, particularly those with udder tuberculosis, must be slaughtered (Blaha, 1990).

Attendants should be checked as they may provide a source of *M. tuberculosis* infection resulting in transient positive reactions in the cattle. Humans may also act as a source of *M. bovis* infection.

Steps should be taken to ensure that reinfection does not result from communal use of watering facilities or pasture, or from inadequate boundary fences. A special problem is created when tuberculosis occurs in cattle run on extensive range country with little

manpower and few fences. It is inadvisable to attempt a control programme until it can be guaranteed that all animals can be gathered, identified, tested and segregated. In some areas it is uneconomic for industry alone to do this (Blood and Radostits, 1989).

Control on an area basis.

The method used to eradicate bovine tuberculosis from large areas will depend on the incidence of the disease, methods of husbandry, attitude of the farming community and the economic capacity of industry and government to stand losses from a test and slaughter programme. Low intensity of management and lack of control of animals were serious constraints to tuberculosis control in the remote and extensive areas of central and northern Australia. Some properties were practising little more than feral and semi-feral cattle harvesting operations. Financial assistance and taxation concessions allowed adequate and updated cattle handling facilities and abattoirs to be built and resulted in a successful control program and a greatly improved cattle industry..

An essential first step in the inauguration of an eradication programme is the prior education of the farming community. Livestock owners must be appraised of the economic and public health significance of the disease, its manifestations and the necessity for the various steps in the eradication programme. Eradication should be compulsory since voluntary schemes, although useful in the initial stages of a national campaign, have never achieved more than limited control and always leave foci of infection. Adequate compensation encourages full co-operation. This may take the form of compensation for animals destroyed or financial incentives for achieving disease-free status or higher payments for milk or beef products.

It is essential at the beginning of a programme to determine the prevalence and distribution of the disease by tuberculin testing samples of the cattle population and monitoring disease levels at meat inspection. Such information helps to identify herds and areas which are disease free or which have a low prevalence. The disease can first be eradicated from these areas, and thus provide a nucleus of tuberculosis-free cattle which can supply replacements for higher prevalence areas as they are brought into the eradication scheme

When the prevalence of tuberculosis is high a routine test and slaughter programme may be economically impossible. Two-herd schemes have been used in Europe and elsewhere but are now of historical interest only.

Ostertag's method

Ostertag believed that the spread of tuberculosis, and therefore its incidence, could be greatly decreased by the detection and slaughter of animals with open tuberculosis as the first step in a comprehensive plan for its complete eradication (Edwards 1942). He advocated regular examination of the herd, identification and slaughter of clinical cases and at the same time removal of the calves at birth and feeding them on either the milk of sound cows or pasteurised milk from infected animals. In a trial in Germany, the number of open cases was only reduced from 20% in the first year to 15% in the third year and the method was totally ineffective. Almost all tuberculous cattle have lesions in the lung, and as discussed in the section on pathogenesis, lung lesions rarely heal and consequently nearly all tuberculous animals are infective. Bang's method utilised the tuberculin test to separate the infected animals into two herds. The most attractive alternative to an immediate test and slaughter programme is gradually to free farms and regions and allow reactors to go into other farms or regions so that fewer cattle are continually being exposed to infection. The infected animals live out their economic lives before they are discarded. It has the advantage of low cost to the farmers and the State and was satisfactory, provided measures were taken to reduce contact and milk-borne spread to man. Whichever preparatory method of reducing an initial high incidence of the disease is used, compulsory testing of residual units is advised when 70-90% of herds are free. In Denmark the general preparatory approach was along the lines proposed by Bang, and the disease has now been eradicated from the country.

Vaccination was used to a limited extent to reduce the ravage of tuberculosis (Burner and Gillespie 1973), particularly when an eradication programme could not be instituted for some time, but it was desired to reduce the incidence of the disease in preparation for eradication. BCG vaccine is the only method available for field use, the vole acid-fast vaccine varying too much in virulence. BCG vaccine has many disadvantages. Vaccination is carried out by the subcutaneous injection of 50-100 ml vaccine and large and unsightly lumps appear at the injection site. Injection by the alternative intravenous route is attended by risk of several systemic reactions.

Vaccination must be repeated annually and the vaccinates remain positive to the tuberculin test. Calves should be vaccinated as soon after birth as possible and do not achieve immunity for 6 weeks. The immunity is not strong and vaccinated animals must not be submitted to severe exposure. In field circumstances where the disease is prevalent, only modest results can be expected. Francis (1947, 1958) reviewed the controlled field trials conducted with BCG and concluded that while it may produce a short lived increased resistance, it has not contributed to the control of bovine tuberculosis. In 1959, the WHO/FAO recommended that BCG vaccination of cattle be stopped.

When overall incidence of tuberculosis is 5% or less, compulsory testing and the slaughter of reactors is the only satisfactory method of eradication. A combination of lines of attack is usually employed. Accredited areas are set up by legislation, and all cattle within these areas are tested and reactors removed. Voluntary accreditation of individual herds is encouraged outside these areas. In some countries, focal points of extensive infection outside accredited areas have been addressed using special legislation. The commonly adopted strategy of encouraging herd accreditation and then introducing compulsory eradication has regularly resulted in the virtual eradication of the disease. When an area or country has been freed from the disease, quarantine barriers must be set up to avoid its reintroduction. Within the region, the recurrent cost of testing can be lessened by gradually increasing the inter-test period to 2 and then to 3 or even 6 years as the amount of residual infection diminishes. Meat inspection services provide an effective monitor should any increase in incidence of the disease occur. Amongst range beef cattle it is usual to check samples of animals at intervals rather than the entire cattle population (Blood and Radostits 1989).

Eradication

Few countries have really achieved complete eradication of tuberculosis. A number of problems arise in the final stage of an eradication programme. The most formidable is the steep rise in the rate of "non-specific" reactors, and this creates administrative and public relations difficulties (Ranney, 1964, 1965). Occasionally, individual herds which have been accredited after a number of free tests are found to have the disease again, often with a very high incidence. Another major problem is that of trace-back of infected animals at packing plants to their herds of origin. Even with major effort it is

often impossible to determine the origin of affected carcasses. There is as yet no highly reliable test to detect poorly sensitised animals in the early or late stages of the disease which are the common cause of recrudescence in herds which have been classified as being free of the disease. Trace-back becomes the principal source of information on the location of infected herds in the final stages of a programme and a major advance would be a suitable method of identifying individual animals which could be utilised on the killing floor.

Public health aspects

Tuberculosis is a major disease problem in the human population of the world, and man is susceptible to infection with human, bovine and avian types of tubercle bacilli. For this reason, the control of tuberculosis in animals forms an integral and essential part of the control of the disease in man, because there are many environmental conditions in which man may derive infection from contact with his animals. One important route is by ingestion of milk infected with mycobacteria. The risk of this kind of infection occurring can be removed by pasteurisation of milk supplies. There is also evidence to show that animals, particularly domestic pets, may contract infection from human sources. The risk of infection are considerable for those who handle and examine infected animals (Buxton and Fraser 1977).

People suffering from *M. bovis* tuberculosis can infect cattle and proven cases of transmission of bovine tubercle bacilli from man to cattle have been reported (Lesslie 1968; Lepper and Corner 1983). Although transmission is often via the respiratory route, a number of cases of cross-infection resulted from attendants urinating on the hay, a common practice in some countries which is said to provide a source of salt for the cattle. Huitema (1969) reported 50 examples of herds infected from human sources and in 24 cases the responsible individuals had renal tuberculosis. It is thought that in regions where bovine tuberculosis has been eradicated, cattle cease being a source for human infection, but man may continue for many years to be a potential source of infection for cattle.

Persons with pulmonary or genitourinary tuberculosis due to *M. tuberculosis* can temporally infect and sensitise cattle. Cattle are very resistant to *M. tuberculosis* and infection does not cause a progressive tuberculosis, but the bacillus can survive for

some time in animal tissues, especially in the lymph nodes, thus sensitising the animal to mammalian tuberculin and complicating interpretation of the diagnostic test (Worthington 1965). Sensitisation can persist for some 6-8 months after the human source of infection is removed.

POSSUMS AND OTHER FERAL ANIMALS AS SOURCE OF TUBERCULOSIS

Cattle were introduced into New Zealand about the time of European settlement and *M. bovis* was undoubtedly introduced concurrently, as happened in many countries (Francis 1958). Tuberculosis was formally recognised as a problem in New Zealand dairy herds in the 1950s and a test and slaughter program was developed to rid dairy herds of *M. bovis* to safeguard market access for dairy products. Testing of factory supply herds began in 1958 (Jamieson 1960) and revealed baseline reactor rates (cattle showing an immune response to tuberculin) of 10.7%. Through repeated annual testing, most individual and regional herds were quickly cleared of *M. bovis*. By 1986, only about 2% (935 herds) of herds of the national total were infected. Of all cattle tested, only 0.05% showed a reaction to tuberculin and were subsequently sent to slaughter (Coleman 1988).

Tuberculosis was first recorded in Australian brushtail possums (*Trichosurus vulpecula*) caught by a trapper on a farm at the mouth of the Mokihinui River, Buller County, in September 1967 (Ekdahl *et al.* 1970). Twenty of the 25 possums which he submitted for examination showed gross lesions of tuberculosis. Attention was further focused on the possible role of the possum when, in 1970, there was a serious outbreak of tuberculosis in a dairy herd at Seddonville, just a few miles upstream from the original discovery of tuberculous possums.

The area in which this outbreak occurred supported a large possum population and was bounded by bush on three sides and by the river on the fourth. A post-mortem survey of possums in the vicinity revealed 12% prevalence of the disease. The isolation of these cattle by physical boundaries from other cattle and the presence of a significant level of disease in possums suggested an hypothesis that possums were

acting as vectors of the disease. Up to this time, it had been considered that cattle contracted the disease from tuberculous in-contact cattle.

The situation faced on the West Coast of the South Island in the early 1970s had presented a new problem to the Ministry of Agriculture and Fisheries. Close examination of testing records for the region revealed unexpected difficulties being experienced in the testing program which could only be explained by the presence of a source of herd re-infection other than cattle. Conversely, and in line with predictions and expectations, many areas of New Zealand were experiencing no difficulties with eradication.

Unfortunately the problem of herd breakdowns was not long confined to the West Coast region. Other regions followed. The northern region of the West Coast was the next area to report tuberculosis in possums, while in 1969, the Wairarapa also identified possums as a factor in intractable herd problems. The Western Bays of Lake Taupo, Central North Island followed in 1972 (Batcheler and Cowan 1988). The situation continued to deteriorate after the primary recognition of the particular problems in these three regions. Other areas in which the disease was initially considered eradicated from cattle have subsequently joined the list of endemic areas. In 1991, there were 19 defined areas wherein tuberculous possums have been found (Livingstone 1991). Since that report, two additional endemic areas were notified, at Waipawa in Hawkes Bay and Otaki in the Manawatu, while at least two other areas were placed under investigation.

Ekdahl *et al.* (1970) and Smith (1972) have reported on the nature of the disease in possums and Smith drew attention in particular to the large numbers of *M. bovis* organisms which were present in lesions in affected animals. Some experimental infection studies were made by O'Hara *et al.* (1976) in New Zealand and Corner and Presidente (1981) in Australia, which indicated rapid progression of disease and a presumably limited host response to counteract the infection, leading to the presence of large numbers of organisms in lesions. In summary, these early studies showed the possum to be a susceptible host with a capability of producing large numbers of *M. bovis* organisms, thus having the potential to be a potent vector of the disease.

There has been a gradual accumulation of evidence during the past 25 years incriminating the possum as the most important source of *M. bovis* infections in cattle and the possum is now considered to be a reservoir host of the disease (Morris *et al.* 1994). Possum control reduces the incidence of tuberculosis in cattle and the species is considered a major source of on-farm infection (Animal Health Division 1986). Eradication of tuberculosis in cattle by control of local possum populations in conjunction with test and slaughter began in 1972, and continues up to the present as an integral part of disease control.

Deer farming was legalised in New Zealand in 1969. *M. bovis* tuberculosis also occurs in farmed deer (mainly *Cervus elaphus*) in New Zealand in areas with tuberculous possums and the eradication program was extended voluntarily to include farmed deer in 1985. Of 179,000 deer tested in 1985, 0.6% were tuberculous (Animal Health Division 1986). The economic value of deer herds as well as of cattle depends on the eradication of tuberculosis.

The greater problem which has emerged in recent years has been the existence of wildlife reservoirs of infection, which are self-maintaining and can also continuously transfer infection to domestic livestock. *M. bovis* can infect quite a variety of other domestic and wild species in addition to man. Numerous wild and feral species have been recorded as hosts for the organism (Allen, 1991), although many of them are probably almost entirely dead-end or spillover hosts as far as transmission of infection back to cattle is concerned. Those known to be of epidemiological importance as reservoir hosts are badgers (*Meles meles*) in Great Britain and brushtail possums in New Zealand. More recently, attention has been drawn to the possible role of tuberculous ferrets (*Mustelo furo*) in transmitting tuberculosis to cattle and the question of whether they are a true reservoir host or a spillover host (Morris *et al.* 1994)

The Australian brush-tailed possum was introduced into New Zealand for its fur potential on many separate occasions from about 1840 until 1920 and it now occupies both mainland and some offshore islands (Pracy, 1974). Possums had spread across half the country by 1950, and occupied over 90% by 1990. Numbers increased dramatically for 3 to 4 decades after each new area was colonised, but by the 1980s

the population was more stable and the total population is now estimated at 60-70 million (Batcheler and Cowan 1988). Naturally acquired *M. bovis* infection in wild brush-tailed possums was first reported in New Zealand in 1970 (Ekdahl *et al.* 1970) and over the next 10 years there was a considerable build up of circumstantial evidence to link tuberculosis in possums to persistent high reactor rates in cattle in some areas of New Zealand (Julian 1981).

In New Zealand, Tb possums are considered the major wildlife reservoir of infection for cattle and farmed deer. Regions are classified as endemic on finding tuberculous feral or wild animals, or their presence based on epidemiological evidence from cattle and deer testing. Although it is possible to remove tuberculosis from infected herds in endemic areas using the conventional test and slaughter programme, reinfection due to contact with tuberculous possums causes sporadic herd breakdowns. However, it has been observed on a number of occasions at different locations that there is decrease in the incidence of infected cattle and herd re-infections following local possum control operations which successfully reduce possum numbers in the immediate area (Livingstone 1991; Pannett 1991; Hoyle 1991).

The evidence incriminating possums as reservoir hosts of *M. bovis* for infections in cattle was recently summarized by Jackson (1995). "Restriction endonuclease typing of *M. bovis* isolates (Collins *et al.* 1986; de Lisle *et al.* 1990) has shown the restriction types of *M. bovis* found in possums to be the same as those found in cattle and deer in the same locality. This is consistent with a cycle of infection between those species and adds further weight to the possum vector theory.

For some time it was questioned whether tuberculosis is self-sustaining in possum populations or whether infected cattle are needed to maintain the disease by re-infecting possums. It is now clear that possums living in the ecological circumstances applying in New Zealand, where they occur in great numbers, have attained reservoir status (Morris *et al.* 1994)

A longitudinal study of naturally occurring tuberculosis in possums in the wild at Castlepoint (Pfeiffer 1994) produced strong evidence to support transmission of infection from mother to offspring and also indicated that transmission between adults

was largely confined to local social groupings, covering as little as 2 to 4 ha of densities. These latter findings point to transmission pathways capable of maintaining the disease in possums independently of cattle.

Some regions of England and Ireland experience a problem with intractable tuberculosis in cattle which is very similar to the New Zealand problem (Nolan and Wilesmith 1994). It was realized that a problem existed towards the end of the 1960s, and in 1971 an infected badger was found on a farm in Gloucestershire which had recently experienced an episode of tuberculosis (Muirhead *et al.* 1974). Following these findings, a strong web of circumstantial and research evidence was gathered, which implicated the badger as a wildlife reservoir of infection. The most conclusive evidence came after two problem areas were depopulated of badgers. This step proved to be successful in preventing re-infection of cattle (MAFF 1979; Little *et al.* 1982). The existence of this proven wildlife reservoir in analogous circumstances to brushtail possums adds plausibility to the possum reservoir theory.

Tuberculosis caused by *M. bovis* occurs in an exceptionally wide variety of warm blooded animals (Francis 1958; Lepper and Corner 1983; Thoen *et al.* 1984; Pritchard 1988) but none of these species which occur in New Zealand is as abundant as possums, nor does any show the consistent overall prevalences of tuberculosis which are found in possums. No other species presents a similar threat in terms of numbers of infected animals capable of transmitting the infection.

In summary, the evidence implicating the possum relies on circumstantial evidence from a variety of sources, viz.

- a) the disease occurs in cattle in contact with tuberculous possums
- b) possums are susceptible hosts capable of producing large numbers of organisms, qualities which are consistent with the concept of their being a successful reservoir
- c) cattle in STCAs are at a higher risk than cattle in surveillance areas
- d) tuberculosis in possum populations is self-sustaining
- e) analogy with badgers
- f) weight of numbers of infected possums, allowing many transmission opportunities

It may be argued that this evidence is not fully conclusive despite its compelling nature. The argument would be strengthened if the tuberculosis status of possums in non-

endemic areas was known and if total removal of infected possums from a region would prevent the recurrence of the disease in cattle. There would be considerable difficulties in obtaining comprehensive evidence regarding the tuberculosis status of possums in non-endemic areas and such an exercise would be an expensive undertaking.”

Tuberculosis in possums

Multiple gross lesions occur commonly in tuberculous possums and recent studies have shown on average about 5 separate gross lesion sites (range 0 to 28) in infected possums (Jackson et al. 1995). The typical form which the diseases assumes in affected animals involves establishment of infection at one or more initial sites, followed by rapid extension to other body sites, probably initially by lymphatic spread but also by the haematogenous route. It would seem that spread to multiple sites commonly occurs before lesions become visible at any body site, as significant numbers of necropsy-negative animals have multiple lesion sites and single site lesions appear to be rare. Tuberculosis in possums is thus a disseminated disease, with a poor host response, often producing small histological lesions in multiple tissues and organs.

It appears that progression of the disease is imperceptible or slow initially but as the number of grossly visible lesions grows, the disease switches at some point from a relatively benign form to a rapidly progressive form and the animal becomes terminally ill. Growth, general body condition and behaviour appear to be unaffected until the terminally ill stage of 1 to 2 months, but for most of the course of the disease, animals are potentially infectious by the respiratory route from open lung lesions established in the early stages of the disease.

Lung lesions are found in 85% of tuberculous possums and axillary and inguinal lymphocentres (which are directly connected in possums by an inguino-axillary trunk) are affected in about 75% of infected animals. In about 95% of all infected animals lesions will be found in either lung or inguinal or axillary lymphocentres. Typical gross lesions are spherical and contain lime-green caseous pus. Infected lymph nodes are enlarged (up to 20 mm in diameter), and occasionally discharge via sinuses to the exterior.

Transmission of the disease between possums

The available evidence suggests that transmission of the disease between possums occurs through two major and one minor pathway (Morris and Pfeiffer 1995). The first major pathway is from mother to offspring during the long rearing process. This may either occur through milk since 12% of tuberculous females had mammary gland infections (Jackson *et al.* 1995) but pathological evidence suggests that it is more likely to be via the respiratory route. This maternal offspring transfer is termed "pseudo-vertical" transmission and a high proportion of the progeny of infected females become infected early in life.

The second major transmission mechanism is direct horizontal transmission between adult possums and the evidence from a long term observational study of a tuberculous possum population in the wild at Castlepoint (Pfeiffer and Morris 1991) strongly suggests that this takes place mainly in the area around where a possum dens. It is thought that horizontal transmission most likely occurs during agonistic behaviour between males particularly through the close contact which occurs at mating, which is frequently polygonous. Den-sharing between adults may also contribute to transmission opportunities but this specific behaviour is not constant among possum populations.

Indirect transmission among mature possums is thought to be only a minor pathway which may take place during sequential den-sharing and through contamination of shared environmental areas, such as tracks, marking sites and feeding grounds.

Spatial distribution of tuberculous possums

In a recent report, Coleman (1988) recorded the highest population density of possums at forest pasture margins. Densities within the forest, indicated by poison catch, followed patterns established for Westland mixed-hardwood forests and were greatest in low-altitude forests adjacent to pasture. Considerably lower densities were found in low-altitude podocarp forest distant from pasture, while medium densities occurred in mid-altitude hardwood forest, and low densities in high-altitude hardwood forest.

Infected possum were also distributed unevenly throughout the habitat. Numbers were highest amongst those foraging on pasture, and lowest in the most remote forest

sampled. High prevalences were thus generally recorded from areas of high possum density. In the Castlepoint (Pfeiffer *et al.* 1995) and other studies (Hickling 1991), distinct localised foci or clusters of tuberculous possums have been identified and these spatial clusters have been observed to persist over extended periods of time. The clusters have become known colloquially as “hot spots”. The best explanation for the formation of clusters is that they start from the arrival of a pseudo-vertically infected juvenile which has dispersed from an existing focus of infection. Most dispersers are juvenile and young males, and they frequently disperse over long distances, usually 2 to 5 km, before establishing their own home range. Horizontal transmission may then establish infection in possums sharing that home range. Maintenance of a focus probably depends on successful pseudo-vertical transmission to female offspring whose normal behaviour is to settle within or close to their natal area. The transmission rate must then exceed a threshold value for the focus to persist over time or be re-seeded from other foci by other dispersers, otherwise it will decline and disappear.

Transmission from possums to other animals

Farmed cattle and deer

Many pastoral areas in New Zealand are bounded by bush margins and many areas of otherwise developed land contain patches of scrub which provide favourable denning sites for possums. In much of the rougher country, there are no fences to prevent cattle wandering into bush areas and indeed it is a deliberate management practice in many parts of the country to graze cattle in bush or scrub areas during winter.

Possums are arboreal folivores but frequently graze on clover and pasture weeds and also some forage crops grown for sheep and cattle feed. Considerable numbers of possums can emerge each night from nearby den sites and traverse and feed on cattle pastures. Although once a popular theory, it is not now considered that pasture contamination by infected possums with *M. bovis* is significant in the epidemiology of the disease. Possums in the final stage of disease act in a disorientated manner and tend to wander about on pasture thereby attracting the attention of cattle and deer. During the vigorous investigation that follows there is ample opportunity for transmission of disease. Behavioural studies of interactions between sedated possums and domestic stock (Sauter and Morris 1995 and Paterson and Morris 1995) support this theory and showed that at other times the species avoid close contact.

Flesh eating animals

Possum carcasses are readily scavenged by feral pigs (*Sus scrofa*), ferrets (*Mustola fera*), cats and stoats weasels and hedgehogs, and all of these species are widespread in New Zealand.

Pigs

Tuberculosis caused by *M. bovis* was found in feral pigs in New Zealand in 1962 and the distribution of infected pigs coincides with those feral pig populations that fall within areas endemic for possum infection. On a national basis the isolation of *M. bovis* from farmed pigs was only common when tuberculosis was prevalent in dairy herds and whey and skim milk feeding of pigs was a common practice. *Mycobacterium bovis* tuberculosis is now extremely rare or does not occur in farmed pigs and the predominant species of *Mycobacterium* isolated from farmed pigs is *M. avium* complex. The distribution of lesions in feral pigs and the coinciding spatial distribution of disease with infected possum populations, strongly indicates that infections in feral pigs are a direct result of transmission from possums to pigs, most likely through the scavenging of dead possums (Allen 1991).

Tuberculosis in a pig herd on the West Coast (MacLaughlin 1989) was linked with the feeding of partially cooked possums, while in another incident (Nuttall 1986) grain contaminated with excreta from tuberculous possums was thought to be responsible. New Zealand legislation now makes the practice of feeding offal to pigs illegal. *Mycobacterium bovis* is not a frequent cause of porcine tuberculosis in areas where the disease in cattle is well controlled. In the United States and Canada, for example, bovine tubercle bacilli are rarely found in lesions of pigs.

In the Central Otago endemic region, a steadily increasing and spreading population of feral pigs was surveyed in 1989-90. In total, some 251 pigs were shot and autopsied with 77 (31%) showing histologically confirmed lesions of mycobacterial infection. Of the lesions cultured, nearly 40% yielded *M. bovis*, and there was only a single isolation of *M. avium* complex (Allen 1991). The frequent occurrence of mycobacteria in lesions that are limited to the cervical and mesenteric lymph nodes in naturally-infected pigs indicates that infection usually occurs by ingestion. Although there is probably a limited amount of horizontal transmission between pigs, experience has shown that the

disease cannot sustain in feral pig populations which do not have access to a separate source of infected material.

Cats

Feral cats are distributed widely throughout the three main islands of New Zealand at widely varying densities. Although recorded as being relatively resistant to *M. tuberculosis*, cats are certainly susceptible to *M. bovis*, and there has been a small but steady stream of diagnoses throughout New Zealand since 1974. Since 1974 up to the present there has been a total of 65 cats diagnosed as having an infection of *M. bovis*. Many of these cases were domesticated cats, and the disease in feral cats has not been intensively investigated. The distribution and nature of tuberculous lesions in cats indicates that they are not likely to be sources of infection to other animals but simply act as dead end hosts.

It is commonly observed that during the lean winter months, cats will scavenge extensively, opening them to transference of infection from infected possum carcasses in some way as pigs (Allen 1991).

Ferrets

Mustelids appear to be susceptible to all forms of tuberculosis. Lesions are poorly fibrosed and contain large numbers of organisms, probably indicative of a poor cell mediated response. Feral ferrets are distributed throughout both islands, and inhabit, rough grasslands and bush margins. They are particularly prevalent in areas where rabbit populations are high.

In New Zealand, *M. bovis* infections have been recorded in both wild and farmed ferrets. Some fitch (pelts of ferrets) farmers used possum carcasses to feed their stock and introduced tuberculosis in this manner (Anon 1984). The oral route of infection appears to be very effective for setting up infection (Allen 1991). Tuberculosis was first reported in a feral ferret in 1982 (de Lisle *et al.* 1993) but more recently high prevalences (up to 17.9%) have been recorded in ferret populations in endemic areas (Ragg *et al.* 1995) prompting widespread discussion on their role in transmitting the disease to farmed stock and whether they are a true reservoir host or a spillover host. The issue is important to resolve so that scarce resources are not expended needlessly to control the species, as has been done on occasions with feral pigs, if maintenance of

the disease is dependent on scavenging on possum carcasses. If the latter is the case, then effort on control of the disease in possums is likely to be more cost effective. Possum flesh forms part of the diet of feral ferrets but is almost certainly derived from dead animals (Fitzgerald *et al.* 1984). Tuberculosis has been reported in wild stoats and weasels in New Zealand and they are also thought to be infected by carrion eating. Their population densities are generally much lower than those of ferrets.

Hedgehogs and Rats

Investigations carried out in the United Kingdom have found occasional infections in rats (*Rattus norvegicus*), but none have been found to date in New Zealand. Lugton *et al.* (1995) recently recovered *M. bovis* from five hedgehogs with gross lesions of tuberculosis in endemic regions of New Zealand and attribute the likely cause of infection to the known scavenging habits of the animals.

Deer

Deer farming is a relatively new farming enterprise in New Zealand and the first feral deer were captured for farming in the late 1960s. They came from many areas and some of these, notably the western shores of Lake Taupo, the Wairarapa, and the northern half of the Westland, are endemic areas with infections of *M. bovis* in their wild deer populations. The national prevalence of tuberculosis observed in wild deer at meat inspection has never been greater than 0.21%, but the true prevalence in these endemic areas is undoubtedly higher than this. Growth of the deer farming industry was very rapid and in the early stages was characterised by high breeding stock prices, and by frequent and widespread of movement of deer throughout the country.

The disease in deer is similar to that in cattle. Lesions may be single or multiple, and are found in most lymph nodes, but are most common in the retropharyngeal, mesenteric and thoracic lymph nodes. Lesions range from the classical granuloma to the liquefactive type. The latter are common in advanced cases and generally contain very high numbers of acid-fast organisms. Any cervine "abscess" with cream-coloured pus is regarded as suspicious of tuberculosis. Resistance to infection is variable. Some clinical-normal deer known to have been infected for a year or more have single

lesions at autopsy, while others die within a short time with generalised tuberculosis (Animal Health Division 1986).

Several tests have been approved by MAF for the diagnosis of tuberculosis in live deer, the standard bovine mid-cervical tuberculin test (MCT) using 0.1 ml of 1 mg/ml tuberculin, the comparative mid-cervical test (CCT), the lymphocyte transformation test (LTT) and more recently a composite blood test (BTB test) incorporating the lymphocyte transformation test and an Elisa. The Elisa test is used as a parallel screening test a minimum of 14 days after reading the MCT, for deer which were MCT negative. The Elisa is only approved for use in this manner. The test may not be used as a serial test on MCT positive deer. The BTB test may be used as a primary test and as a serial test on deer that are positive to the MCT. For the last five years both the ST and CCT have been used with increasing frequency while the use of the LTT has diminished since its approval in 1986. When the CCT was approved for use in 1986, its primary function was as an ancillary test to the ST, i.e. for testing of ST positive deer where there was no epidemiological data to support a diagnosis of tuberculosis (Carter 1991).

Whole herd testing was promoted by the Deer Farmers Association, and pre-sale testing became generally accepted as standard practice. A voluntary tuberculosis accreditation scheme was introduced in August 1985 and became compulsory in 1990. Herds are accredited tuberculosis-free when there have been 3 consecutive whole herd tests with no tuberculin reactors over a period of not less than 2 years (Animal Health Division 1986). The number of herds placed on movement control at 31 January 1991, were 278 (4.4%) out of a total of 6298 herds (Carter 1991). Herds with suspicious reactions are placed under movement control, and permits are required before deer may be moved off the property. No compensation is paid for reactors but they may be sent to a Deer Slaughtering Premises (DSP) for salvage of the carcass in the absence of signs of generalised disease.

At the end of June 1995, 4% of New Zealand's 5245 farmed deer herds were on movement control. About 70% of farmed deer herds are located in Surveillance areas where only 0.8% of herds are on movement control. As is the case with cattle, the majority (66%) of deer herds on movement control are located in endemic areas. There

has been good progress in freeing deer herds from infection, especially in non-endemic areas where the initial level of herds on movement control was higher than for cattle. The difficulties still being experienced in endemic areas are due to continuing re-infection from feral sources (O'Neil and Pharo 1995).

Although approximately 75% of deer farmers have cattle on their properties, the probability of farmed deer infecting cattle with tuberculosis is considered to be very low, as there have been only four incidents recorded where there is evidence to support this. In three of these incidents, cattle became infected by grazing in rotation behind deer. In the fourth, five cattle on a property that was agisted to an infected deer herd contracted tuberculosis. A common feature of these incidents was the high prevalence of tuberculosis in the affected mobs of deer, which presumably caused a very high degree of environmental contamination with tuberculosis organisms. In addition, these incidents occurred before DSPs accepted reactors, an unsatisfactory situation which now does not apply. It is important to note that, in general, cattle are very rarely grazed with deer, and also that it is uncommon for cattle to be grazed on pasture that has been grazed by deer.

Deer with tuberculosis may also be an indirect source of infection for cattle. In 1985, four cattle herds in the Mackenzie Basin which were previously free of tuberculosis were shown to have infected cattle. Two tuberculosis-infected possums were captured in the locality. One of these was found on deer farm known to have tuberculosis-infected deer. There is strong circumstantial evidence to show that possums acquired tuberculosis from the deer, as it was considered very unlikely that the possums could have travelled from other areas in the South Island known to have tuberculosis endemic in possums.

Although there is no evidence that *M. bovis* has been directly transmitted from cattle to deer, there are no reasons, apart from husbandry practices which restrict contact, why it should not occur. However, the probability of transfer is low in non-endemic areas where tuberculosis is rare in cattle. Within endemic areas, it is more likely that deer would acquire the disease from possums rather than cattle (Animal Health Division 1986).

Morris and Pfeiffer (1995) have raised the possibility that deer may be reservoir hosts and may have had more responsibility for infecting wild possum populations than has been previously acknowledged. They point to the highly infectious nature of tuberculous deer and their opportunities for contact with possums in the wild to allow transmission to take place, albeit very rarely. They call for more investigations to be made to clarify the epidemiological role of deer.

CHAPTER 2

**An outline of some epidemiological
methods relevant to studies of
disease in herds**

INTRODUCTION

Epidemiology has been described as the study of the frequency, distribution and determinants of health and disease in a population (Martin et al. 1987). The fundamental task in epidemiology is to quantify the occurrence of illness and then to evaluate hypotheses about the causation of disease and its sequelae, and to relate disease occurrence to various characteristics of populations and their environment. It is the branch of empirical science that deals with the occurrence of disease (Rothman, 1986).

Measuring disease occurrence

Measures of disease frequency may take the form of counts, proportions, ratios or rates. **Counts** of individuals that are infected, diseased, or dead may be used to estimate workload, costs, or the size of facilities required to provide adequate health care for a specific animal population. However, unless the size of the population is known, counts are of very limited use.

Proportions. A proportion is always defined as a *part* divided by a *whole* and is useful for describing nominal ordinal and numerical data especially when the observations have been placed in a frequency table. Proportions are calculated as the number (a) of observations with a given characteristic (e.g. disease state) divided by the number of observations with (a), and without (b), that characteristic of interest in the population. Proportions may be converted to percentages by multiplying the proportion by 100%.

$$Proportion = \frac{a}{a + b}$$

Ratios. In a ratio the numerator is not a subset of the denominator and is the number (a) of observations with the characteristic of interest divided by the number (b) without that characteristic.

$$Ratio = \frac{a}{b}$$

Rates. A rate is a number in which the denominator is the number of animal-time units at risk, thus taking into account the time which elapses before disease occurs. A measure of the rate of disease describes the speed and extent to which cases of disease are occurring. Strictly

speaking, “rate” should only be applied to disease measures which include a measure of animal-time units in the denominator, but in general use “rate” is commonly used to refer to other measures of disease frequency. For example, mortality rates are a standard method for describing and comparing the numbers of deaths in different populations but in the strict sense, a mortality rate is a proportion and not a true rate.

$$\text{Mortality rate} = \frac{\text{number of animals which died during a given time period}}{\text{number of animals at risk of dying during the same period}}$$

Morbidity rate is a general term for those measures of disease occurrence (as distinct from mortality rate where the outcome is death) which are used to provide direct measures of health in populations. The epidemiological measures of morbidity are incidence and prevalence.

Incidence

Incidence, as applied to disease, measures the rate at which new cases of a disease occur in a population and measures of incidence allow investigators to study factors associated with an animal becoming ill. Morgenstern *et al.* (1980) and Rothman (1986) state that, for acute events of limited duration, an incidence rate can be calculated for first events by use of those who have never had the disease as the denominator, or a total incidence rate can be calculated for all events, including first and recurrent events within the same individual. For these reason, the definition of incidence rate in terms of "new events" rather than "new cases" would seem preferable (Last, 1983).

There are two measures of incidence, cumulative incidence and true incidence. Incidence measures the flow of individuals from a disease-free to a diseased state. The two essential components of an incidence value are the number of new cases and the period of time over which the new cases occur.

Incident cases

Incident cases are animals which develop new episodes of the disease of interest within the observational period. This means that only animals free from the disease in question are candidates for incident cases. These disease free animals are used in the denominator either as individuals at risk, when calculating risk (cumulative incidence), or as individuals' time at risk, when calculating the true incidence rate (Bendixen 1987).

Cumulative incidence

The cumulative incidence is the proportion of non-diseased individuals at the beginning of a period of study that become diseased during the period. Cumulative incidence is dimensionless (i.e. has no units), and can range between 0 and 1. It is uninterpretable without specification of the time period to which it applies. A cumulative incidence of disease of 3% may be low if it refers to a 5 year period whereas it would be high if it applied to a 5 month period. In general the longer the period of observation, the greater the cumulative incidence. Cumulative incidence is an indication of the average risk of an individual developing the event of interest, which may be disease or death, during a particular period.

$$CI = \frac{\text{\# of individuals that develop the event of interest during a particular period}}{\text{\# of healthy individuals at risk of the disease during the period}}$$

Cumulative incidence is the measure of choice in a fixed or stable population, e.g. a population in which no animal is added after the beginning of the observational period and with few losses, and when the period of risk is short relative to the base time period. In veterinary medicine, such populations are common in production units with all-in all-out systems, e.g. fattening pigs, beef calves and broiler flocks (Bendixen 1987). To establish the correct denominator for cumulative incidence, requires that each animal initially at risk be observed for the full duration of the base time period, or until the event of interest occurs. If there are withdrawals (losses from the study for reasons other than the event of interest) the denominator is commonly determined by subtracting one half of the withdrawals from the initial number at risk or by measuring the population at risk at various interval during the study period and calculating an average. Alternatively, a denominator for an unstable population may be calculated from actuarial methods such as Life Table Analysis or estimated from true incidence rate. For a risk rate to be valid, the number of withdrawals should be minimised. Cumulative incidence may be interpreted at either the population or individual level (Martin, 1990).

True incidence rate

A true incidence rate, also referred to as incidence density (Miettinen 1976) or force of morbidity (or force of mortality in reference to deaths) expresses the velocity at which new events are occurring per unit of time in a defined population size. True rates are used when the animal population being studied is very dynamic (with additions, and/or a large number of withdrawals relative to the initial population at risk) during the base period of the rate. True

rates are also used if the period of risk is long relative to the base time period. True rates have a minimum value of zero and a maximum value of infinity. Because incidence rate is a quotient with a frequency in the numerator and a measure of time in the denominator, its dimensionality is time^{-1} , i.e. the reciprocal of time. The numerical value of an incidence rate in itself has no interpretability because it depends on the arbitrary selection of the time unit. It is essential in presenting incidence rates to give the appropriate time units e.g. as $\# \text{ month}^{-1}$ or $\# \text{ year}^{-1}$. Thus it is preferable not to use the term “annual incidence rate of”; this use is analogous to describing a velocity of 60 miles/hour as “an hourly velocity of 60 miles” (Rothman, 1986). An incidence rate refers strictly to a population and therefore has no direct interpretation at the individual level, as does risk (Kleinbaum *et al.* 1982).

The denominator of a true rate is not just the number of animal at risk, but rather the animal-time at risk. The incidence rate of disease occurrence is the instantaneous potential for change in disease status (i.e. , the occurrence of new cases) per unit of time at time t , relative to the size of the candidate (i.e., disease-free) population at time t (Morgenstern *et al.* 1980).

The general rule for calculating the denominator is to multiply the number of animals times their average period at risk to obtain the animal-time of risk. If the data are available, an exact denominator for a true rate is formed by summing each individual time period at risk for all animals in the study. Each individual animal's contribution is exactly equal to the length of time that individual is followed from start to the onset of the event of interest, which may be disease or death. True incidence rates can either be calculated using only the first occurrence of disease for any given animal (and from then on they are not considered at risk) or using all occurrences of disease (Bendixen 1987).

$$\text{Incidence rate} = \frac{\# \text{ of cases of the event of interest in a defined time period}}{\text{the \# of animal - time units at risk during the time period}}$$

In practice, that level of detail for animal-time is often not available for animal populations and this limitation requires an average value for the denominator to be calculated. There are two methods for calculating the average number of animals at risk. One method is to add the number of animals at the beginning of the time period to the number at risk at the end of the time period (excluding all animals which develop the disease of interest), and then divide the sum by two to estimate the average number of animals at risk. A second method is to add one-

half the number of additions, and subtract one-half the number of withdrawals from the initial number at risk. The number one-half assumes that on average, these movements or events occur at about the midpoint of the base period. If this assumption cannot be reasonably applied, then the period of observation may be divided into shorter base periods during which this assumption is more valid. In either case, the number of animal at risk is then multiplied by the average base period (days, weeks, months, etc) to obtain the animal-time at risk (Martin *et al.* 1987).

Prevalence

Prevalence refers to the amount of disease in a known population, at a designated time, with no distinction made between old and new cases. Observation of existing (i.e., prevalent) cases of disease in a population is the primary design feature of cross-sectional studies. Because prevalent cases represent survivors of a disease, they are not as well suited to identifying risk factors as are incident case identified from a well defined candidate population. Prevalence is appropriate and relevant for testing an etiologic hypothesis and is less costly to determine than incidence. Knowledge of disease prevalence is most important in planning health services and administering medical care facilities, since the number of prevalent cases at any time is an important determinant of the demand for health care (Kleinbaum *et al.* 1982).

There are two types of measures to quantify disease prevalence in a population: point prevalence and period prevalence (MacMahon and Pugh 1970; Mausner and Bahn 1974; Zeigami *et al.* 1979). The most common of these measure is the simple *point prevalence* (usually simply called prevalence), which is the probability that an individual in a population will be a case at a particular point in time.

$$\text{Prevalence} = \frac{\# \text{ of cases of disease at a point in time}}{\# \text{ of animals in the population at the same point in time}}$$

A second and less frequently used prevalence measure is *period prevalence*. When dealing with large populations, point prevalence can be difficult to obtain, since it may not be possible to examine all the individuals in that population at a particular point in time. In such circumstances, measurements of prevalence have to take place over a period of time and this measure is known as period prevalence. Provided that the time taken to measure the prevalence remains reasonably short, then the estimate of the parameter retains a fair degree of precision.

If, however, the time interval becomes too long, then a significant number of new cases of the disease will occur after the start of the measurement period. The estimate then becomes a mixture of point prevalence and incidence, loses precision, and becomes an unsatisfactory measurement.

The terms incidence and prevalence are frequently confused and misused (Martin *et al.* 1987) and many examples of this confusion are found in older literature. By definition, incidence rates require two test, one at the start of the period of observation and the other at the end of that period. Rates based on one test by definition measure prevalence. An incidence rate should always be expressed in terms of a unit of time. Prevalence does not involve a period of time. It is essentially a proportion although it has been frequently called a rate. The distinction between prevalence and incidence can be more easily appreciated by considering the factors affecting prevalence. In general, as incidence increases, so does prevalence. Duration of disease also has a marked effect, however: the longer the average duration (D) of an attribute or event, the higher the prevalence. In a steady state population in which the incidence rate of a disease remains constant, the relationship between prevalence and incidence rate can be shown as follows:

$$Prevalence = \frac{Incidence\ rate \times mean\ duration}{(Incidence\ rate \times mean\ duration) + 1}$$

Thus prevalence is proportional to the product of incidence and average duration. The average duration of any characteristic is dependent on two primary determinants, viz:

- its mortality
- its rate of disappearance (either spontaneously or in response to some treatment or other intervention)

A disease with a high incidence may therefore have a low prevalence if it is of short duration. Conversely, a disease of low incidence can attain a high prevalence if it is incurable but nonfatal.

In populations in which the occurrence of attributes or events is stable,

$$Prevalence (P) \propto Incidence\ rate(I) \times Duration(D)$$

and if the prevalence is small then

$$P = I.D$$

"Stability" here means that incidence and average duration remain constant over time. Thus, if the incidence of a certain characteristic remains stable, and no change occurs in its rate of disappearance, its prevalence will also remain unchanged (Miettinen 1976). In such situations any two of the three quantities, if known, can be used to calculate the third. This is frequently the case for non fatal, chronic illness. It is also true for fatal diseases for which no effective treatment is available (Kramer 1988).

It is important to differentiate incidence rates from prevalence proportions. First, their magnitude may differ greatly, particularly with chronic diseases. Prevalence has limited value in epidemiological investigations of disease aetiology, since factors associated with acquiring a particular disease may differ from those associated with survival with that disease. On the other hand, knowledge of the time period when the disease was acquired assists in demarcating the time period during which causal factors may have operated and, hence, assists in the identification of these factors (Martin 1987).

In summary incidence measures are used in studies based on incident cases of disease to study factors associated with an animal becoming ill. Cumulative incidence is used in studies in which making individual predictions is the objective. Incidence density is used in studies to determine what factors are related to diseases and what the effects of those diseases are. Prevalence measures have special application in studies of congenital malformations since incident cases cannot be measured due to early and unrecognised termination of many cases of this class of disease. Prevalence is also used in studies where there is no clear moment of onset and inferences have to be made about the duration of illness or infection.

A subtype of an incidence risk is an attack rate (AR). The attack rate is used when the period of risk is limited, as in the simultaneous exposure of a group of animals to a noxious substance or contaminated water or food.

$$\text{Mortality rate} = \frac{\text{\# of deaths from all causes in a defined time period}}{\text{the \# of animal - time units at risk during the time period}}$$

Because the biologic period of risk is limited, an attack rate represents the total incidence rate; no new cases would arise from that exposure even if the period of observation were lengthened.

A further modification of morbidity rates, primarily used to study the spread of infectious diseases in defined subgroups (e.g., households) of the population, is the secondary attack rate (SAR), which is calculated as:

$$SAR = \frac{\text{\# of animals exposed to prob. and which develop disease within range of incubation period}}{\text{Total \# of animals exposed to prob and}}$$

Secondary attack rates are usually applied to natural groupings of animals such as pens or farms. They may also be used evaluate the communicability of diseases of unknown aetiology in an attempt to see if infectious agent might be involved. For infectious disease, the higher the second attack rate the more contagious the agent. However, some non-infectious diseases can occur in a manner that may result in a high secondary attack rate. This may occur if there is a variable latent period following a common exposure of individuals within the group, and hence the disease may appear to spread from animal to animal (Martin *et al.* 1987).

Mortality measures

Mortality measures are analogous to incidence measures where the outcome event of interest is death instead of the occurrence of a new case of disease (Kleinbaum *et al.* 1982). Two frequently used measures of mortality are the mortality rate and cause-specific mortality rate.

The mortality rate is the total number of deaths occurring in a specified population during a specified time period divided by the average number of individuals in that population during the specified time period. It is analogous to incidence rate except that the outcome of interest is death and it is calculated in the same way as incidence rate.

$$\text{Mortality rate} = \frac{\text{\# of deaths from all causes in a defined time period}}{\text{the \# of animal - time units at risk during the time period}}$$

Cause-specific mortality rate is a useful rate of death measure and is defined as the total number of deaths occurring from a specified cause in a specified population during a specified

time period divided by the average number of individuals in that population during that time period. The formula for the cause-specific mortality rate is:

$$\text{Cause - specific mortality rate} = \frac{\text{\# of deaths from or with a disease in a defined time period}}{\text{the \# of animal - time units at risk during the time period}}$$

The probability of dying in a specified time period may be determined by restricting the denominator to those alive at the start of the time period and adjusting this number for any withdrawals. All animals must be observed for the full time period, or until death or withdrawal occurs.

The risk of death in animals with a specific disease can be calculated as the case fatality rate. The formula for a case fatality rate(CFR) is:

$$CFR = \frac{\text{\# of deaths from a specific disease}}{\text{total \# of animals with the disease}}$$

Case fatality rates are of greater value in studies of acute rather than chronic diseases and are used to describe the virulence of the agent and/or the severity of the disease.

EPIDEMIOLOGICAL RESEARCH METHODS WHICH CAN BE USED TO STUDY HERD DISEASE DATA

Case Control Studies:

In case-control studies, the outcome is the starting point and prior exposure is investigated to try to detect possible causes or risk factors for the disease outcome. Reasoning is inductive, from effect to cause (Kramer 1988). The cases in case-control studies are animals selected on the basis of some disease or outcome and the controls are animals without the disease or outcome. Case-control studies may also involve incident cases of disease; i.e. cases and non-cases may be identified over time, as is done in a cohort study. The history or previous events of both cases and controls are analysed in an attempt to identify a characteristic or risk factor present the cases' histories but not in the controls' history. The central condition in case-control studies is that cases must be selected independently of exposure. They may include

every identified case of disease in a geographic region or alternatively only include those identified by a particular veterinary clinic. Controls are selected on the basis that they would have become identified as cases had they developed the disease, i.e. they would have navigated the same pathway of selection forces as the cases had they developed disease (Rothman, 1986).

Case-control studies may also be based on prevalent cases rather than incident cases. If the duration of the illness is unrelated to exposure, then a case-control study based on prevalent cases will provide an odds ratio that is an unbiased estimator of the incidence rate ratio. If exposure affects the duration of illness, then a case-control study based on prevalent cases will be unable to distinguish an etiologic role for the exposure from its effect on duration unless the effect on duration is known.

Although matching (i.e., partial restriction) is seldom used in cohort or cross-sectional studies, it is used often in case-control studies. The widespread use of matching in case-control studies is explained by practical considerations, such as the convenience or cost of subject selection and, perhaps, by the intuitive appeal of the procedure for dealing with extraneous risk factors (instead of relying solely on complex statistical techniques).

The case-control design has been used by biomedical researchers for more than a century (Lilienfeld and Lilienfeld 1980) and is generally considered to be a major contribution by epidemiologists and their predecessors to the general area of research methods. In relatively recent years, the number of published case-control studies has increased dramatically (Cole 1979), generating many insights into the aetiology of chronic diseases (Pearson 1979). These past successes are largely the result of the practical advantages of conducting case-control studies when other basic designs would be much less feasible. Not only does the case control design afford the opportunity to study diseases that occur very infrequently, but it also allows for the investigation of diseases with any latent period or duration of expression. The convenient sampling strategy and the relatively short study period usually make case-control studies less time-consuming and less expensive than cohort or cross-sectional studies. Nevertheless, the use of the case-control design has generated a good deal of controversy among epidemiologists (Ibrahim 1979, Mann *et al.* 1979) because it is most different, methodologically, from the classical experiment (Schneiderman and Levin 1973).

The principal limitations of case-control studies derive from two key features of the study design: study factor information is obtained after the occurrence of the disease; and the compared groups of cases and non cases are selected from two separate populations. Consequently, it is difficult to ensure that cases and non cases in the study population are comparable with respect to extraneous risk factors and other sources of distortion.

Aside from the above selection problems, case-control studies have other practical limitations. The disease must be measured as a categorical variable (e.g., case *versus* non case), and it must be recognised as the one health outcome of interest before subjects are selected. Thus, the case control design may not be appropriate for exploring the possible health effects of a certain study factor. Without additional information, this design is also not appropriate for estimating the frequency of the disease in a population. Furthermore, because case-control studies cannot have forward directionality, the ability of the investigator to distinguish antecedent from consequent depends on the retrospective ascertainment of study factor information. Either these data must be collected from records, which are often inadequate or incomplete, or they must be obtained from the recall of past events and habits, which is subject to substantial human errors. The use of subject recall is particularly problematic when respondents are aware of the association being tested or when the study factor involves a very subjective assessment. In these latter situations, self reported study factor level is likely to be influenced by the disease status of the subject. Such select recall would distort results, usually making the observation between study factor and disease stronger than the actual association (Kleinbaum *et al.* 1982).

When the outcome is continuous and the exposure is categorical, the results can be analysed by comparing the mean outcomes in each exposure group.

The usual method of analysing the results of a case-control study uses dichotomous outcomes and categorical (usually dichotomous) exposures. Continuous exposures can be categorised to permit the use of this strategy. When both outcome and exposure are dichotomous, the result can be displayed in a 2 x 2 table (Kramer 1988).

Cohort Study

In cohort studies, separate samples of exposed and unexposed units are selected. The groups are observed for a predetermined period, and the rate of disease in each is compared (Martin *et*

al. 1987). The population at risk of developing the outcome event, either disease incidence or mortality, is followed for a given period through re-examination or population surveillance, during or after which new cases or deaths are identified. A cohort study may be completely prospective or retrospective (using secondary data). Since a prospective cohort study is the type of observational design that most closely resembles an experiment, it is generally preferred for making causal inferences. Yet, a retrospective cohort study may be more feasible for studying a rare disease or a disease with a long latent period. The retrospective design, of course, depends on the availability of previous study factor information on a well-defined population that has been followed for detection of new cases or deaths. A potential problem in any type of cohort study, particularly those involving disease incidence and long follow-up periods, is the loss of subjects because of migration, lack of participation from the owners, and death.

The cohort study involves either a fixed cohort, which gets followed for a given period, or a dynamic population, for which the individual follow-up period of every subject is known (or assumed) by the investigator (Kleinbaum *et al.* 1982).

Selection of exposed and unexposed groups:

In most cohort studies, special exposure groups are purposively selected for comparison. This could include comparing rates of disease in different breeds; comparing rates of pneumonia in animals on different rations; comparing rates of disease in animals with and without serum antibodies to selected antigens; or comparing disease rates and production levels in herds on preventive medicine programs to similar herds not on these programs. The sampling units are frequently obtained through purposive sampling, not probability sample from a defined sampling frame. Because of this and in order to extrapolate results beyond the study groups, some indication of how representative the study groups are of exposed and unexposed segments of the population should be obtained.

A further concern in selecting the cohorts is that they should be comparable (i.e., not differ in ways other than the exposure). This may require the measurement of ancillary variables so that analytic control can be used to adjust for known differences between the groups, although matching may be used to increase the similarity of the groups. More than one unexposed group may be selected as the referent if it is thought that the information provided will be useful.

Although the exposure status of selected units may seem obvious, the probability of misclassification of exposure status can be reduced by clear, concise descriptions of what constitutes exposure (possession of the factor). Specific tests may be used to help assess exposure status in a manner similar to their usage as diagnostic aids.

If pre-recorded data on exposure history are used to define the cohorts, investigations into the meaning, validity, and completeness of the data should be performed.

Whenever possible, all the sampling units entering the study should be examined for the presence of the disease of interest at the start of the study. By starting the study with disease-free units, the investigator can determine incidence rates, and this also establishes a clear temporal relationship between the factor and disease. Sometimes such an examination is very difficult; thus the sampling units are assumed to be disease-free at the start of the study. This assumption is frequently made in retrospective cohort studies.

The cohorts should be observed for the occurrence of disease at regular periods throughout the study. Both groups should be followed with equal rigor, and withdrawal of sampling units from the study should be minimised. Withdrawals can bias the results if the losses are related to both exposure and disease status. Obviously, this problem is more severe in studies spanning many years. If a high percentage (e.g., 95%) complete the study, potential biases from withdrawal will be minimised. Care is also required when cohorts are defined retrospectively, because many withdrawals (due to culling, sale, or death) will have occurred before the study begins.

Bias assessment

As in other types of analytic studies, minimising analytic bias is essential in assuring the internal validity of observational cohort studies. Three of the four general types of analytic bias are important considerations in cohort studies, viz. information bias, sample distortion bias, and confounding bias. The fourth type, reverse causality bias, is less of a concern, because the forward directionality of cohort studies indicates that exposure precedes outcome, particularly if the study subjects are known to be free of the outcome in their baseline state.

The best protection against information bias in cohort studies is in their design. Measurements should have proven reproducibility and validity and should be performed by observers who are blind to the subjects' exposure status.

Sample distortion bias can occur in assembling the cohort as a result of non-representative sample selection from the target population. It may also occur during follow-up if losses occur preferentially in some exposure-outcome combinations or if the duration of follow-up varies according to exposure and is independently related to the outcome. It can be guarded against by using a sample selection procedure that ensures representation of the target population, by standardising follow-up procedures, and by minimising losses.

Confounding bias may result from exposure selection, unequal susceptibility at baseline, or exposure contamination.

Exposure selection bias can be controlled for only to the extent that the reasons for subjects' choice of exposure can be reproducibly and validly measured.

Measurable differences in susceptibility that vary according to exposure can be controlled at either the design or the analysis stage. When sample selection is by exposure, the resulting exposure groups can be matched, during the design, according to the suspected confounding susceptibility factors. When matching is included in the design, the analysis should take account of the matching. When the exposure is dichotomous, the outcome is continuous, and the matching is by pairs, paired tests of group means can be performed (Kramer 1988).

The usual 2 x 2 table format may be used to display and analyse the data if the duration of the study is relatively short, the average period of risk is equivalent in both cohorts, and the losses to follow up are minimal. The rates of disease in each cohort can be calculated and compared directly, or the Mantel-Haenzel technique, or standardisation of rates may be used to control the effects of extraneous qualitative variables.

Often the duration of the period at risk may differ greatly between cohorts. This is particularly likely when the cohorts are not completely formed at the start of the study.

Two analytic approaches are used to adjust for the differing periods of observation. The first method is based on the calculation of true rates and the concepts of animal-time of risk. The total unit-time of risk in each group is used as the denominator for calculating true rates in the usual manner. Although these data may be summarised in a 2 x 2 table, the regular chi-square test should not be applied to these data. Thus, although using true rates is useful for removing biases from differences in period of risk, it does not allow the evaluation of the role of chance by standard statistical methods. If group sizes are very large sampling variation is not of great importance and may be ignored.

The second analytic approach is the follow-up life table method that allows the investigator to calculate risk rates. This is accomplished by taking into account the different periods of risk, and the technique also allows formal statistical evaluation of observed differences.

Cross-sectional Study

Also called a survey or prevalence study, a cross-sectional study-involves a non-directional or backward design of a study population that has been selected from a single target population. A cross-sectional study design involves disease prevalence, not incidence, and usually involves random sampling of the dynamic target population. After the selection process, all sampled animals are examined.

A cross-sectional study has limited value for describing the frequency of disease or other characteristics in a population and has serious limitations for making causal inferences if a random sampling design has not been used. Carefully conducted probability sampling substantially increases the likelihood that the study population is representative of the target population. While this feature does not ensure the validity of our comparisons for making causal inferences, it does make cross-sectional studies particularly useful for describing characteristics of a target population (Feinstein 1978).

Because the cross sectional design does not involve a follow-up period, it is often used to generate new etiologic hypotheses regarding study factors and/or diseases. However, cross-sectional studies are not appropriate for studying rare diseases of short duration (Kleinbaum *et al.* 1982).

Cross-sectional studies usually measure prevalent outcomes. Fatal cases, dropouts, and migrants are not counted, nor are cases that were successfully treated or that resolved spontaneously. Consequently, cross-sectional studies are best suited to chronic, nonfatal conditions.

Exposure is measured at the same point in time as outcome. Since exposure and outcome have usually been present for some time prior to the study, the investigator cannot be certain that exposure preceded outcome. Consequently, any inference that exposure caused outcome rests on the unknown true temporal sequence of events.

The analysis of the results of a cross-sectional study depends on the method of sample selection and on the measurement scales for exposure and outcome. When selection is by exposure, analysis is similar to that used for cohort studies. For continuous outcomes, mean outcomes are compared between the groups defined by exposure status. When selection is by exposure and outcomes are prevalent rather than incident, the relative risk derived from a cross-sectional study is often referred to as a prevalence rate ratio.

When sample selection is by outcome, analysis is similar to that used for case-control studies. Outcome is usually dichotomised (cases vs. control) and odds ratios can be calculated. The classification of outcome status as "case" vs "control" does not render the design truly case-control, however, since the exposure ascertained in cross-sectional studies is simultaneous with, rather than prior to, the outcome.

Cross-sectional studies are generally prone to the same sources of sample distortion bias, and confounding bias as case-control studies. Since ascertainment of exposure is based on contemporaneous exposure, however, there is less opportunity for information bias in the exposure measurement. Since cross-sectional studies do not rely on the subject's memory of exposure, its measurement is less likely to be randomly erroneous or differentially selective (those with the outcome being more likely to recall exposure) than in case-control studies. Adequate blinding of investigator and clear a priori criteria for exposure are usually sufficient to guard against this form of bias in cross-sectional studies.

For reducing sample distortion bias, restriction, matching, stratification or multivariate adjustment techniques can be used to control for confounding bias in cross-sectional studies.

The potential for reverse causality bias is of crucial importance in cross-sectional studies and is the major reason why causal inferences are more tenuous than in cohort or even case-control studies (Kramer 1988).

The major advantages of cross-sectional studies are their rapidity and low cost, compared with cohort studies, and their relative freedom from memory bias. Since exposure and outcome are both ascertained at a single point in time, no follow-up is required. Data can therefore be obtained quickly and at little expense to the investigators. Furthermore, the potential for information bias in ascertaining exposure is less than in case-control studies, since subjects do not have to rely on their memory of past exposure.

The main contribution of the cross-sectional design is in descriptive, rather than analytic, epidemiologic studies. Disease or other phenomena can be classified by species (age, sex), place (nation, region, province, district, etc) or time. Cross-sectional studies are also useful for describing the clinical spectrum (symptoms, signs, laboratory test results, pathologic findings) of a given disease entity.

Cross-sectional design also have a role in analytic studies. Because they can be done quickly and inexpensively, cross-sectional studies can often provide the first clue to an exposure-outcome association, which can serve as a stimulus for more definitive cohort or case-control studies.

The major disadvantage of cross-sectional studies are their frequent inability to distinguish cause from effect and their potential for sample distortion bias. The latter problem is one shared by case-control studies. The problem of distinguishing the horse from the cart, i.e., whether exposure preceded outcome or vice versa, is unique to cross-sectional studies and constitutes their major limitation in analytic research (Kramer 1988).

The proportions being compared (the proportion of cases that are exposed and the proportion of non-cases that are exposed) in the case-control study should be calculated and displayed together with the results of statistical analysis and the appropriate epidemiologic measures of association.

If the factor has more than two levels on the nominal scale (e.g., breed) the level of factor that makes the most biologic or practical sense should be chosen as the reference group (odds ratio = 1). A series of 2 x 2 tables each containing the referent group is constructed, and the strength of association assessed in the usual manner (Martin *et al.* 1987).

MULTIVARIATE ANALYSIS FOR EPIDEMIOLOGICAL STUDIES:

Regression analysis

Regression analysis is a statistical tool for evaluating the relationship of one or more independent variables X_1, X_2, \dots, X_k to a single, continuous dependent variable Y . It is most often used when the independent variables cannot be controlled (Kleinbaum *et al.* 1988).

There are three objectives that may be met by elaborating the functional relationship implied in regression:

- i) finding an optimal equation for predicting the outcome. This may involve using all predictor variables, but more frequently it requires the identification of a subset of predictor variables whose predictive power is nearly as high as that of all of the predictor variables.
- ii) discovering which variables are related to the outcome and possibly ranking them in terms of their importance. Related as used here means "statistically associated", although in some models (path models) the relationship usually is presumed to be causal.
- iii) identifying the effects of one or more variables when the effects of potential confounding variables are removed (controlled) by analysis.

A regression is called simple, if only one predictor variable is present. A regression equation can be shown as $Y = a + bX$, where the point where the line intercepts with the Y axis is a and the slope of the line is b . The slope of the line measures the amount of change in Y for each one unit change in X . In the regression equation the slope in the population is generally symbolised by β_1 , called the regression coefficient; and β_0 denotes the intercept of the regression line. Thus β_0 and β_1 are the population parameters in regression. The values of Y obtained from the regression equation are predicted values, rather than actual values of Y , and as such are conventionally represented by Y' . The regression equation is given by

$$Y' = \beta_0 + \beta_1 X$$

Not all predictions for Y will be precise since in most applications the points do not fall exactly along a straight line. For this reason, the regression model contains an error term e to represent the amount that must be added to, or subtracted from $\beta_0 + \beta_1 X$ to obtain Y' . The error term, e , is difficult to quantify since it varies for each sampling unit (case).

The usual assumptions of least squares regression are:

- At each value of the X variable, the Y variable is assumed to have a normal distribution, and the mean of the distribution is assumed to be the predicted value Y' . In addition, no matter what the value of the X variable is, the standard deviation of Y is assumed to be the same.
- For each sampling unit the e 's are independent, normally distributed, with a mean of zero, and with a common variance. An assumption of normality of residuals is quite reasonable because there are usually many components to the residual (eg. biological variation, measurement errors, errors in the test, etc.) and the sum of these components will tend to follow the normal distribution
- The linear assumption requires that the mean values of X fall on a straight line
- The values of Y are assumed to be independent of one another.

Logistic Regression

Logistic regression is a mathematical modeling approach that can be used to describe the relationship of several independent variables to a dichotomous dependent variable. It is commonly used in veterinary epidemiology to decide which factors are predictive of disease. It can produce useful epidemiological measures in the form of estimates of odds ratios from prevalence or case-control data and relative risk estimates from follow-up studies.

In logistic regression the independent variables need not be qualitative. The main advantages of its application in case-control studies are that it allows control of confounding and evaluation of interaction with a great deal of statistical efficiency. Logistic regression models can contain more variables for a given number of cases than the Mantel-Haenzel procedure and it is the preferred and more precise technique when the effect of numerous variables and their subgroups are being evaluated, since stratified data may be spread too thinly in subgroups, and there may be too many combinations to evaluate efficiently.

The procedure allows precision to be retained while controlling for many variables and allows efficient use of the data to evaluate many effects simultaneously. However it can have the disadvantage of distancing operator from the data with less awareness of deficiencies also suffers because many readers are unfamiliar with the technique by readers and poor summary explanations are often presented by authors

In its most common use, the dependent variable takes only one of two possible values. As a key example, a study of the development of a particular disease in population could use logistic regression to describe in probabilistic terms whether a given individual in the study group will ($Y = 1$) or will not ($Y = 0$) develop the disease in question over the follow-up period of interest (Kleinbaum *et al.* 1988).

The logistic model has also been used both extensively and successfully to describe the probability (or risk) of developing some disease over a specified time period as a function of certain risk factors X_1, X_2, \dots, X_k (Kleinbaum *et al.* 1982).

Discriminant Analysis

Logistic regression is used almost exclusively in the biological sciences. A related technique, discriminant analysis is used less frequently in medicine than in the social sciences and is similar in that it is used to predict a nominal or categorical outcome. It differs from logistic regression in two important ways:

- it assumes that the independent variables follow a multivariate normal distribution, so it must be used with caution if some X variables are nominal
- it can be used with a dependent variable with more than 2 values

It is particularly useful to determine factors which distinguish subjects into several particular conditions. The statistical problem is to develop a rule, or discriminant function, based on the measurements obtained on each of these individuals, that will help us to assign some new individual to the correct population when it is not known from which populations the individuals comes.

The data obtained on each individual will invariably consist of observed values of a set of mutually correlated random variables, and the presence of these inter-correlations will necessitate consideration of the variables together rather than one at a time. The general

approach, as with regression analysis, is to construct in some optimal way a linear combination of these variables that is then used for classification. This transforms the basic problem from a complex multivariable one to an easier to handle univariable one, and the assignment of an individual to a population is then based simply on the value of the linear combination for that particular individual.

CHAPTER 3

Tuberculosis control programmes in New Zealand

INTRODUCTION

The Animal Health Board (AHB) administers a national programme to reduce the incidence of bovine tuberculosis and eventually to eradicate the disease from cattle and deer. The AHB is made up of representatives of the farming industry and organisations plus government. The cattle disease control program was first developed and administered by the Ministry of Agriculture and Fisheries (MAF), but for some years, an arm of the MAF, MAF Quality Management (MAFQUAL) has been contracted by the AHB to be the main service provider for the tuberculosis disease control program. MAFQUAL develops regulations for the program, manages national testing and maintains the National Livestock Database which was specifically developed for the tuberculosis control scheme. Most of the cattle testing is carried out by MAFQUAL livestock officers. Farmers are compensated for slaughtered reactors and are not charged directly for testing but are levied on all cattle killed to provide most of the annual working capital for the operation of the scheme. Compensation, calculated as a percent of fair market value (fmv), is paid for reactors. Initially this was 85% of fmv, but it has now been reduced to 65%; it may be reduced further in the future.

The program for deer was developed by the deer industry, the New Zealand Veterinary Association and MAF. In this scheme, farmers pay directly for the costs of all tests and can choose between competing veterinary practitioners or MAFQUAL livestock officers for routine testing of deer. Deer farmers receive no compensation for reactors but are paid for salvageable carcasses.

At the end of the 1990/1991 testing season (31 August 1991), 98% of cattle herds were classified as accredited free of tuberculosis and 2% as infected or on movement control because of possible infection. At the end of the 1995 testing season, 2.4% of the 59,796 cattle herds were on movement control (O'Neil and Pharo, 1995). Although only 12% of cattle herds are located in endemic areas, these herds make up 77% of the movement control herds. The situation regarding the current disease status of deer herds was described in the deer section in Chapter 1.

National Livestock Database

This database was established by MAF in 1985 to assist the administration of tuberculosis testing. It was designed using the software package, Scientific Information Retrieval (SIR) contains information on all herds that have been included in the testing programme. Included in the data stored is a profile of the herd, its location and its testing history and this information is used to plan and organise the testing programme and maintain a record of the results.

The data can be organised on the basis of three different 12 month periods, viz. testing year, calendar year and financial year.

The testing year runs from the 1st of September through to the 31st of August. It is used for technical purposes and for all technical reports. It is the time period used for calculating annual morbidity rates and in particular, annual incidence and prevalence.

The calendar year runs from the 1st of January to the 31st of December. Various international agencies require disease surveillance statistics per calendar year. This is the main use of this measure.

The financial year runs from the 1st of July to the 30th of June. It is necessary for financial purposes and coincides with the official government financial year for budgeting and taxation purposes.

In this thesis the testing year period is used to calculate cumulative incidence and true incidence (incidence density).

Herd classification

Herds are officially classified into 5 categories according to their disease status:

- 1) Infected herd for herds on movement control that are considered infected
- 2) Clear herd when the herd is on movement control but has achieved a valid negative whole herd test
- 3) Transitional herd: - the herd is classified as transitional status when either the tuberculosis status is unknown (as is the case with a new herd), or there is some suspicion about the tuberculosis status of a herd

- 4) Free herd – A herd which has been released from movement control (after two negative whole herd tests a minimum of 6 months apart) but has not been accredited (see below). Non-breeding herds where there is a constant turnover of stock, say, in this status (ie, they do not progress to accredited-free).
- 5) Accredited for breeding herds which have achieved three clear whole herd tests with a minimum of 6 months between test 1 and test 2, and 12 months between test 2 and test 3
- 6) At the time of writing discussions were going on as to whether this herd status classification should be changed. It is apparent that many farmers found the terms ‘clear, free and accredited’ confusing.

Area classification

The Ministry of Agriculture has classified the control area of New Zealand into two major categories, viz. special tuberculosis investigation areas (STCAs) and surveillance areas.

Special tuberculosis control areas are geographically defined areas comprised of a central endemic and a surrounding fringe zone. Beyond this lies the surveillance zone.

Initially a further zone, a so-called non-endemic zone, was also defined outside the fringe area.

There are moves to eliminate this because of the confusion between it and surveillance areas (both non-endemic). At the time of writing this change has not been approved and therefore it has been included.

The boundaries between endemic and fringe areas are set so that tuberculosis testing of cattle and deer will highlight any changes in disease incidence in feral/wild animal populations if the central endemic area expands.

Endemic areas are defined as those areas where tuberculous feral or wild animals have been found or are implicated in the transmission of the disease on the basis of epidemiological evidence.

The epidemiological findings linking infection in cattle or farmed deer with tuberculosis in feral/wild animal populations are in order of importance:

- a) an infected herd within a 5 km radius of another herd that has had an infected MC status within the last 2 years
- b) infection which cannot be linked to the movement of cattle or deer onto the property

- c) a test and slaughter programme which does not eliminate infection
- d) low annual incidence and no obvious spreaders of tuberculosis detected at slaughter
- e) habitat in vicinity of infected herd which is a potential source of infected possums

Fringe areas separate the central endemic from the non-endemic areas. They are set sufficiently wide so that the probability of migrating tuberculous feral/wild animals establishing new foci of infection in the non-endemic area is negligible.

The potential spread of tuberculosis into and across fringe areas is monitored by testing herds within the fringe zone with the intention of control before the disease becomes established in the feral/wild animal population. The objective is geographical containment of disease within the endemic area.

The placement of the outer boundary of the fringe area takes account of possum habitat and geographical features that are judged to affect the movement of wild/feral animals including

- expanses of open clear farmland area
- areas of pine plantations, bush and scrub
- good possum habitat-lined catchment drainage
- major rivers
- mountain ranges

It is also important that the fringe zone contains sufficient deer or cattle herds to allow adequate monitoring of any potential spread of tuberculosis in feral/wild animals.

In those parts of the endemic area where the incidence of tuberculosis is high, the probability of movement of infected juvenile possums into the fringe area may also be high. In such situations the fringe area should be approximately 10 km wide. Other factors may cause the area to be set wider e.g. the presence of tuberculous feral pigs or deer.

The fringe area may extend for 20 km along a waterway bordered by good possum habitat and so include all farms adjacent to the waterway. It should also encompass all farms adjacent to stands of bush/scrub close to the waterway.

If the endemic area is bounded by a major river with no bridge or wire to allow the passage of possums, the fringe area may only need to be set at one farm wide on the opposite side of the river.

In open farm land devoid of possum habitat, the fringe area may only need to be 3-5 km wide, especially where there are high cattle stocking rates to aid detection of the presence of tuberculous possums.

In regions infested with feral pigs or deer, the width of the fringe area should take account of size of the home range of those wild animals.

The *non-endemic areas* separate the fringe from the surveillance area. In a non-endemic area, monitoring of farmed deer and cattle to detect the spread of tuberculous feral/wild animals in the area may be less intense. If the design of the fringe area has been thought through carefully, and the testing recommendations fully implemented, the probability of a feral or wild source of infection moving into the non-endemic area should be low.

The non-endemic zone is the second line of defence after the fringe area and provides an added insurance against infected possums spreading through the fringe zone undetected. The design of the non-endemic area should take account of the likely movements of feral or wild animals from the endemic and fringe areas. The boundaries of the non-endemic area should be dynamic and adjusted to reflect disease in adjacent parts of the fringe area. The placement of boundaries for the non-endemic area should take into account vegetation, geographical and stock density details using principles similar to those used for the fringe area. As a general guideline, the non-endemic area will be set at about 3-5 km wide.

Surveillance areas are those areas outside the STCAs, where there is believed to be no tuberculosis in the possum population and when there are only sporadic outbreaks of the disease.

Testing considerations within the various areas

The test programme for tuberculosis varies with the classification of the area in which a herd is located. The aims of testing are to remove infected animals from a herd and prevent cattle to

cattle transmission, and also to prevent the disease from establishing in feral and wild animal populations. In general, herds are tested annually in endemic and fringe areas, biennially in non-endemic areas and triennially in surveillance areas.

TEST CLASSIFICATION

A **whole herd test** is defined as a test performed on all test eligible animals in the owner's herd and which has been completed in one day.

Whole herd testing of beef breeding and dairy herds

In accredited Tb-free herds, all breeding cattle over 24 months of age should be tested. Where the number of test eligible cattle exceeds 250, a whole herd test should comprise of a minimum of 250 cattle randomly selected by either the testing inspector or veterinarian. This recommendation is modified on farms where large groups of cattle are effectively grazed in isolation, and in such cases, 250 test eligible cattle are selected from each group.

In Tb-free herds, transitional herds and movement control herds, all beef cattle over three months of age and dairy animals over 6 weeks should be tested.

Whole herd testing of beef dry-stock herds

For Tb-free herds where 90% or more of animals go direct to slaughter, no testing is required. For transitional herds and movement controlled herds, all cattle over three months of age are eligible for testing. However the MAFQUAL veterinarian may elect to use works surveillance where all cattle remaining in the herd at the time tuberculous cattle were diagnosed, plus any subsequent in-contact cattle are destined to be slaughtered within the following 12 months. A combination of testing and work surveillance may also be used.

Whole herd testing for miscellaneous herds

No testing is required in Tb-free in Tb-free dealer herds where 90% or more of animals go direct to slaughter. In all other Tb-free miscellaneous herds all breeding cattle over 24 months should be tested.

All dairy cattle over six weeks of age and all beef cattle over 3 months should be tested in miscellaneous herds with transitional or movement control status.

Whole herd test programmes

For accredited Tb-free herds, the interval between successive herd tests should not exceed 3 years. For Tb-free beef breeding and dairy herds, the interval between successive herd tests should not exceed 12 months. For Tb-free miscellaneous herds the requirement is as for accredited herds, i.e. the interval between successive herd tests should not exceed three years.

Where any herd has been under a "movement control herd" notice, it is required to be tested at least every 12 months until it has been free of tuberculous cattle for a minimum of three consecutive herd tests over a period of not less than two years. The first test after a diagnosis of disease will be less than 12 months, since for movement control herds, with the exception of those herds which are monitored by work surveillance, a whole herd test must be performed within six months of the date of diagnosis of any tuberculous cattle.

Herds with an infected movement control status may be tested at a two to six month interval after the diagnosis is made, and once a clear movement control status is obtained, the interval between whole herd tests must not be less than 90 days and no longer than 12 months.

Tests other than whole herd tests

A *partial herd test* designation is used when all the animal can not be tested on the same time. The test at which the final animals are tested after partial herd tests is designated a *final herd test*.

The term, *miscellaneous test*, is used where any number of animals are tested but the test does not count towards a whole herd test. It is mostly applicable to small herds of less than ten adult animals and miscellaneous dairy dry stock operations e.g. heifer rearing or dairy stock dealing.

A *sale test* is a miscellaneous herd test, where the purpose was to clear animals for sale.

Other episodes

A *Tb cull* entry is made in the database when a Tb infected non-reactor animal is found at routine meat inspection.

A *veterinary direction* is entered when a veterinarian sets or alters the status of a herd. It is used when a new herd is registered and has not been tested.

Tuberculin testing: dose and interpretation

The following doses of bovine tuberculin and test interpretation are applied in a caudal fold tuberculin test:

Accredited Tb-free herds -0.1 ml of 1 mg bovine tuberculin (5000 IU) is injected into the caudal fold. The test is read 72 hours after injection and a positive reaction constitutes a swelling (4 mm or greater for modified interpretation) at the injection site.

Tb-free herds -0.1 ml of 1 mg tuberculin is injected into the caudal fold. The test is read 72 hours after injection and a positive reaction constitutes a swelling at the injection site.

Movement control herds -0.1 ml of 2 mg/ml tuberculin (10000 IU) is injected into the caudal fold. The reaction is read 72 hours after injection and a positive reaction is any visible or palpable reaction at the injection site.

Introduction or removal of cattle from a herd

If outside a declared movement control area, cattle leaving Tb-free, accredited Tb-free and transitional herds may be removed without testing. But animals leaving movement control herds, unless moving directly to slaughter, must be negative to a test not more than 60 days prior to their removal, and if so directed, be re-tested no sooner than 60 days and no longer than 120 days after the pre-movement test. To minimise the potential spread of tuberculosis it is advisable that these cattle are kept separate until the result of the re-test are available. Movement control was introduced in 1977 and rules have become progressively tighter with time.

Area movement control was introduced in 1992, in recognition of the importance of tuberculous vectors in the transmission of tuberculosis in certain areas. In some endemic areas and STIAs, it was considered that tuberculous vectors could have introduced infection into the herd since the last whole herd test, and that in such areas it was not wise to move animals without first testing them. They are known as declared movement control areas. Initially

movement control restrictions applied to only animals 12 months and over, but in 1993, the regulations were amended to include all animals 3 months of age and over.

Tuberculosis declaration cards were introduced in 1992 on a voluntary basis and in 1994 became compulsory so as to alert cattle purchasers to the status of livestock and to force vendors to reveal that status.

Movement control herds

A movement control herd notice will be issued where tuberculous cattle are either suspected or diagnosed within a herd. For the movement control herd notice to be revoked the herd needs to be free of tuberculous cattle for a minimum of two consecutive whole herd tests over a period of not less than six months. In herds monitored by works surveillance, the notice may be revoked twelve months after the last tuberculous cattle was slaughtered. All cattle requiring a permit to move (except reactors and tuberculous cattle) must have been negative to a test within 60 days of the proposed movement.

Suspension or cancellation of a herd's Tb status

The tuberculosis status of a Tb-free or an accredited herd may be suspended where

- tuberculous cattle are suspected
- the herd is associated with animals of another species which are affected with tuberculosis
- cattle, which in the opinion of the MAFQUAL controlling veterinarian are of a lower tuberculosis status, are introduced

The tuberculosis status of a Tb-free or accredited Tb-free herd may also be suspended or cancelled if any rules of the scheme are violated. The re-instatement of a herd's tuberculosis status is at the direction of the MAFQUAL veterinary officer responsible for the district.

Approved tests

The caudal fold test (CFT) is an intradermal test using 0.1 ml of either 1 mg/ml or 2 mg/ml bovine PPD tuberculin applied to the right hand caudal fold of the tail and read at 72 hours after injection, using a standard or modified interpretation, depending on the herd status.

The comparative cervical test (CCT) is an intradermal test using 0.1 ml of 0.5 mg/ml avian PPD tuberculin applied at a clipped site in the mid-cervical area of the neck and 0.1 ml of 1mg/ml bovine PPD tuberculin applied at a mid-cervical clipped site at least 12 cm from the site of the avian tuberculin injection. The test is be read at 72 hours after injection. A positive interpretation of this test will be where the reaction to bovine tuberculin is 4 mm or greater than the reaction to avian tuberculin.

Testing requirements for movement control herds

- A) For those infected movement cattle herds outside STCAs or in fringe or non-endemic areas, apart from those monitored by slaughter house surveillance, a whole herd test must be performed within six months of the date of diagnosis of any tuberculous cattle. The ideal re-testing interval for these infected movement control herds is three or four months, but if this is impractical for reasons of cattle management, such as late pregnancy or early lactation, the cattle should be re-tested within two to six months. Where the period prevalence is 2% or greater, the farmer is strongly advised to test at three to four months since this will help to overcome problems of test sensitivity
- B) The re-test interval may be between two and twelve months for infected movement control herds within endemic areas. The interval chosen will be influenced by any current or proposed possum control programme.
- C) The ideal re-testing interval for clear movement control herds, irrespective of area designation, is three to twelve months. In determining the interval, the following points are considered, viz. whether tuberculous non-reacting cattle are still likely to be in the herd (large number of tuberculous cattle at the test before the last; or a tuberculosis problem that has persisted over twelve months), the cumulative incidence and the current, or any proposed, possum control programme.

Tb-free herds outside special Tb control areas

- A) For beef breeding and dairy herds with a Tb-free status, the standard re-test interval is twelve months. However, where these herds have a history of chronic tuberculosis or a high incidence of tuberculosis, a re-test interval of six months is considered.
- B) For miscellaneous herds other than dealer herds, the interval between successive tests should not exceed three years. Where the herd has been on movement control it should be tested every twelve months until it has been free of tuberculous cattle for a minimum of three consecutive herd tests over a period of not less than two years.

- C) When tuberculosis is traced back to a dealer herd, identifying the source of infection is costly and inefficient. Typically there is a high turnover of stock in dealer herds. Cattle are brought from and sold to a variety of outlets, with the aim of maximising returns. To this end cattle are variously slaughtered, sold either at public or private sale, or kept to raise calves. In the interim, cattle may be mixed with other mobs and grazed on a variety of properties. Some dealers purchase and slaughter large numbers of cull dairy cows. The inability to trace back infected dairy cows in particular, is a small but costly hole in the tuberculosis surveillance system. Unless trace back investigations implicate them as a source of tuberculosis, it is not cost-effective to monitor dealer herds by testing.
- D) The tuberculosis status of beef dry-stock herds is monitored by routine meat inspection of cattle sent for slaughter. Each year, the number of lesioned non-test animals is equivalent to about 25% of the number of reactors slaughtered. Less than 50% of lesion non-tested come from beef dry-stock herds. These herds, however, provide poor surveillance information because identifying the source of tuberculosis is costly and has a low probability of success because:
- dry-stock farmers often do not know the herd of origin or how long the cattle have been on their property.
 - slaughter plants cannot record animal identification or earmarks.
 - all cattle from these herds will be sent for slaughter and the herd will be identified as infected from slaughterhouse inspection.

Provided beef dry-stock herds are not under movement control, there is no real need to monitor these herds by live animal testing. There are generally only two age classes of cattle on beef dry stock properties as weaners are bought in and fattened for slaughter at 20 -28 months. Because of the short time animals spend on these properties, tuberculosis is unlikely to become established.

Ancillary testing

The objective of ancillary testing is to assist the veterinarian to correctly identify infected and non-infected animals. Ancillary tests are used to increase specificity but inevitably will have lower sensitivity than the primary screening test. Therefore the major risk in using these tests is that truly tuberculous animals will be test negative (false negatives) and will remain as a potential source of infection to the rest of the herd and feral/wild animals.

The following guidelines assist veterinarians in the use of ancillary tests.

1. Designated area status. The justification for using an ancillary test is stronger if the herd is located in a surveillance area because the risk of an animal becoming infected with tuberculosis is lower. The opposite is true in STCAs. However, there are situations in STCAs where other factors are such that ancillary testing may be warranted.
2. Current herd status. In general, ancillary tests should not be used in movement controlled herds because ancillary tests have reduced sensitivity. Nevertheless, the cautious use of ancillary testing may be justified in herds which have mixed infection of *M. bovis*, *M. avium*, or organisms (e.g. actinomycetes) which may sensitise the animals to bovine tuberculin. Evidence of non-specific sensitisation includes location, previous testing history, clinical factors (e.g. skin Tb) and culture results (*M. avium* isolates). If the lesion reactor rate of the screening test for the herd, or a particular age group, is consistently less than 20%, then a non-specific sensitisation to bovine tuberculin probably exists.
3. The number of standard test positives. The lower sensitivity of the ancillary tests (75%-90%) means that the probability of identifying an infected herd is lower. Therefore, except in surveillance areas, ancillary tests should not be used in herds where there are only a small number of standard test positive animals. In contrast, where there are four or more standard test positive animals which are tuberculous, the probability of identifying the herd as infected is almost 100 %. If the number of standard test positive is significantly larger than expected in herds for that area, a sample should be slaughtered.
4. Clustering of test positives by age and breed. In certain localities, non-specific sensitisation in cattle occurs predominantly in animals one to three years old. This is known as the "heifer reaction". On the other hand, sensitisation caused by skin Tb is usually distributed through all ages of adult dairy cows. The prevalence of skin Tb should be considered before using ancillary tests on adult dairy cows. Dairy cattle are more likely to have non-specific sensitisation than beef cattle.
5. Previous ancillary tests. In general, the probability of an animal with non-specific sensitisation being standard test positive, ancillary test negative and then standard test positive at the following whole herd test is low. It probably continues to react to the standard test because it is tuberculous. It is important that animals passing an ancillary test have their identification noted. Any animal found to be standard test positive after having a preceding ancillary negative test should be slaughtered.
6. Sampling. If there is a large number of standard test positive and evidence of tuberculosis in the herd, a sample (six to ten) of test positive animals should be slaughtered. If there are

all classed as non visible lesion reactors, then ancillary testing should be considered for the remaining animals.

7. Tuberculosis identified in ancillary test positive animals. In most herds where tuberculosis is found in ancillary test positive animals, all ancillary test negative animals should be designated reactors and slaughtered.
8. Assessment of other risk factors. Other risk factors taken into consideration are:
 - were test positive introduced into the herd since last test?
 - were test positive grazed apart from the rest of the herd?
 - proximity of the property/run-off from a potential tuberculous possum habitat

Use of a comparative cervical test less than 60 days after caudal fold test:

In extra-ordinary circumstances, such as the sale of a herd which precludes waiting the required 60 days before applying the CCT, the responsible veterinarian may authorise the test to be applied earlier. Such authorisation should only be given where there are four or more test positive cattle, using the rationale that if four are tuberculous then there is a reasonable probability that at least one will react positively to the CCT.

In cases where there are less than four test positive cattle and the test result is required in less than the required 60 days, the animals have to be slaughtered as reactors.

Use of transitional status

Transitional Tb status may be applied either to herds with no previous testing history, or to herds whose current tuberculosis status has been suspended.

Clear MC herds which might normally proceed to a Tb-free status may also have their status set to transitional where the responsible veterinarian has insufficient epidemiological information to declare the herd as being Tb-free. Under these conditions the status would be regarded as a suspension of a Tb-free status.

When determining the final status the following factors are taken into consideration:

- current herd status
- number of reactors post mortem
- type of post mortem (nominal or critical)

- number of animals re-tested
- previous testing and Tb history
- status of in-contact farmed and feral animals
- status of neighbouring deer and cattle herds
- origin of reactors
- farm management practices

Tuberculous cattle

The veterinarian with responsibility for the herd determines whether reactors shall be deemed to be "tuberculous cattle". A full post mortem examination and report is normally done for each reactor. Where reactors are slaughtered but no necropsy is performed, these are deemed to be "tuberculous cattle" and a movement control herd notice is issued (herd status becomes infected MC).

Lesions which have a gross appearance of *M. bovis* are required to be submitted for histological examination. If histology is suspicious or positive for tuberculosis from cattle in non-movement controlled herds located in surveillance, non-endemic and fringe areas, mycobacterial culture should be requested in all cases from stored tissues collected postmortem.

When lesions are consistent with *M. bovis*, either by histology or culture, these reactors are deemed to be tuberculous, and an infected MC status applied to the herd.

TESTING POLICIES FOR SPECIAL TUBERCULOSIS CONTROL AREAS

The testing of non-movement controlled herds located in STCAs is programmed to maximise the identification of infection in feral or wild animals populations, and is not necessarily related to the herd's tuberculosis status.

The following guidelines are the recommendations for the approach taken when testing non-movement controlled herds in endemic, fringe and non-endemic areas.

Endemic Areas

Where tuberculosis eradication is being attempted

Eradication may be attempted for an entire endemic area or as part of a planned roll-back operation.

Computer models predict that eradication may be achieved by large scale possum control operations which reduce possum numbers by 70% or over initially, and where there is no immigration of tuberculosis infected possums. The possum population must then be maintained at about 20% of its original size by possum control operations applied annually or biennially (maintenance control) for the next seven to ten years.

A well considered cattle and deer testing policy linked to farm management and grazing practices is the most cost-effective way of identifying the probable location of tuberculous possums.

Within an enterprise, linking tuberculous reactor and non-reactor herds with the paddocks they grazed helps to define areas where tuberculous possums are likely to be located. Grazing patterns of non-reactor herds are also important in defining possible clean areas. This analysis should ideally be completed within one month of the last testing results becoming available from neighbouring farms.

Associations may also be investigated on a locality or district; e.g. all farms adjacent to a stream or patch of bush had tuberculous reactors. This information allows pest control agencies to target suspected tuberculous possums during their maintenance work and where grazing management and weather allows, pest control is usually undertaken within 6 to 8 weeks of finding tuberculous reactors.

The following basic policy is applied to all herds in the area for the first five years of possum control:

When all herds in the control area have been free of tuberculosis for five years, testing can revert to that of a non-endemic area. After seven years of tuberculosis-freedom, surveillance testing can be re-instated.

Veterinarians are aided in the assessment of the risk of cattle to cattle transmission by conducting full post mortem examinations on all reactors. If a "tuberculous spreader" (open case) is identified at post mortem this must be considered as a source of infection, although it does not rule out possums as a source unless there is clear evidence that possums are not involved.

The area livestock officers endeavour to keep in regular contact with farmers of beef dry-stock and miscellaneous cattle dealers, and by so doing, can make reasoned judgements as to whether reactors originated from within the control area. Good farmer records showing the origin of all cattle are of considerable assistance, and farmers are increasingly encouraged to keep accurate records as more responsibility for control devolves to them. If tuberculosis is identified at a later date, information is then available to judge whether or not the infection originated on that property.

Testing policy immediately prior to possum control

Clear tests immediately prior to any possum control gives a reasonable assurance that herds are clear of infection. Any infection identified at a subsequent test is more likely to have originated from an external source (e.g. possums) than from within the herd. This assumption is tempered by the number of reactors at the test prior to possum control and the number of "spreader" animals found at post mortem.

All herds in the area being controlled for possums should be tuberculin tested within 3 months of the initial possum control operation.

Testing policy options following an initial possum control operation

Option 1 - The ideal testing frequency

Ideally all herds in the possum control area should be tested within six months of the operation being completed. Regardless of status, all herds should be tested twice yearly for the first two years. Testing however has to take into account management constraints and partial herd tests may need to be employed in some herds.

Option 2

This option is sometimes used in areas where farms are extensive. Only MC herds and their close neighbours are tested twice yearly until the MC herds reach accredited Tb-free status.

The balance of herds in the area are only tested on annually.

Testing policy two to five years after an initial possum control operation.

Experience has shown that the number of tuberculous possum may increase during the late part of this period, particularly if hot spots re-emerge or possum maintenance control fails and in these circumstances increased numbers of reactors and/or the number of herd breakdowns can result.

Testing policy for herds not under movement control nor a close neighbour to a MC herd

All herds which are neither under movement control nor a close neighbour to a MC herd must be tested annually, with the exception of beef dry-stock in which all animals are slaughtered annually. If dry stock herds have animals which are held over for longer than 12 months then they are also tested annually, preferably at a time when stock numbers are lowest.

Testing policy for herds under movement control or a close neighbour of movement controlled herd

It is important to try to identify the source of infection in cases where herds are either on movement control or coming on and off movement control. If the infections are possum related then testing in conjunction with collaboration with the pest control agency in their maintenance programme will facilitate the elimination of infection.

Until the breakdown herd attains an accredited Tb-free status, the movement controlled and neighbouring herds tested twice yearly.

If tuberculosis testing and directed possum control maintenance fails to prevent herd infection within three years of initial control starting, an in-depth epidemiological study should be undertaken on these infected herds. Some simple epidemiological analyses, in which herds which have been successfully cleared of infection are contrasted with herds which have failed, can produce reasonable hypotheses about the cause of the infections. If possums continue to be implicated, the MAFQUAL veterinarian, the consulting veterinarian and possum control

experts should explore other ways to eradicate tuberculous possums. A further intensive possum control operation may be required and consideration should be given to other potential sources of infection (ferrets, feral pigs, deer etc).

Testing policy five years after initial possum control operation

In depth epidemiological investigations are particularly warranted when a breakdown occurs five years after an initial possum control. It is important that the disease is contained if infection is possum-related and intensive possum control should therefore be directed at all favoured possum habitats within 4-5 km, and maintenance work should be targeted on infected herds. In such cases, the breakdown herd and its neighbouring herds should be tested twice yearly. Herds that are contiguous with good possum habitats targeted for special attention should be tested on an annual basis.

Under normal circumstances accredited herds should have breeding stock tested annually or biennially, with decisions on which classes of animals to test and the frequency of testing based on:

- distance from any infected herd
- the relative density of cattle grazing between the herd in question and any infected herds
- time that the herd has been accredited
- number of animals under test
- proximity of the herd to favourable possum habitat
- seasonal possum food availability on the property

Testing requirements for beef dry stock are unchanged from those applied in the early stages of the area campaign.

Where tuberculosis eradication is not being attempted

In large endemic areas, it is not possible to eradicate tuberculosis from the possum population. However, a realistic goal is to restrict the spread of tuberculous possums from the endemic area. Control policies state that localised possum control operations will be undertaken within these areas if they can be shown to be cost-effective in terms of savings in reactor compensation (by a benefit cost analysis), or are required to reduce the probability of tuberculous possums crossing buffers

Testing policy for herd in localised possum control areas

All herds in localised possum control areas are required to be tested annually, with the exceptions of beef dry-stock and miscellaneous herds, to which the usual conditions apply.

HERD BREAKDOWN INVESTIGATION

Herd Breakdown Investigation Outside Endemic Areas

Investigation of suspect tuberculosis cases and confirmed breakdowns in herds which lie outside STCAs and those within fringe and non-endemic areas are given high priority. It is recommended that this task should be initiated within two weeks of receiving histopathology results using the following guidelines:

Investigation where *M. bovis* is not suspected

Where the controlling veterinarian has good evidence that a suspect tuberculosis lesion is unlikely to be caused by *M. bovis* (e.g. those herds in surveillance areas where *M. bovis* has never been cultured from lesion non-tuberculous animals) a movement control herd notice need not be issued, nor a herd breakdown investigation undertaken. However, the herd status is changed to transitional and all breeding animals and animals of the same age, or management cohort, as those with lesions should be tested within 90 to 120 days. The normal testing procedure for transitional herds is then followed.

Investigation where *M. bovis* is suspected or confirmed

A herd breakdown investigation is required to be undertaken for all breakdowns where *M. bovis* is suspected. The objective of the herd breakdown investigation is to provide the supervising veterinarian with sufficient information to produce a plan to efficiently eradicate or control the infection. In order to do this, he needs to develop and rank hypotheses as to the source and means of spread of tuberculosis within the herd. This allows him to then put into action a plan to provide the necessary trace-back and trace-forward contacts. A livestock officer (LO) is normally assigned to the herd to assist the investigation since LOs usually have a sound knowledge of the physical characteristics of the region and a close understanding of farmers and farming practices in the vicinity of the herd.

The herd breakdown investigation is looked at on two levels; the first is on the basis of receiving histopathology results and the second when *M. bovis* is confirmed either through testing or histopathology/culture. The second level may also be undertaken where the number of reactors generalised cases or locality of the herd points to a need for a more detailed investigation.

The first level of the herd breakdown investigation is undertaken by the livestock officer within two weeks of receiving the first indications of disease e.g. test positive or histopathology. He then develops a plan which includes:

- testing of in-contact cattle on the property where appropriate
- assigning the next herd test date
- detailing the test eligible cattle on the property and making sure that the farmer understands the need for a complete muster
- determining whether tuberculous animals had been purchased into the herd, and if so, identifying their origin. Where possible he records the dates of purchase and names of vendors or saleyards from which cattle were purchased. Sale yard records may be used to identify vendors
- determining whether the owner has any remaining in-contact animals or animals from the same source, and if so, where they have been grazed
- determining whether any in-contact animals or animals from the same source, have been sold, and if so to whom where and when. These animals are then forwards traced and tested within 90 days of contacting the purchaser

At the second level of herd breakdown investigation the controlling veterinarian and livestock officer prepare an epidemiology report. It is a regulatory requirement that this report be completed within four weeks of receiving confirmation of the disease.

The epidemiology report should:

- include a map showing the locality of the farm and all close neighbours defined by farm type, stock held and tuberculosis status
- provide background information on the herd infection
- outline the probable source of infection

- outline probable means of spread within the herd if more than one animal was found infected
- outline a plan which includes:
 - the ongoing herd testing programme and policy on ancillary testing
 - casing and testing all cattle herds on properties that have contiguous or near contiguous boundaries with the infected herd, and also taking account of geographic features and feral animal habitat. Neighbouring herds not on movement control may have their status changed to transitional until check testing is complete, although this is not required where there is unequivocal evidence that infection has been brought in with cattle
 - a recommendation for a possum survey control if necessary. If the investigation indicates that in all probability, infection did not occur as a result of brought-in stock, tuberculous possums must be considered as a likely source of infection
 - give an estimate of the number of reactors expected at the next whole herd test and an estimated date for eradication. These predictions should be based on the most likely source of infection, the nature of the lesion sites, the number of tuberculous animals, test sensitivity (tuberculous cattle are more likely to have passed a caudal fold test where a modified interpretation has been used).
 - provide for alternative actions to be taken if the actual results differ significantly from predicted results

This epidemiology report becomes part of the herd history. It is a dynamic report and should be continually updated. The hypotheses will need to be re-evaluated as actions are completed.

Advice or assistance are sought from the consulting veterinarian when:

- herds fail to reach "clear movement" status after two re-tests
- infection is re-identified in herds that have reached "clear movement control", "Tb-free", or "accredited" status
- infection is identified in herds neighbouring a MC herd.

Testing of neighbouring herds

If a herd breakdown investigation cannot rule out tuberculous feral/wild animals as the possible source of infection, neighbouring herds should be tested to assist in confirming the source and defining the extent of the tuberculosis problem.

All neighbouring herds to the infected herd are tested within three months following the outbreak unless they have been tested within the previous six months. Test requirements are that all dairy cattle over six months of age and all beef cattle over three months of age are tested, unless, as may happen in beef herds, the total herd is to be slaughtered within the next three months. If this latter action is prescribed by a farmer, the procedure is audited. Any cattle remaining after three months are then tested.

Unless already on movement, cattle are caudal fold tested using 1 mg/ml tuberculin. Two mg/ml tuberculin is used for movement control cattle herds. All tests should use standard interpretation. All neighbouring herds are required to remain under an annual testing programme until the breakdown herds have reached a Tb-free status. If any of the neighbouring herds are found to be infected, a herd breakdown investigation is then performed.

HERD BREAKDOWN INVESTIGATION WITHIN ENDEMIC AREAS

A herd breakdown investigation must be undertaken for each new breakdown i.e. where the herd was not on movement control. The level to which the investigation is taken depends on the possum control programme for the herd's location.

A complete herd breakdown investigation is required when area eradication is being attempted and where an emerging pattern of disease requires clarification.

A herd breakdown investigation which is limited to on-farm information is required when a localised possum control operation is being performed and data from the herd breakdown investigation may assist in this work.

Possum control programmes

Possum control programmes are an integral part of tuberculosis control. The routine testing of cattle, especially within the STCA is designed to identify sources of feral/wild animal infection or the success of feral/wild animal control.

Possum control programmes are designed to achieve one or more of the following objectives:

- preventing the establishment of infection in feral/wild animal populations which had been clear of the disease previously

- containing the geographical spread of disease
- determining prevalence of tuberculosis in feral/wild animal populations
- eradication of disease in feral/wild animal populations
- reducing expenditure on compensation for cattle reactors

Possum survey

Possum surveys are normally undertaken in surveillance, fringe and non-endemic areas but only with the approval of the consulting veterinarian since the cost of surveys are usually met by MAFQUAL.

A sample of possums from a defined population are autopsied to determine, with a specified degree of confidence, the prevalence of tuberculosis in that population. The size of the sample is calculated as in the following example:

Area of farmland = 100 ha

Estimated possum per ha = 6.4

Therefore the total no of possums = 640

The prevalence of disease is assumed to be 0.5%.

The sample size for autopsy can be estimated from tables, Carnon and Roe (1982) who use the following formula for calculation for sample sizes.

$$n = [1 - (1 - a)^{1/D}] [N - (D - 1)/2]$$

where n is the required sample size

a = probability (confidence level) of observing at least one diseased animal in sample when the disease affects at least D/N in population

D = number of diseased animals in population

N = population size

Example calculation:

$$n = [1 - (1 - a)^{1/D}] [N - (D - 1)/2]$$

$$n = [1 - (1 - 0.95)^{1/3.2}] [640 - (3.2 - 1)/2]$$

$$n = [1 - (0.05)^{0.3125}] [640 - 1.1]$$

$$n = [1 - .392] [638.9]$$

$$n = [0.608] [638.9]$$

$$n = 388$$

Thus approximately 400 possums would need to be autopsied to be 95% confident of detecting disease if it existed at a level of 0.5%. This calculation assumes that the diseased animals are randomly distributed throughout the population, and for possum surveys, the estimate should be regarded as the minimum number required.

Surveys are an insensitive technique for detecting disease when the prevalence of disease is low and/or the feral/wild animal population density is low.

Brief locally initiated possum control

Brief locally initiated possum (BLIP) control may be undertaken in surveillance, fringe and non-endemic areas in response to an in-charge veterinarian's request where a herd breakdown cannot be attributed to introduced farmed cattle, or the breakdown may lead to the transfer of infection into the wild/feral animal populations.

Brief locally initiated possum control will reduce the probability of local wild/feral animal infection occurring and reduce the infected population if disease has already established. Brief locally initiated possum control therefore provides breathing space to test surrounding herds to define the extent of a possible problem while minimising the risks. Brief locally initiated possum controls are funded by regional councils.

POSSUM CONTROL STRATEGIES

Possum control strategies for STCAs are based on benefit-cost analyses. Computer simulation modelling of tuberculosis in the possum population suggest that tuberculosis can be eradicated from possum populations if they are maintained at 40% of their carrying capacity for up to ten years and tuberculous possums do not migrate into the area (Barlow, 1991). Accordingly, a 70% reduction in possum numbers needs to be achieved in the initial year of the operation and thereafter 20-30% of the population should be removed during annual maintenance programmes.

Eradication of Tb from the possum population in endemic areas

The non-endemic areas contain minor endemic areas which are well defined geographically and these areas are termed Special Tuberculosis Investigation Areas (STIAs) (Livingstone, 1992). It is conceded that the disease cannot be eliminated from STCAs at this stage but

eradication of disease from STIAs is considered technically feasible and is currently underway in some of these areas.

Containment of spread from endemic areas

In major endemic areas it is currently not feasible, either technically or financially, to eradicate tuberculosis from the feral/wild animal population. One of the strategies for these areas is to restrict the spread of disease out of the endemic area by the placement of "buffers".

Buffers

Natural geographical features (mountain ranges and major rivers) are used as barriers wherever possible. However, where these are absent, low density possum buffers within the fringe areas may be established to protect areas from infection. Normally these buffers are 3-5 km wide but their effectiveness may be improved by extension to include areas of favoured possum habitat, e.g. possum populations may be reduced for 10 to 15 km along all catchments that drain the endemic area. Where the prevalence of infection is high, reducing the density of tuberculous possums on the endemic side of the buffer reduces the risk of tuberculosis spreading.

It is not possible to place buffers around all endemic areas, especially where they adjoin large tracts of Department of Conservation estate and exotic forest. Movement of the tuberculosis front in these areas is monitored by checks on feral deer going through Game Depots and information from private hunters and trappers.

Reducing reactor numbers

Possum control is also undertaken to reduce reactor numbers in endemic areas if a benefit-cost analysis shows this to be economic (i.e. the projected savings in compensation for cattle reactors outweighs the cost of possum control). This is normally undertaken on a localised area basis as defined by testing and associated grazing management information.

FUNDING

Initial operations are funded entirely by Ministry of Agriculture and Fishery (MAF). Maintenance operations are funded 67% by regional councils (ratepayer) and 33% by MAF. All possum control work must comply with MAF specifications. As at 1995 \$16.1 million per

annum is spent on vector control of which the Crown funds \$10 million, the regional councils \$3.5 million and the remainder by the slaughter levy (O'Neil and Pharo, 1995).

STRATEGIC PLANNING

In October 1992, the AHB released a draft strategic plan (AHB 1992), since adopted, in which 4 major objectives were identified for realization by the end of 1998:

- To reduce the percentage of movement control (MC) herds, deer and cattle combined by 30 - 50% in each endemic area.
- To reduce the percentage of MC herds in the non-endemic areas to 0.2%, which is the internationally recognized level for official freedom from tuberculosis.
- To prevent the establishment of new endemic areas and expansion of existing endemic areas into farmland free of tuberculous feral/wild animal vectors.
- To encourage individual farmers to take responsibility for the control of tuberculosis within their herds.

At October 1992, only 2 regions, Northland and Hawkes Bay had less than 0.2% MC herds, although Taranaki and Gisborne were approaching that level.

The definition of specific objectives to be completed within a set time frame is a positive action which corrects a serious weakness in past administration of the scheme. The adoption of time limited goals is expected to lead to regular reviews of progress with prompt attention to problems, and improve commitment in personnel working in the field of tuberculosis control.

CHAPTER 4

The epidemiology of bovine tuberculosis in cattle herds in the Masterton and Taumarunui veterinary districts and Surveillance areas

INTRODUCTION

Herd disease history data are routinely collected as part of the conduct of the national tuberculosis eradication program and are stored in a central data base. The data includes herd and individual animal test results, necropsy results, cases detected at slaughter and alterations to the disease status of individual herds. The database is useful for procedural operations of the testing program such as generating testing instructions for technicians, but a variety of statistical reports can also be generated from the database for use in analyses which focus on key elements of the disease control strategy. These key elements include indications of new infections in wildlife and cost benefit analyses of possum control operations, in which the benefits of the expected reduction in risk of disease to farmed cattle and deer are balanced against the estimated costs of the pest control programmes. The information system is constantly reviewed and updated when necessary to keep it relevant to the needs of staff and to maintain the overall efficiency of the control program.

A case control study (Pfeiffer et al., 1991, Pfeiffer, 1995) used national database information to identify risk factors associated with the establishment of tuberculosis in herds (breakdowns), but apart from that study and occasional ad hoc analyses during investigations of local problems, the potential epidemiological value of the records has seldom been exploited.

The objectives of this study were:

- a) to describe and compare the epidemiology of the disease in herds with a history of tuberculosis in a long established endemic area, (Masterton veterinary district) a recently established endemic area (Taumarunui veterinary district) and surveillance areas throughout New Zealand.
- b) to assess the value and adequacy of routinely collected data for epidemiological analyses which could be used to improve the understanding of the disease and improve the quality and effectiveness of the national disease control program.

MATERIALS AND METHODS

Source of data

The data used in these studies were supplied by the Ministry of Agriculture and Fisheries, Ruakura Agricultural Centre, Hamilton, New Zealand, and were derived from movement control herd histories in the Taumarunui and Masterton veterinary districts and surveillance

areas throughout New Zealand. The data were collected during the conduct of the national bovine tuberculosis control and eradication scheme to measure the progress of the scheme and for various analytical purposes associated with the conduct of the program. The data were stored in a SIR (Scientific Information Retrieval, SIR) relational database. Data from the Taumarunui, Masterton and surveillance areas were taken from records for the period 1984 to 1990.

Taumarunui veterinary district data base

The tuberculosis herd history data set for Taumarunui comprised records from 484 herds. Three hundred and thirty (330, 68%) were beef breeding herds, 133 (28%) beef dry-stock herds and 14 (3%) dairy herds. There was 1 miscellaneous herd and 6 others which were currently noted as containing no animals that required testing for tuberculosis.

The distribution of herds by tuberculosis area class was 418 (86%) in endemic areas, 50 (10%) in fringe areas, 3 (1%) non-endemic areas and 13 (3%) in surveillance area.

Masterton veterinary district data base

The herd history data from Masterton comprised records of 582 herds, of which 342 (59%) were beef breeding, 64 (11%) were beef dry stock, 140 (24%) were dairy herds. There were 35 miscellaneous herds and one with non-eligible stock.

The distribution of herds by tuberculosis area class was 556 (96%) in endemic areas and 26 (4%) in fringe areas.

Movement control herds in surveillance areas throughout New Zealand

The 287 herds located in surveillance areas throughout New Zealand comprised 94 (33%) beef breeding herds, 36 (13%) beef dry stock herds, 140 (49%) dairy herds, 9 (3%) miscellaneous stock, 4 (1%) miscellaneous herds, and 4 (1%) non-eligible stock.

Calculation of measures of disease occurrence and data analysis

The data were transferred from SIR into the data base program, Panacea (Pan Livestock Services, Reading, England). Analyses were conducted in this program and in SOLO, (BMD Statistical Software, Inc., USA), in Statistix (Analytical Software, St. Paul, Minnesota, USA), and in SAS (SAS Institute Inc., Cary, North Carolina, USA). Figures were constructed in

Quattro Pro (Borland International, Scotts Valley, California, USA) and in Excel (Microsoft Corporation).

Epidemiological measures of interest were calculated using the following formulae:

Cumulative Incidence (CI)

$$\text{Average annual CI per 100 cattle} = \frac{\# \text{ of reactors}}{\# \text{ of animals tested} \times \# \text{ of years}} \times 100$$

The denominator used for the calculation of annual cumulative incidence was based on the average number of animals tested. The term cumulative incidence, as used throughout the text, refers to average annual cumulative incidence per 100 cattle and the notation used is (CÎ) per 100 cattle or (CÎ) $\times 10^{-2}$. Unless otherwise stated, animals tested for sale or for miscellaneous tests were not included in the calculation of the denominator. All animals that reacted to the caudal fold intradermal tuberculin test were included in the numerator for cumulative incidence.

Other cumulative incidence parameters were corrected cumulative incidence and the annual corrected cumulative incidence of reactors with lesions per 100 cattle. These were calculated using the following formulae:

$$\text{Corrected cumulative incidence} = \frac{\text{Cumulative incidence}}{\# \text{ of days between whole herd tests in consecutive years}} \times 365$$

$$\text{Percentage of reactors with lesions} = \frac{\# \text{ of reactors with lesions}}{\# \text{ of reactors}} \times 100$$

$$\text{Annual CI of lesioned reactors per 100 cattle} = \frac{\# \text{ of reactors with lesions}}{\# \text{ of animals tested} \times \# \text{ of years}} \times 100$$

Incidence density (ID)

Because many incidence densities were very low, it was decided that for ease of interpretation they would be best expressed as rates per 100 cattle. Notations used throughout the text include (ÎD) $\times 10^{-2} \text{ year}^{-1}$, (ÎD) $\times 10^{-2}/\text{year}$.

$$I D \text{ per } 100 \text{ cattle per year} = \frac{\# \text{ of reactors}}{\# \text{ of animal} - \text{ years}} \times 100$$

The numerator used in the calculation of incidence density was the same as that used for the calculation of cumulative incidence.

Cumulative incidence and incidence density were calculated for herd size groups (herd sizes, 1-100, 101-200, 201-300, 301-400, 401-500 and <500 animals), for tuberculosis area classes (endemic, non-endemic, fringe and surveillance) and for herd types (beef breeding, beef dry stock and dairy herds).

The incidences of lesioned reactors and incidence densities in the periods one, two and three years after TB breakdown were calculated.

Student's paired t-test was used to determine whether incidence measures calculated using different test years, viz. calendar, financial or test year, were significantly different.

Linear regression was used to evaluate relationships between incidence statistics and the distance to the nearest infected herd, and for case herds, to evaluate the relationship between the distance from the Rangitoto buffer and incidence statistics.

Logistic regression was used to examine the simultaneous effect of the independent variables, herd type, area class, veterinary district (Taumarunui, Masterton and surveillance), number of tested animals and the year of test on cumulative incidence (dependent variable). The same explanatory variables were examined using Poisson regression to model their effects on incidence density.

Approximate 95% confidence limits for Odds Ratios and Relative Risks were calculated according to the following equation:

$$95\% \text{ confidence limits} = e^{\beta_i} \pm [1.96 \times \text{Std. Error}^{(\beta_i)}]$$

The length of time individual herds were on movement control was studied using survival analysis. The statistical relationships between the probability of becoming clear of infection and potential risk factors were analysed using Cox's proportional hazard regression model. For survival analysis calculations, only herds which had no reactors at the beginning of the study

period were included. Herds which remained on movement control over the whole period were considered as right censored information. To deal with the problem of herds which experienced multiple movement control episodes, only the first episode was included in the analysis

Pearson product and Spearman rank correlation coefficients were calculated as measures of the relationships between the various measures of disease occurrence. The Spearman rank correlation is the preferred measure when data are not normally distributed (Dawson-Saunders and Trapp, 1990) and this was the case for the most of the data reported herein.

RESULTS

DESCRIPTIVE EPIDEMIOLOGY

Cumulative incidence in herds classified by herd size: Taumarunui region

The distributions of cumulative incidence levels varying from 0 to 7.0 per 100 cattle for six categories of herds classified by size of herd in the Taumarunui district are shown in Figure 4.1. The annual cumulative incidence was less than 1.0 per 100 cattle for 30-50% of herds in categories other than the smallest herd size of 1-100 animals.

The smallest herd size category (1-100 cattle) showed a strikingly different pattern, with over 30% of herds having an annual incidence > 6.0 per 100 cattle.

Figure 4.2 shows in more detail the distribution of cumulative incidence between 0 and ≥ 2.0 per 100 cattle, classified by herd size for the same herds in the Taumarunui district. About 60% of herds with 1-100 cattle had an annual cumulative incidence ≥ 2.0 per 100 cattle. About 30-40% of other herd sizes had very low cumulative incidences, produced by only 1 or 2 reactors per herd per annum.

Some herds are shown as having no reactors. They represent those herds which started out on movement control and subsequently had no reactors at later tests. Under the policy rules of the national tuberculosis control program, herds on movement control were required to have two consecutive clear whole herd tests before they could have their movement control status revoked.

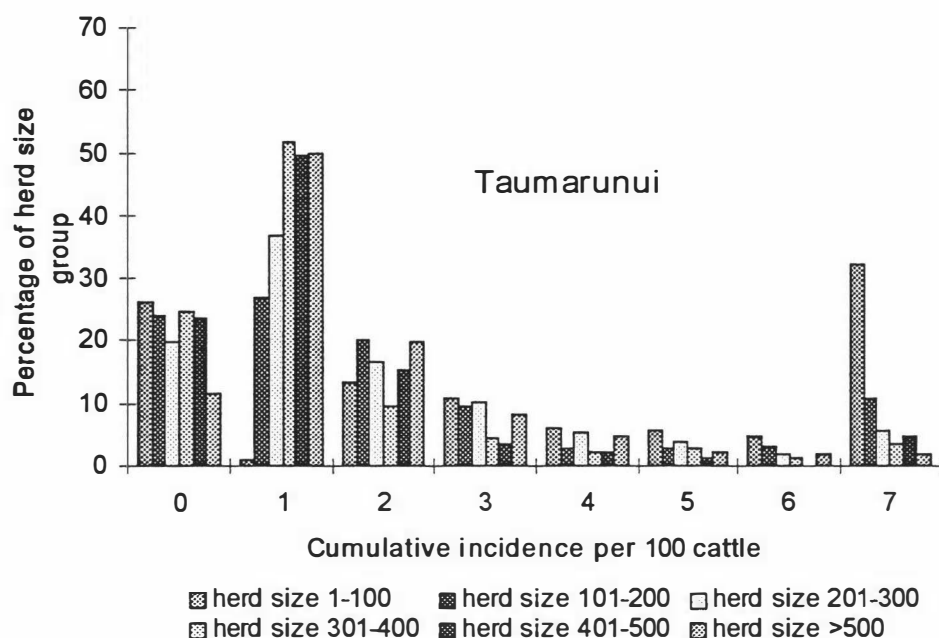


Figure 4. 1. Taumarunui district. The relative frequencies of all levels of cumulative incidence from 0 to 7 per 100 cattle in six categories of herds classified by size of herd

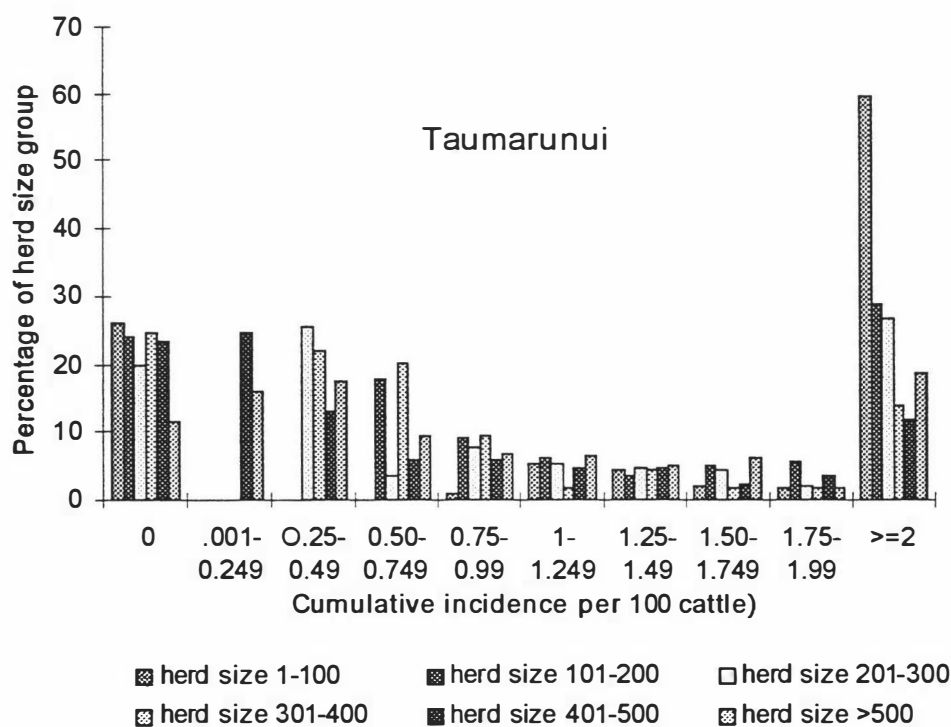


Figure 4. 2. Taumarunui district. The relative frequencies of all levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in six categories of herds classified by size of herd

Incidence density classified by herd size: Taumarunui district

The pattern of the distribution of incidence density for the range of herd sizes illustrated in Figure 4.3 is similar to that for cumulative incidence seen in Figure 4.1. Twenty-five to 55% of herds with more than 100 animals had an incidence density less than $1.0 \times 10^{-2} \text{ year}^{-1}$. The proportion of herds reduced linearly with increasing levels of incidence but smallest herd size category of 1-100 cattle again followed a different pattern to herds with greater numbers of cattle by having about 35% of herds with an incidence density $> 6 \times 10^{-2} \text{ year}^{-1}$.

The distribution of incidence densities from 0 to $2 \times 10^{-2} \text{ year}^{-1}$ for the different herd size categories is shown in Figure 4.4. The pattern is similar to that for cumulative incidence with the smallest herd size category of 1-100 cattle again poorly represented. Sixty percent of that herd size category had an incidence density $\geq 2 \times 10^{-2} \text{ year}^{-1}$. About 30-40% of herds in the other herd size categories had very low incidence densities, representing 1 to 2 reactors per annum.

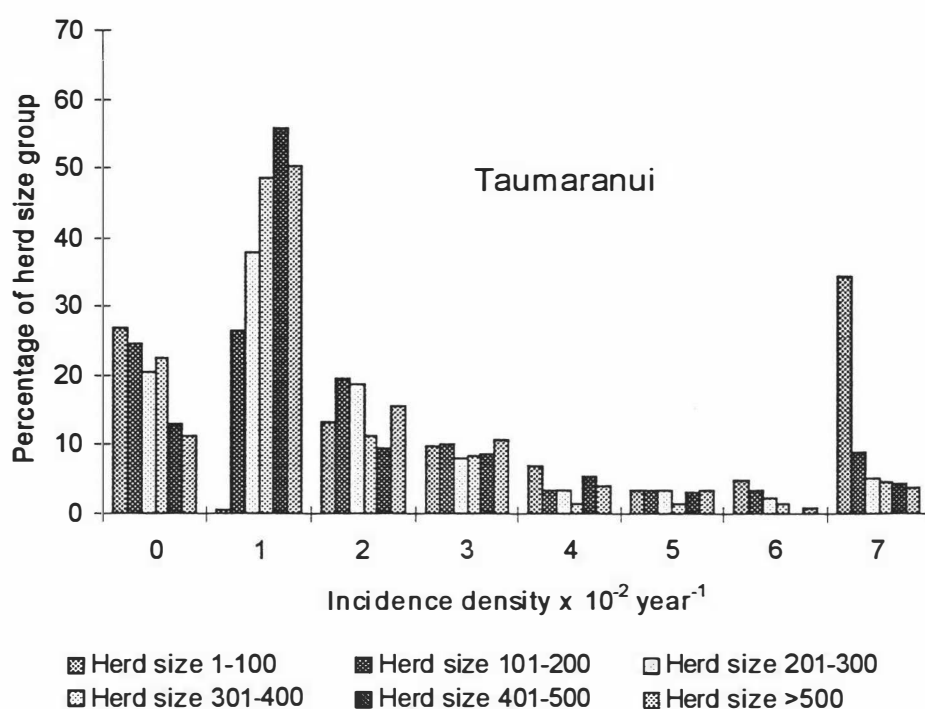


Figure 4.3. Taumarunui district. The relative frequencies of all levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by size of herd

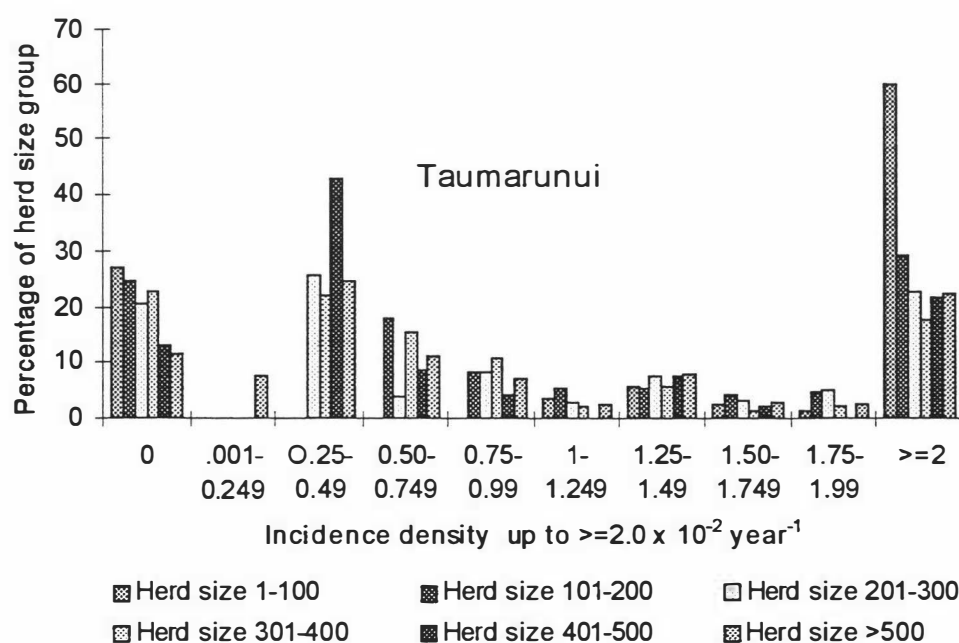


Figure 4.4. Taumarunui district: The relative frequencies of all levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by size of herd

Cumulative incidence classified by herd size: Masterton district

With the exception of the two smallest herd size categories of 1-100 and 101-200 cattle, cumulative incidence for the Masterton district herds broadly followed the same pattern found in the Taumarunui district. Thirty to fifty percent of herds with more than 200 cattle had an annual cumulative incidence < 1.0 per 100 cattle. The percentage of herds steadily decreased in line with increasing levels of cumulative incidence. In the Masterton district, 34% of herds had an annual cumulative incidence < 1.0 per 100 cattle whereas there were 28% of Taumarunui herds of that same level of cumulative incidence. The cumulative incidences in Taumarunui and Masterton herds of 101-200 cattle were significantly different (chi-squared = 34.9, $P < 0.05$).

The distribution of cumulative incidence from 0 to 2.0 per 100 cattle for different herd sizes is shown in Figure 4.6. As in the Taumarunui district, more than 50% of herds with 1-100 cattle had an annual cumulative incidence ≥ 2.0 per 100 cattle. For the other herd sizes, 50-60% of herds had low annual cumulative incidences < 2 per 100 cattle.

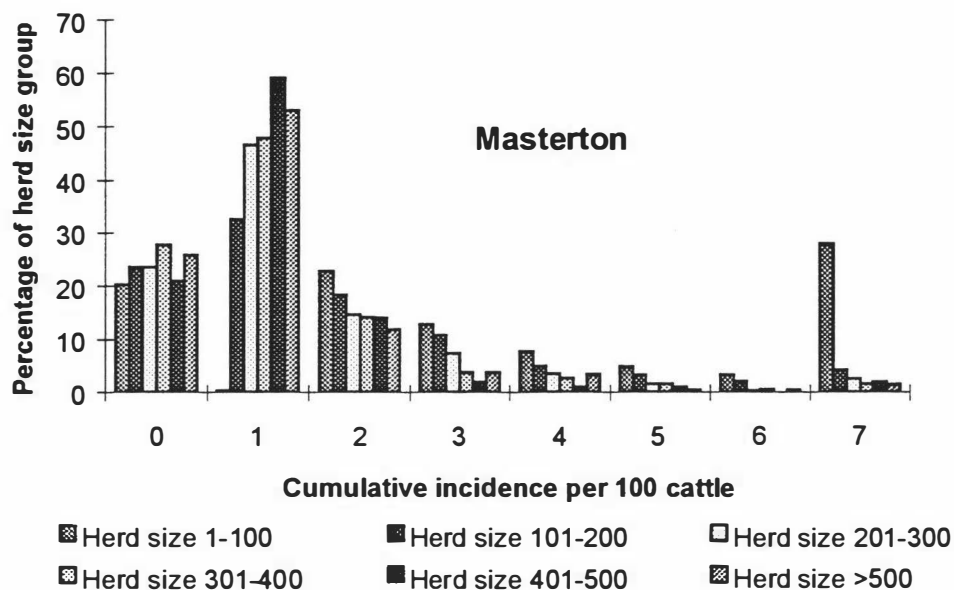


Figure 4.5. Masterton district. The relative frequencies of all levels of cumulative incidence from 0 to 7.0 per 100 cattle in six categories of herds classified by size of herd

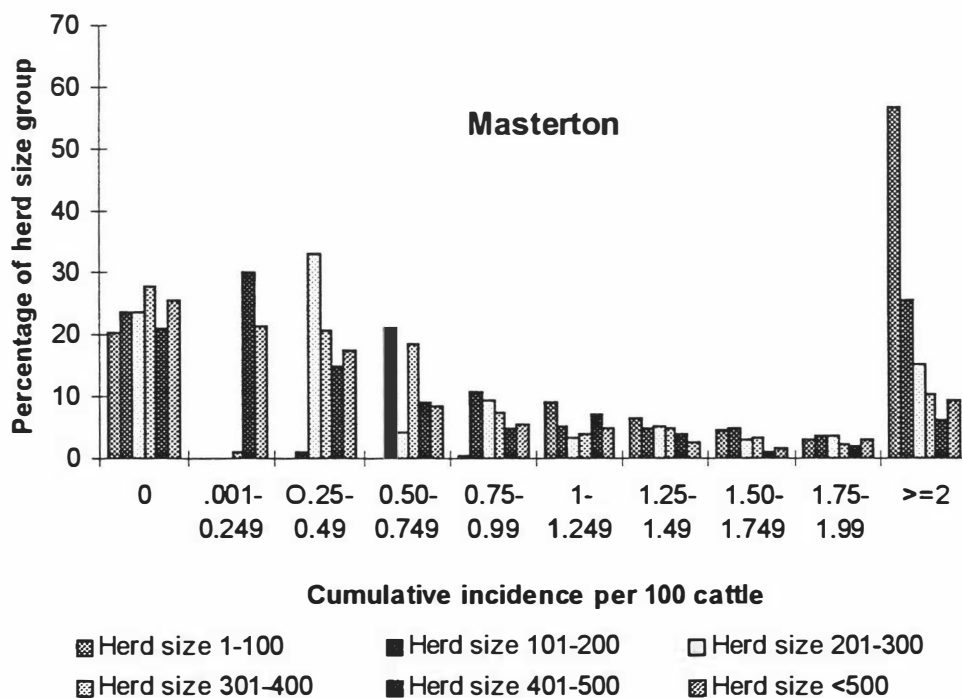


Figure 4.6 Masterton district. The relative frequencies of all levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in six categories of herds classified by size of herd

Incidence density $\times 10^{-2}$ year⁻¹ classified by herd size: Masterton district

Incidence densities for the range of herd sizes in the Masterton district are shown in Figure 4.7 and follow a similar pattern to that displayed for the Taumarunui district. More than 25% of herds with less than 100 cattle had an incidence density $> 6.0 \times 10^{-2}$ year⁻¹. In the other herd size categories, 30-58% of herds had an annual incidence density of $< 1.0 \times 10^{-2}$ year⁻¹. The Masterton district had 32% of herds in the smallest herd category at the 1.0×10^{-2} year⁻¹ incidence rate level, whereas in the Taumarunui district there were 27%. Incidence densities for herds of 101-200 cattle in the Taumarunui and Masterton districts were significantly different (Chi-square = 19.0, $P < 0.05$). In general, the proportion of herds affected steadily decreased as the level of incidence rate increased.

The distributions of incidence density from 0 to 2.0×10^{-2} year⁻¹ for the range of herd sizes are shown in Figure 4.8. Slightly more than 55% of herds with 1-100 cattle had an incidence density $\times 10^{-2}$ year⁻¹ ≥ 2.0 . The incidence density was less than 2.0×10^{-2} year⁻¹ in 50 to 60% of herds in the other herd size categories.



Figure 4.7. Masterton district. The relative frequencies of all levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by size of herd

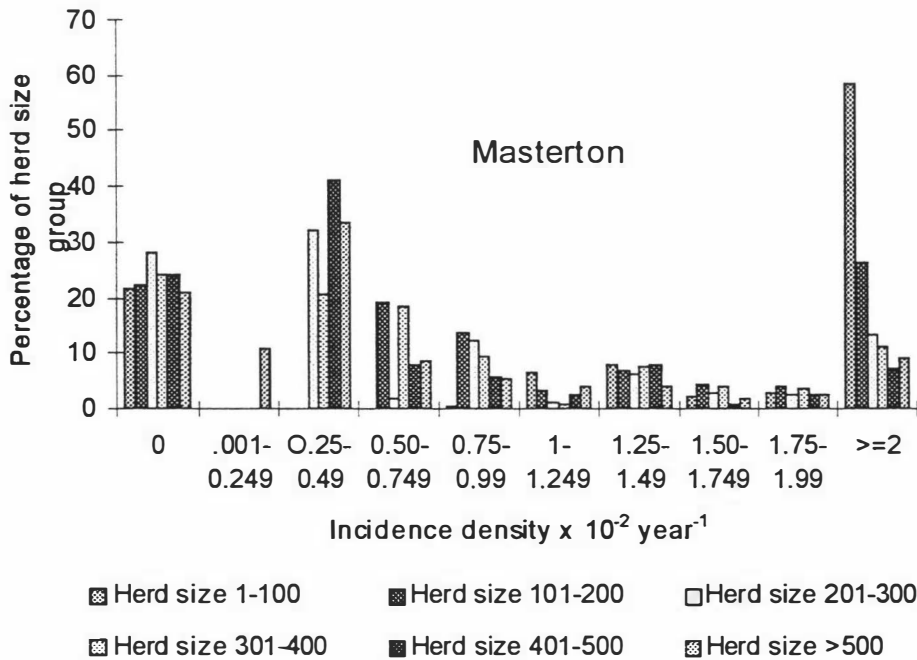


Figure 4.8. Masterton district. The relative frequencies of all levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by size of herd

Distributions of annual cumulative incidence in six categories of herd classified by size of herd: Surveillance areas

The distributions of cumulative incidence in six categories of herds classified by herds size in surveillance areas are shown in Figure 4.9. It should be noted that the scale for the Y axis is 0-100%. The annual cumulative incidence was less than 1.0 per 100 cattle for 50-70% of herds with more than 100 cattle, which contrasts with the Taumarunui and Masterton districts where only 30-50% of herds with more than 100 cattle had annual incidences less than 1.0 per 100 cattle. As in the other districts, the proportion of herds steadily declined as the level of cumulative incidence increased. In surveillance areas, only 13% of herds of 1-100 cattle had cumulative incidences > 6.0 per 100 cattle, whereas in both Taumarunui and Masterton districts 30% of herds of that size had incidences > 6.0 per 100 cattle.

The distribution of cumulative incidences between 0 and 2.0 per 100 cattle for the range of herd sizes in Surveillance areas is shown in Figure 4.10. Apart from the herd size category of 1-100 cattle, the patterns differed from those of the Taumarunui and Masterton districts, with most of the categories highly clustered about the lowest levels of incidence. The pattern for herds containing 1-100 cattle was similar to those for the Taumarunui and Masterton districts, although the proportions of herds of that size with annual cumulative incidences ≥ 2.0 per 100 cattle were different (40% in surveillance areas *versus* 60% in Taumarunui and Masterton districts).

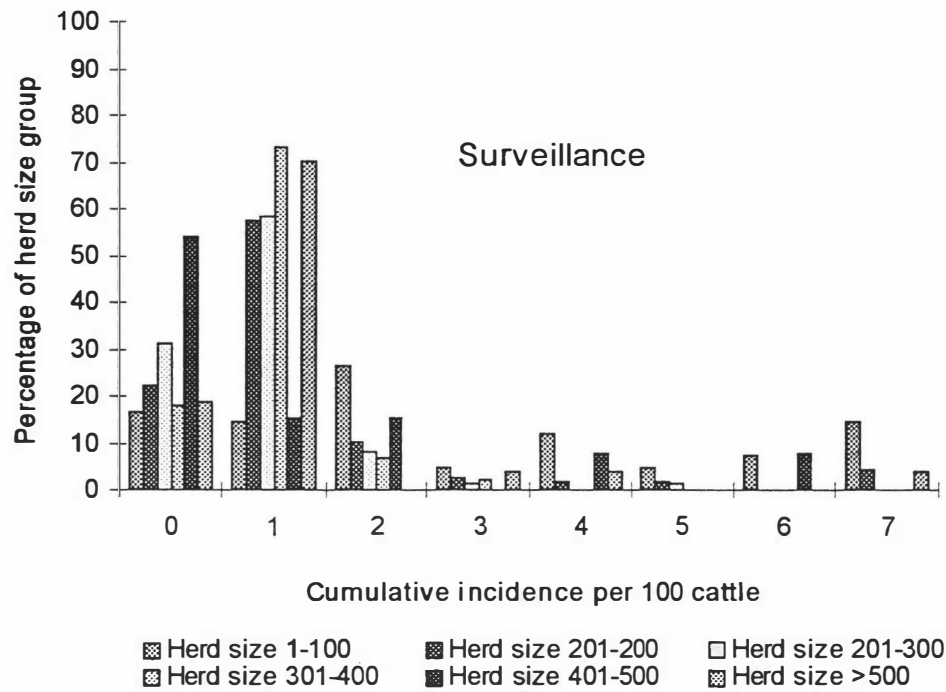


Figure 4.9. Surveillance areas The relative frequencies of all levels of cumulative incidence from 0 to 7.0 per 100 cattle in six categories of herds classified by size of herd

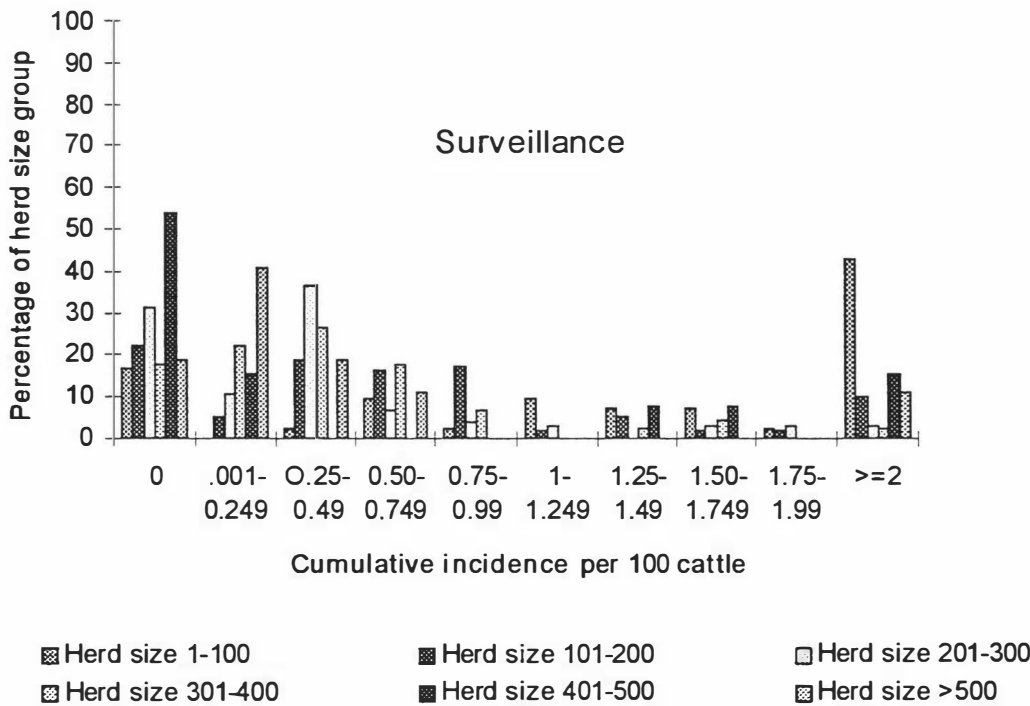


Figure 4.10. Surveillance areas. The relative frequencies of all levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in six categories of herds classified by size of herd

Distribution of incidence density in six categories of herds classified by size of herd: Surveillance areas

The distributions of incidence density in six categories of herds classified by herd size are shown in Figure 4.11. With the exception of the smallest herd size category of 1-100 cattle, 38-75% of herds in other categories had an annual incidence density $< 1.0 \times 10^{-2} \text{ year}^{-1}$. There were proportionately fewer herds at higher incidence rates, producing a pattern similar to that for cumulative incidence. Within the smallest herd size group, more than 27% of herds had zero incidence (these herds started on movement control and had no more reactors). This contrasts with the Taumarunui and Masterton districts where less than 20% of herds with 1-100 cattle had had zero incidence.

The distribution of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ for the range of herd sizes is shown in Figure 4.12. As with the distribution of cumulative incidence, there were relatively few herds of 1-100 cattle with incidence density less than $2.0 \times 10^{-2} \text{ year}^{-1}$. Forty to fifty percent of herds with more than 100 cattle had an incidence density $< 2.0 \times 10^{-2} \text{ year}^{-1}$. Within the smallest herd category of 1-100 cattle, 35% of herds had an annual incidence $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$, whereas 42% of herds in this category had cumulative incidences ≥ 2.0 per 100 cattle.

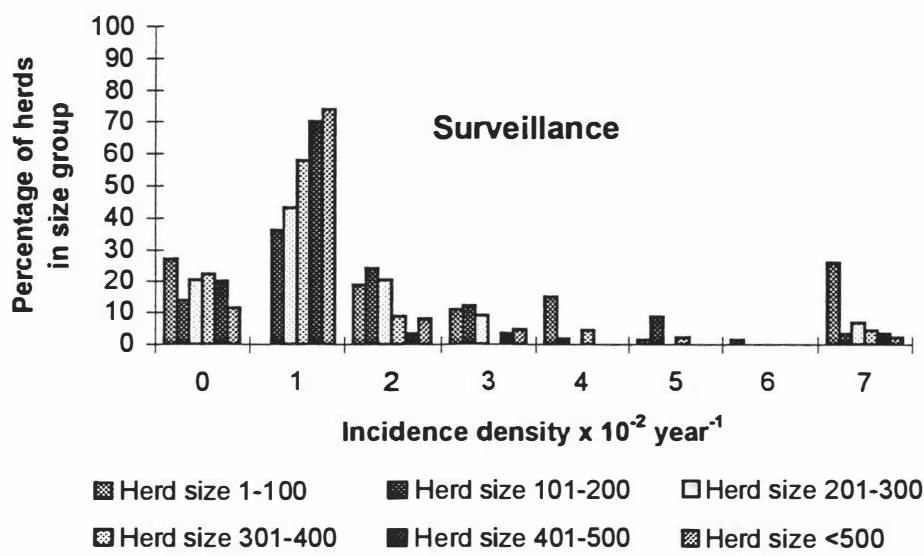


Figure 4.11. Surveillance areas. The relative frequencies of all levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$

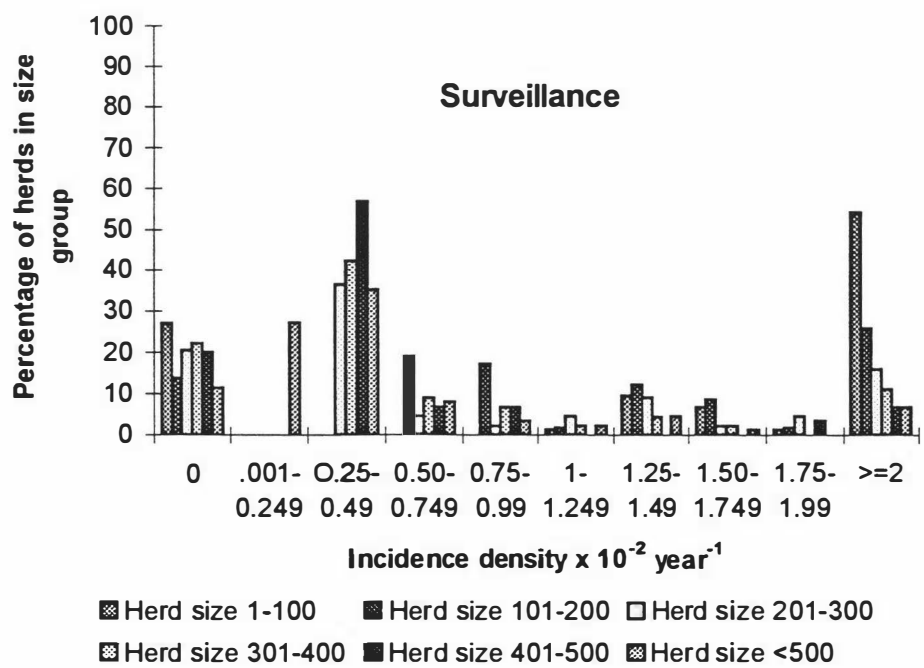


Figure 4.12. Surveillance areas. The relative frequencies of all levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by size of herd

Distribution of cumulative incidence in herds in endemic and fringe zones: Taumarunui district

The distributions of cumulative incidence from 0 to 7.0 per 100 cattle in herds within the endemic Taumarunui region and its fringe zone are shown in Figure 4.13. The predominant level of cumulative incidence was 1.0 per 100 cattle with 46% of fringe zone and 34% of endemic zone herds at that level of risk. The figure indicates that herds in the endemic zone were at greater risk than herds in the fringe zone at all levels of cumulative incidence except at the 1.0 per 100 cattle level

The distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle for the two zones are shown in Figure 4.14. Over 40% of fringe area herds which were on movement control at the start of the period under consideration had no further reactors, whereas only 19% of herds in the endemic area had no further episodes of infection. There were more fringe herds than endemic zone herds at the two lowest levels of cumulative incidence and more endemic herds than fringe herds at higher levels of risk. There were 29% of herds in the endemic area with cumulative incidences ≥ 2.0 per 100 cattle, compared with only 5% in the fringe zone.

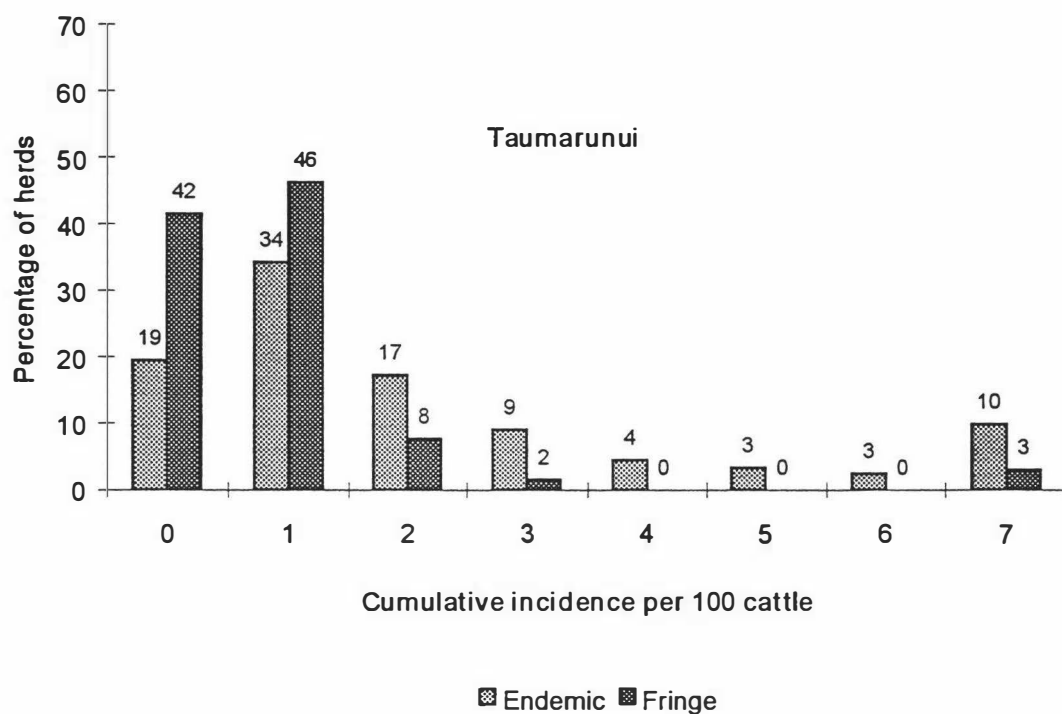


Figure 4.13. The distribution of levels of cumulative incidence from 0 to 7.0 per 100 cattle in herds in the endemic Taumarunui area and its fringe zone

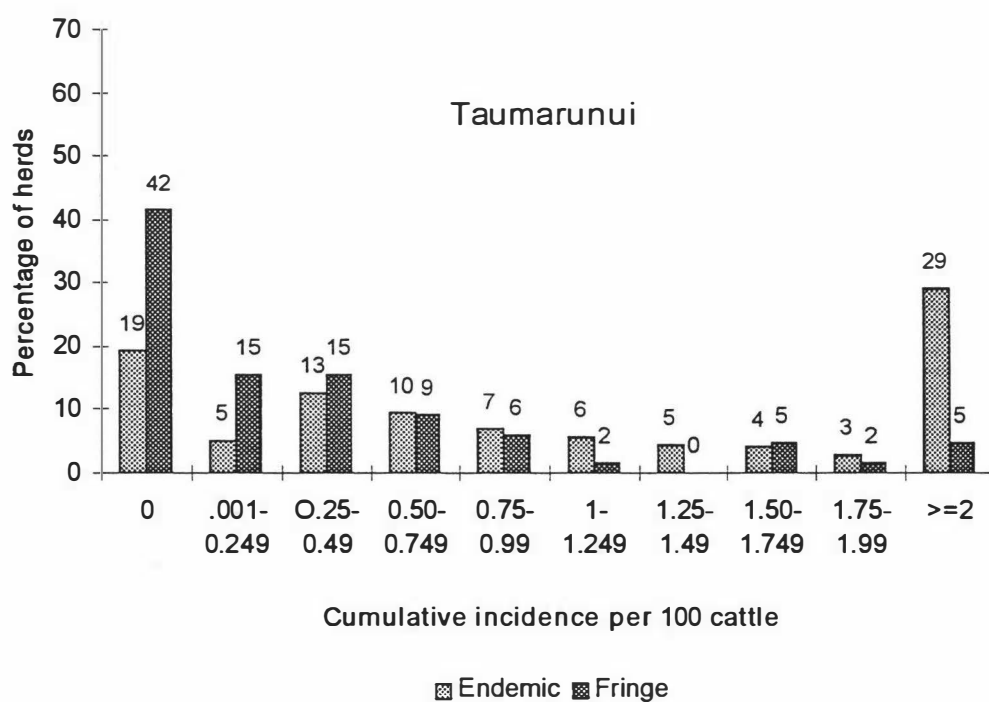


Figure 4.14. The distribution of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds in the endemic Taumarunui area and its fringe zone

Distribution of incidence density in herds in endemic and fringe zones: Taumarunui district

The distributions of incidence densities in herds within the endemic and fringe zones of Taumarunui are shown in Figure 4.15. The pattern is generally similar to that shown for cumulative incidence with 44% of fringe zone and 34% of endemic zone herds having an incidence density of $1.0 \times 10^{-2} \text{ year}^{-1}$. The figure clearly shows that considerably higher proportions of endemic herds were involved at incidence rates from 2.0 to $7.0 \times 10^{-2} \text{ year}^{-1}$.

The distributions of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ for both zones are shown in Figure 4.16. Again the pattern was similar to that for cumulative incidence with 43% of endemic zone and 20% of fringe zone herds having no new infections. In the endemic area, 30% of herds had incidence densities $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ compared to only 7% in the fringe zone.

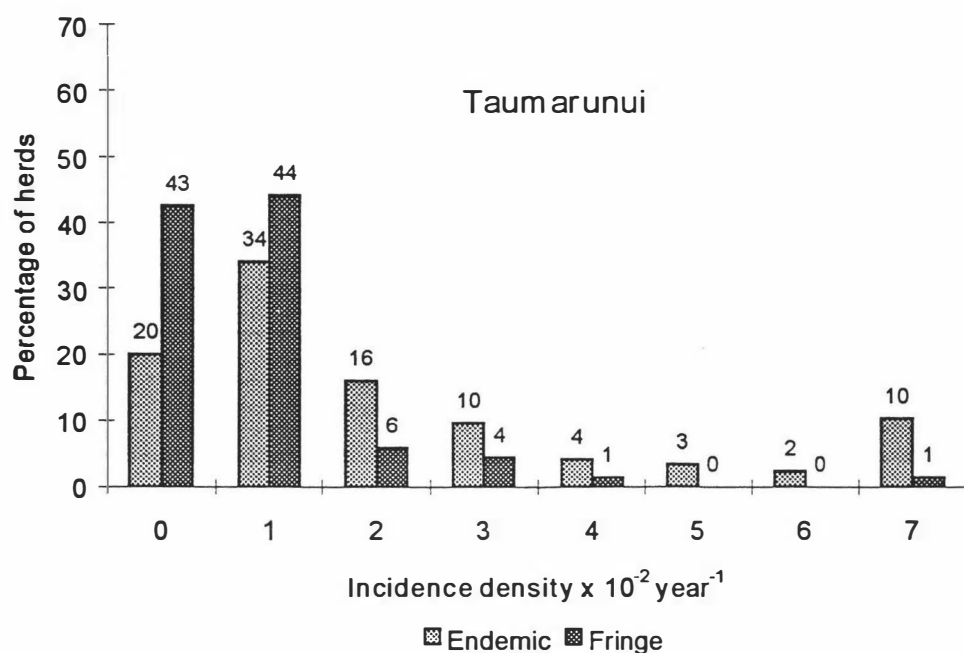


Figure 4.15. The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in herds in the endemic Taumarunui area and its fringe zone

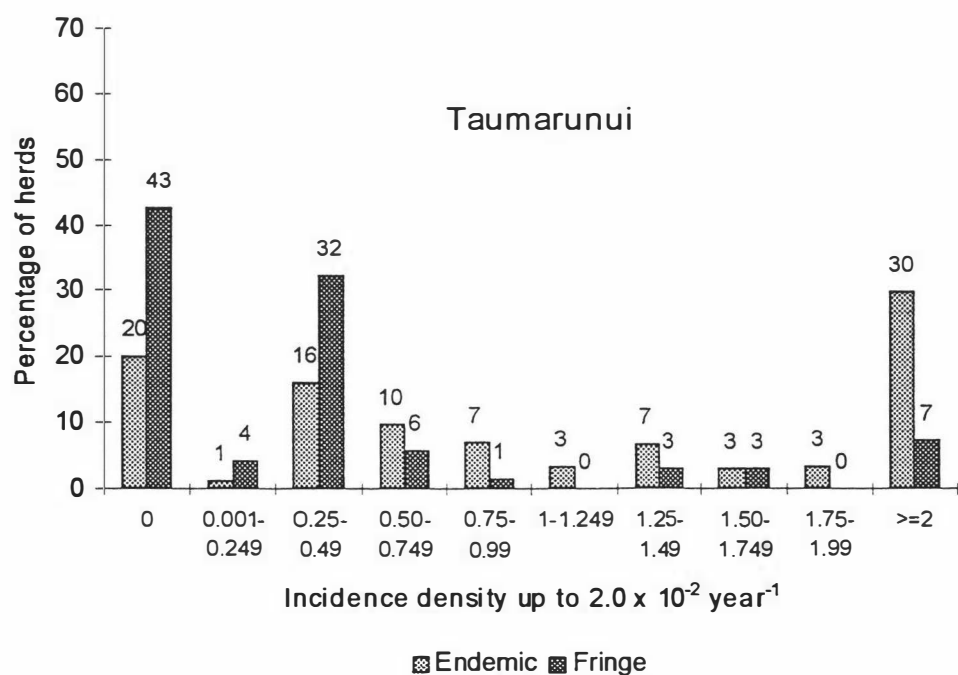


Figure 4.16. The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in herds in the endemic Taumarunui area and its fringe zone

Distribution of cumulative incidence in herds in endemic and fringe zones: Masterton district

The distributions of cumulative incidence in herds within the endemic Masterton region and its fringe zone are shown in Figure 4.17. The pattern is similar to that found for cumulative incidence in the Taumarunui district. The most common level of risk was 1.0 per 100 cattle with 46% of fringe zone herds and 36% of endemic area herds having cumulative incidences at that level. Only 17% of herds in the endemic area and 7% in fringe area herds had cumulative incidences between 1.0 and 2.0 per 100 cattle. As the level of cumulative incidence increased, the proportion of herds steadily declined

The distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds from the two zones are shown in Figure 4.18. The pattern is different from that for Taumarunui where zero incidence predominated, whereas in the Masterton region, 25% of fringe zone herds and 10% of endemic area herds were at the 0.50-0.749 per 100 cattle level. There were 24% of endemic area herds and 17% of fringe zone herds at levels of cumulative incidence ≥ 2.0 per 100 cattle.

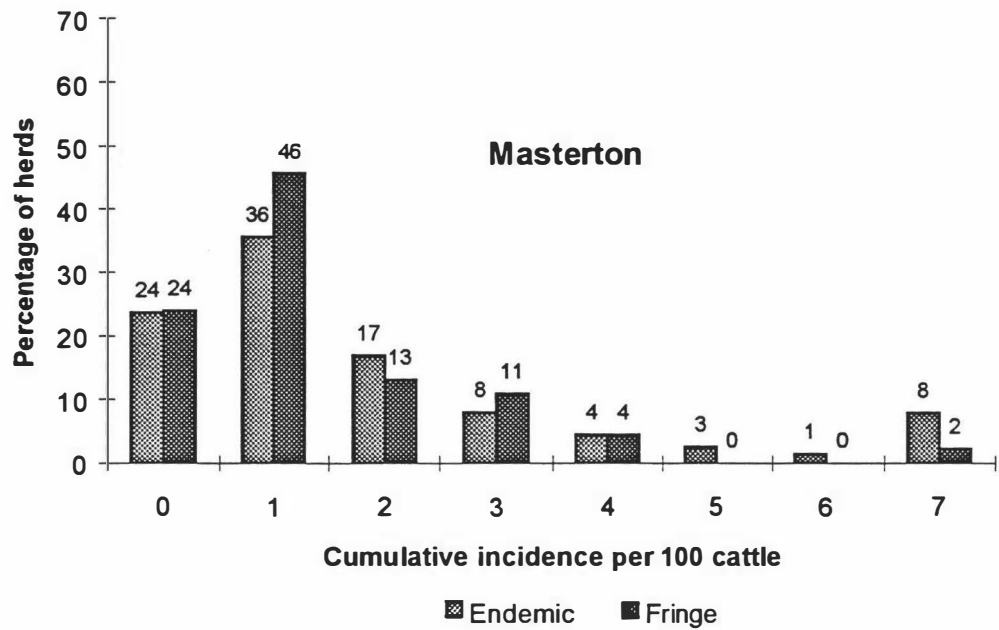


Figure 4.17. The distribution of levels of cumulative incidence from 0 to 7.0 per 100 cattle in

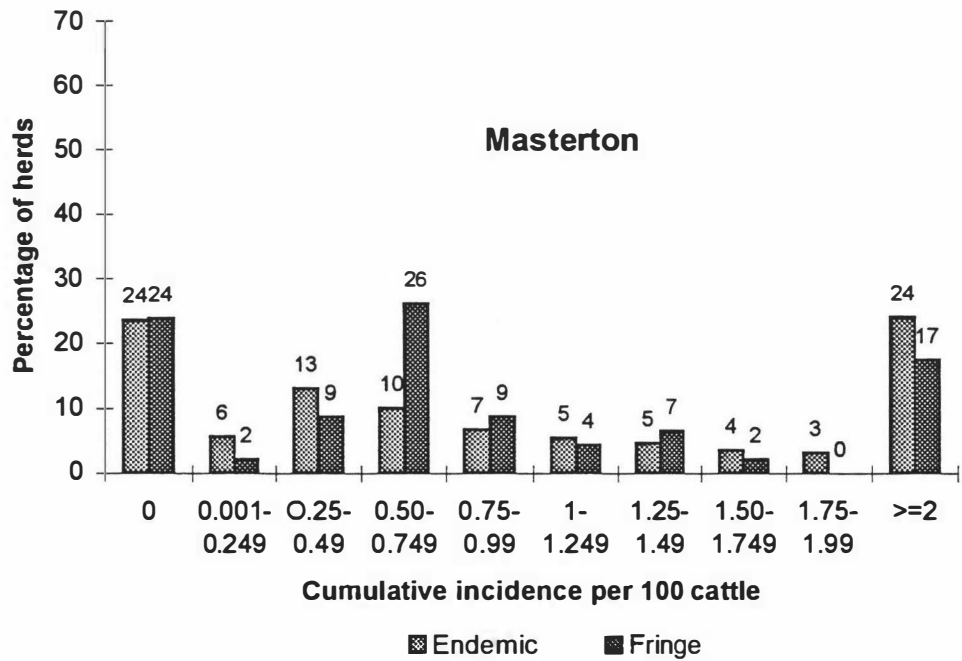


Figure 4.18 The distribution of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds in the endemic Masterton area and its fringe zone

Distribution of incidence density in herds in endemic and fringe zones: Masterton district

The distributions of incidence density in herds in the endemic area and the fringe zones of the Masterton district are shown in Figure 4.19. The pattern is similar to that for cumulative incidence with 50% of fringe zone and 38% of endemic area herds having less than 1.0×10^{-2} year⁻¹ with a steady decline in the proportion of herds with increasing levels of incidence. Ten percent of endemic area herds and 2% of fringe area herds had incidence densities $\geq 6.0 \times 10^{-2}$ year⁻¹. The Taumarunui data showed a more obvious difference between endemic and fringe zones at 2.0 - 4.0×10^{-2} year⁻¹ levels with more endemic herds becoming infected than fringe herds

The pattern of incidence density from 0 to $\geq 2.0 \times 10^{-2}$ year⁻¹ in herds from both zones displayed in Figure 4.20 closely resembles the cumulative incidence pattern with 27% of endemic and 26% of fringe zone herds having no new cases. Most herds had low rates of infection and the proportion of newly infected herds reduced as the incidence rate level increased up to $\geq 2.0 \times 10^{-2}$ year⁻¹, where 24% of endemic area and 11% of fringe zone herds were involved. The pattern was similar to that for incidence density in the Taumarunui endemic and fringe zones, although in that district, 20% of endemic and 43% of fringe zone herds had no new cases compared with 24% of endemic and fringe herds in the Masterton region.



Figure 4.19. The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in herds in the endemic Taumarunui area and its fringe zone

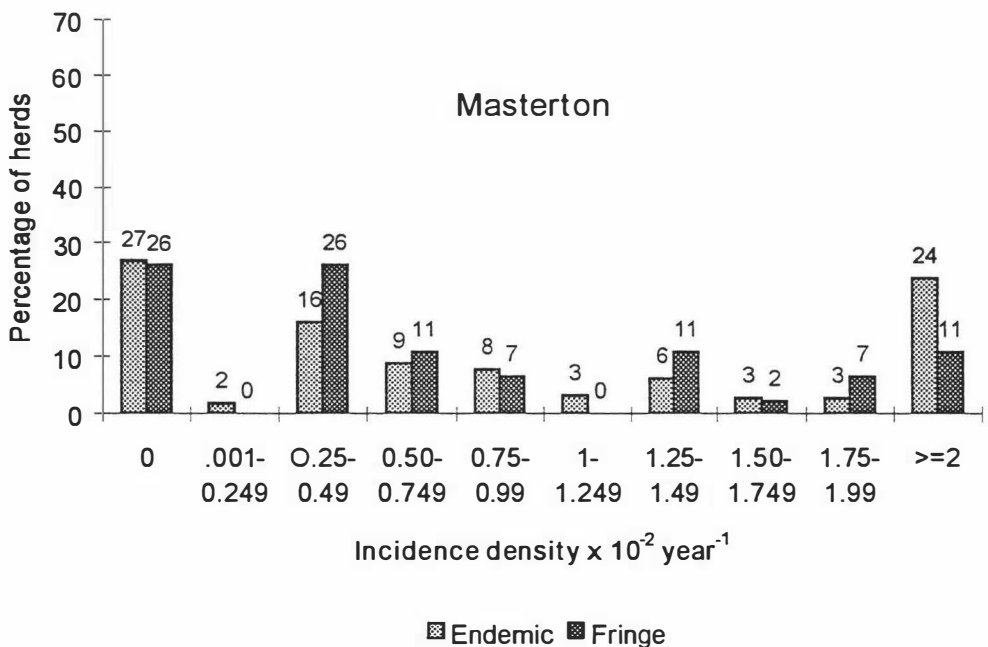


Figure 4.20. The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in herds in the endemic Taumarunui area and its fringe zone

Distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in endemic areas within herd size categories of 1-100, 101-200 and >500 cattle

The distribution of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in the Taumarunui and Masterton endemic areas for herds with up to 100 cattle is shown in Figure 4.21. In the Taumarunui district, 25% of herds had no reactors during the period under consideration compared to 20% of herds in the Masterton district.

The distribution of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in the Taumarunui and Masterton endemic areas for herds with 201-300 cattle is shown in Figure 4.22. Masterton had more herds infected at the <1.0 per 100 cattle incidence level, (46% vs 35% for Taumarunui) but at higher levels of incidence, the situation was reversed.

The distribution of cumulative incidence over the same levels of cumulative incidence for herds with 500 or more cattle is shown in Figure 4.23. More herds (25%) in the Masterton endemic area had no new infections than in the Taumarunui district (11%).

The general pattern in Figures 4.21 to 4.23 was similar for the three herd sizes examined.

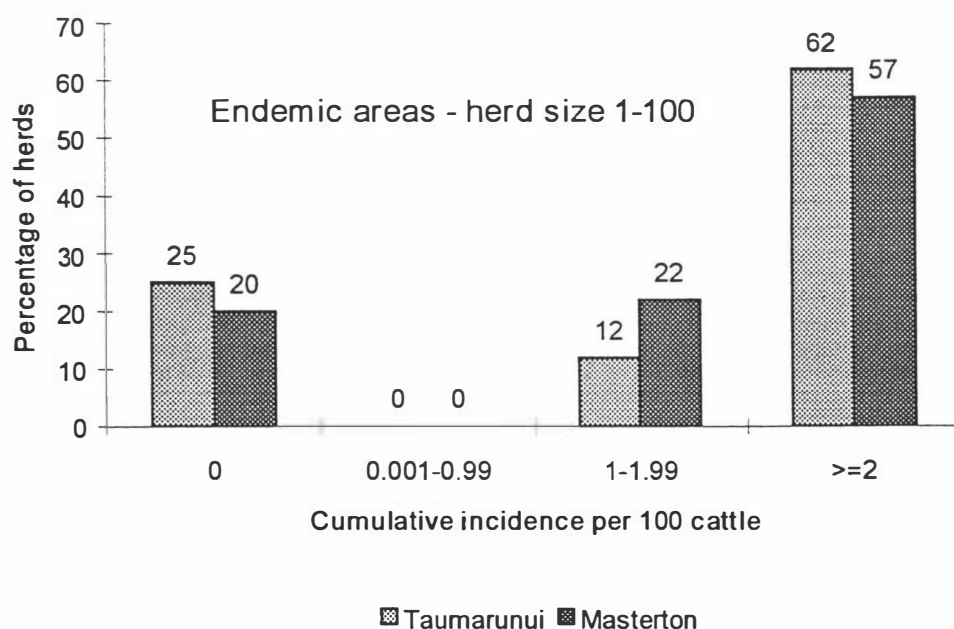


Figure 4.21. The relative frequencies of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds of 1-100 cattle in the Taumarunui and Masterton endemic areas

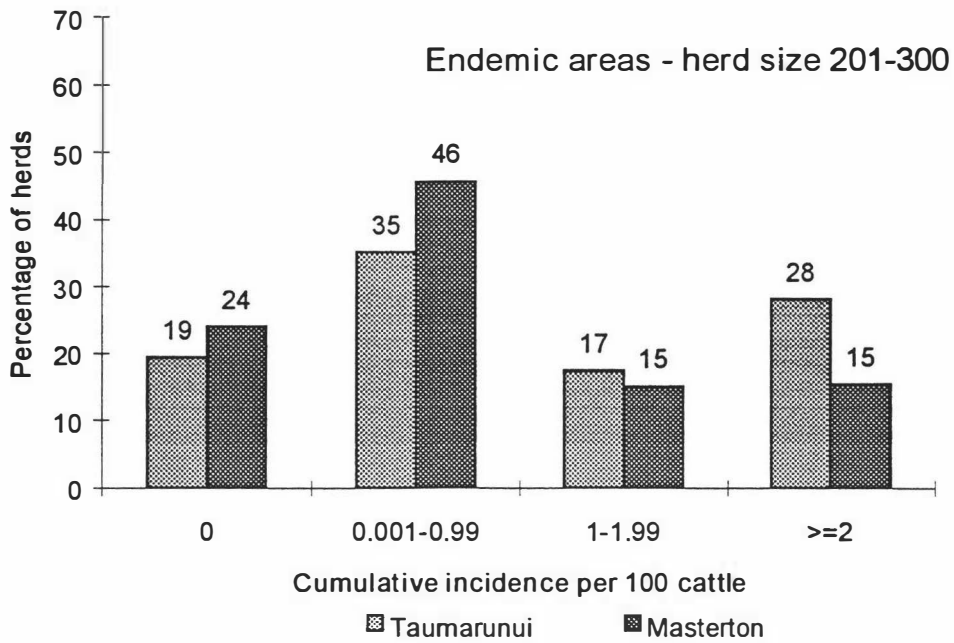


Figure 4.22. The relative frequencies of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds of 201-300 cattle in the Taumarunui and Masterton endemic areas

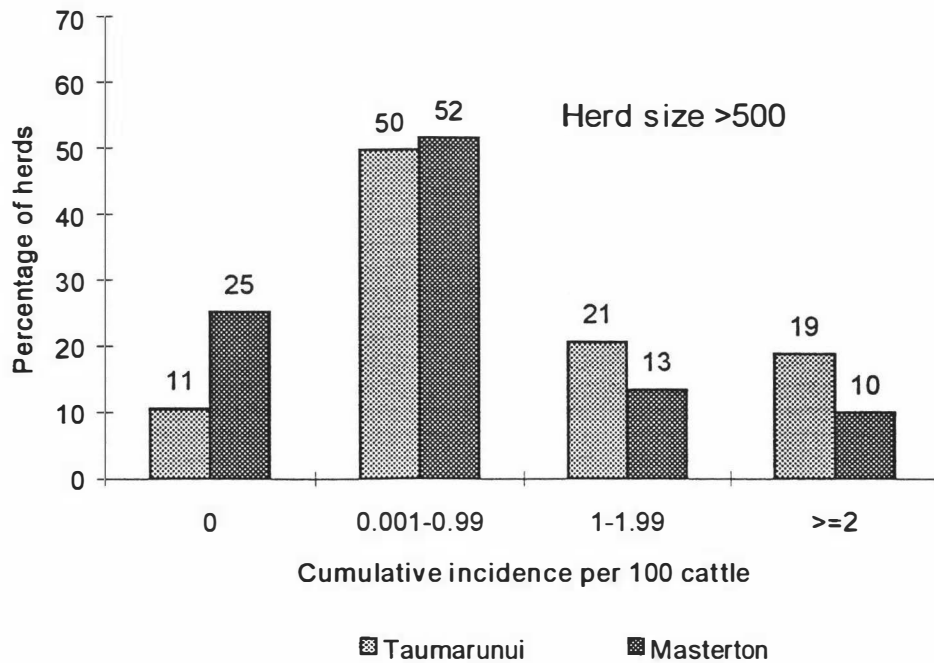


Figure 4.23. The relative frequencies of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in herds of >500 cattle in the Taumarunui and Masterton endemic areas

Distribution of cumulative incidence in beef breeding herds in the Taumarunui and Masterton endemic areas

The distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in beef breeding herds of 1-100, 201-300 and >500 cattle in the endemic areas of Taumarunui and Masterton are shown in Figures 4.24, 4.25 and 4.26 respectively. The patterns of incidence for the beef breeding herds closely resemble those shown in Figures 4.21, 4.22 and 4.23 for all herds with 1-100 and 201-300 cattle in the same districts. The patterns for >500 cattle are identical due to the predominance of beef breeding herds in that herd size category.

Distribution of cumulative incidences according to the type of herd

The distributions of cumulative incidences from 0 to 7.0 per 100 cattle for beef breeding, beef dry stock and dairy herds in the Taumarunui district are illustrated in Figure 4.27. The differences at levels of ≤ 1.0 were small, but at the 3.0 per 100 cattle level, 7 % of beef breeding, 13% of beef dry stock and 21% of dairy herds were affected. There was no apparent trend or pattern in the range of levels between 4.0 and 7.0 per 100 cattle.

The distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle for the three types of cattle operations are shown in Figure 4.28. No cases were found in 21% of beef breeding and beef dry stock and 16% of dairy herds. There was no apparent pattern through the intermediate levels of incidence, but 25% of beef breeding, 36% of beef dry stock and 34% of dairy herds had average annual cumulative incidences ≥ 2.0 per 100 cattle in the Taumarunui district.

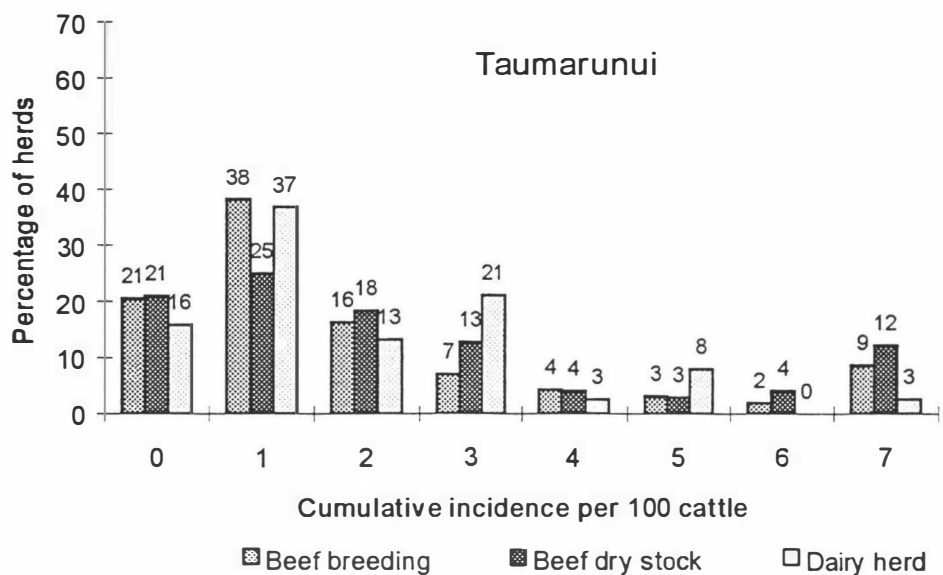


Figure 4.27 The distribution of levels of cumulative incidence from 0 to 7.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Taumarunui district

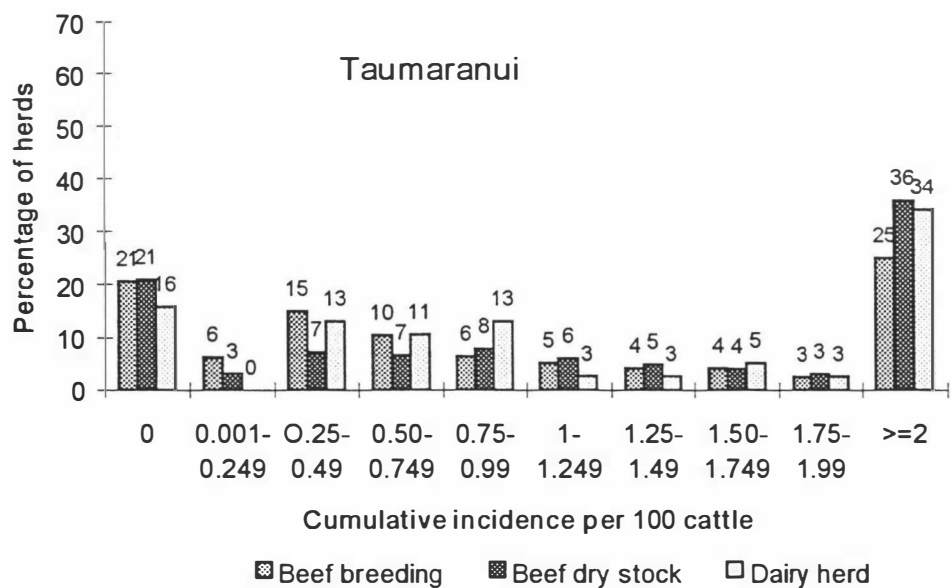


Figure 4.28 The distribution of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Taumarunui district

Distribution of incidence density according to the type of herd: Taumarunui district

The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ for all types of cattle enterprise in the Taumarunui district are shown in Figure 4.29. Apart from some small differences at the $1.0 \times 10^{-2} \text{ year}^{-1}$ level, there were no noteworthy patterns in the distributions.

The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ are shown in Figure 4.30. A total of 62% of beef breeding, 61% of beef dry stock and 56% of dairy herds had incidence densities ≤ 1.0 to $1.249 \times 10^{-2} \text{ year}^{-1}$.

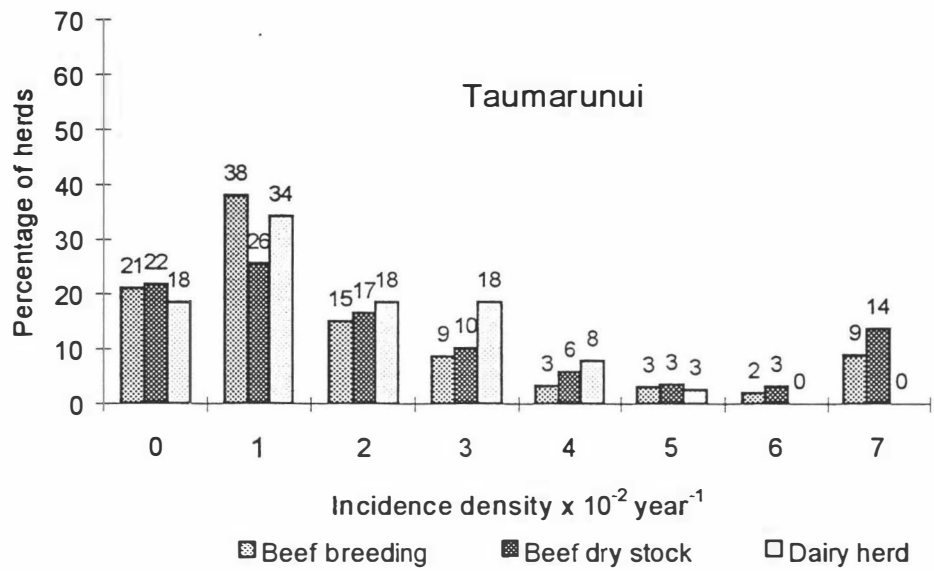


Figure 4.29. The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Taumarunui district

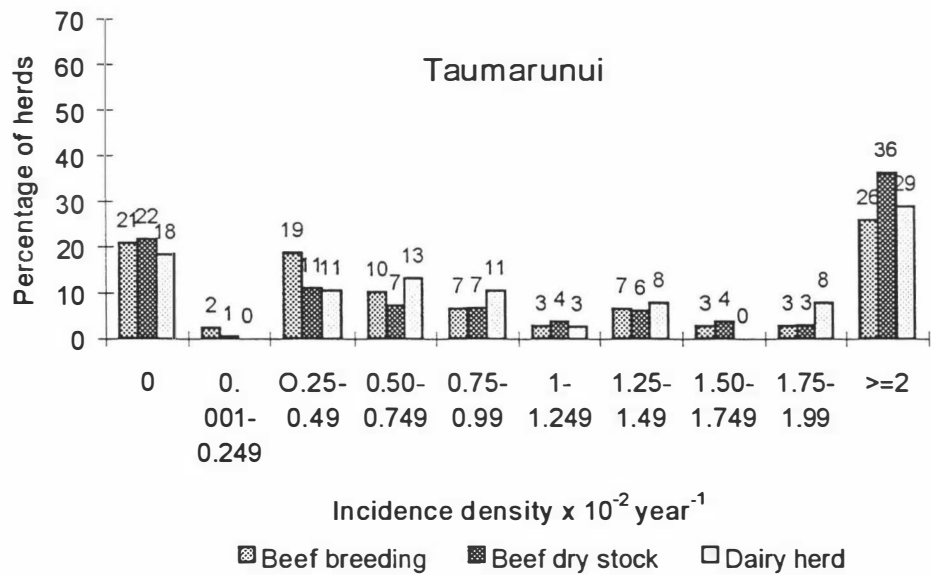


Figure 4.30. The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Taumarunui district

Distribution of cumulative incidence according to type of herd: Masterton district

The distributions of cumulative incidence from 0 to 7.0 per 100 cattle for all types of herds are shown in Figure 4.31. At the 1.0 per 100 cattle level of incidence, there were relatively few beef dry stock herds but they were prominent at the highest levels of incidence. The pattern was similar to that for Taumarunui.

The distributions of cumulative incidence from 0 to ≥ 2.0 per 100 cattle for all types of herds are shown in Figure 4.32. Once more, the pattern was similar to that for Taumarunui with relatively more dry stock herds at levels of ≥ 2.0 per 100 cattle, where there were 22% of beef breeding, 41 % of beef dry stock and 18% of dairy herds.

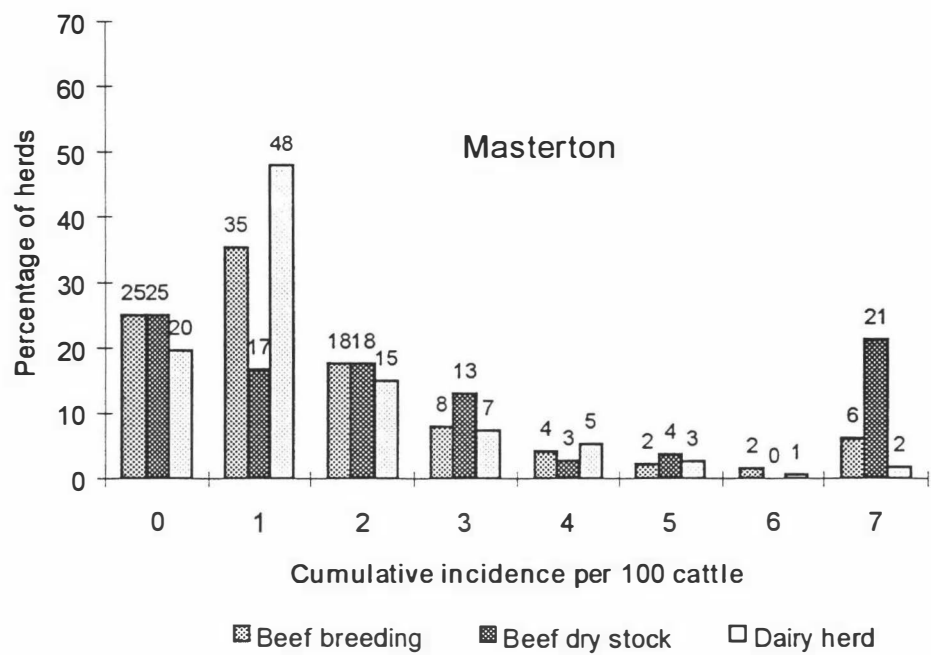


Figure 4.31. The distribution of levels of cumulative incidence from 0 to 7.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Masterton district

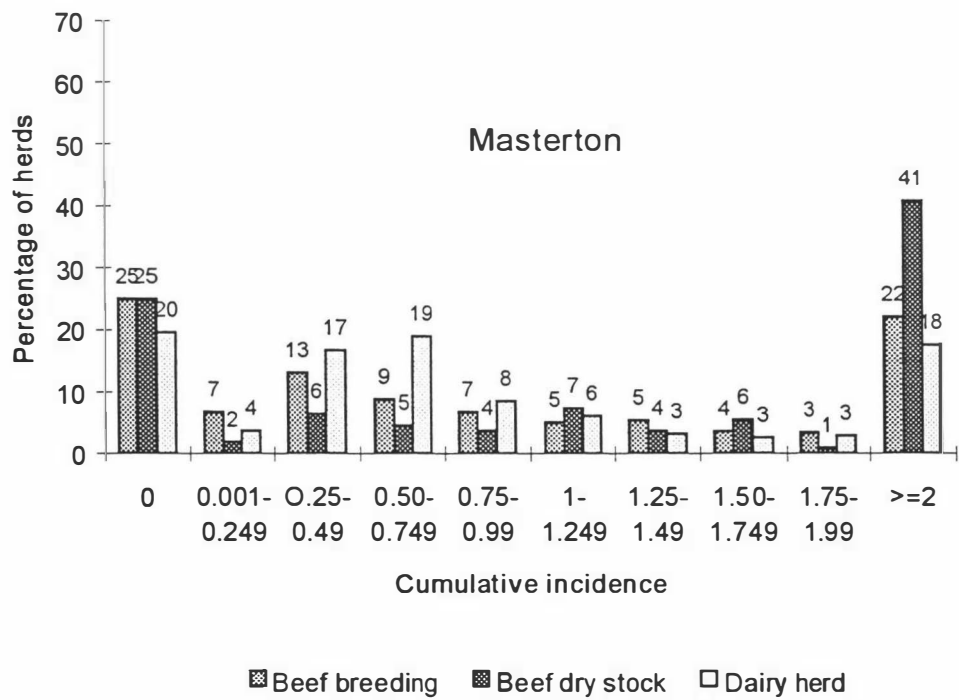


Figure 4.32. The distribution of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Masterton district

Distribution of incidence density according to type of herd: Masterton district

The distributions of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ for all types of herds in the Masterton district are shown in Figure 4.33. At the $1.0 \times 10^{-2} \text{ year}^{-1}$ level, there were fewer beef dry stock than beef breeding and dairy herds. The pattern was similar to that for Taumarunui with a relative predominance of beef dry stock herds at the $7.0 \times 10^{-2} \text{ year}^{-1}$ level.

The distributions of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ for all types of herds in the Masterton district are shown in Figure 4.34. A total of 66% of beef breeding, 50% of beef dry stock and 69% of dairy herds had incidence densities ≤ 1.0 to $1.249 \times 10^{-2} \text{ year}^{-1}$. The pattern was similar to that for Taumarunui.

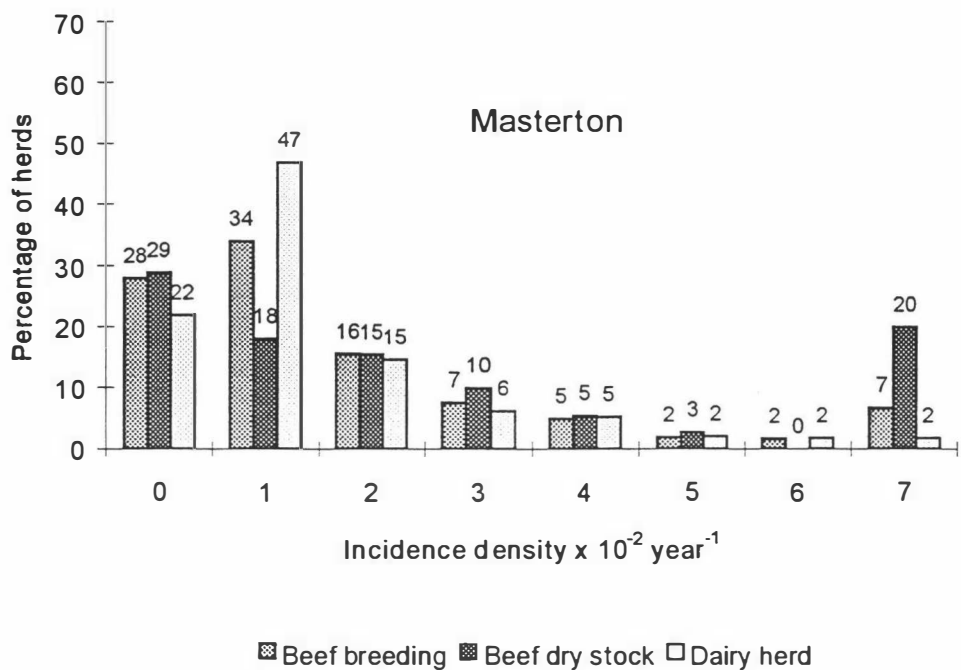


Figure 4.33. The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Masterton district

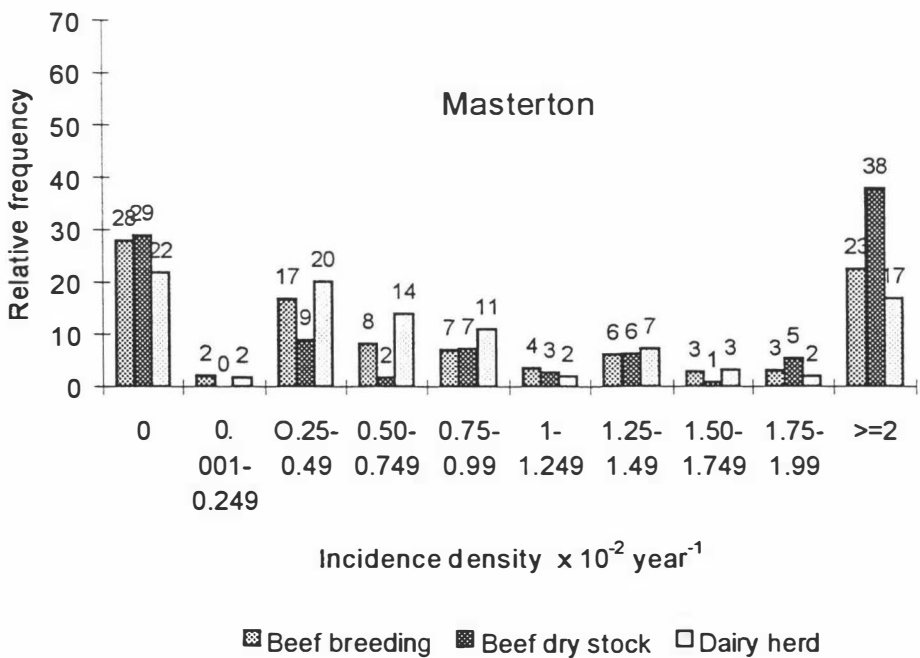


Figure 4.34. The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Masterton district

Distribution of cumulative incidence according to type of herd: Surveillance areas.

The distributions of cumulative incidence between 0 and 7.0 per 100 cattle for all types of herds in Surveillance areas are shown in Figure 4.35. Surveillance areas had relatively more clear status beef herds and fewer clear dairy herds than did Taumarunui and Masterton districts.

The distributions of cumulative incidence between 0 and ≥ 2.0 per 100 cattle for all types of herds are shown in figure 4.36. In the Surveillance areas there were proportionately few dairy herds with no incident cases but dairy herds predominated at cumulative incidence levels from 0.001 to 1.0 per 100 cattle, with little difference in herd type above that level.

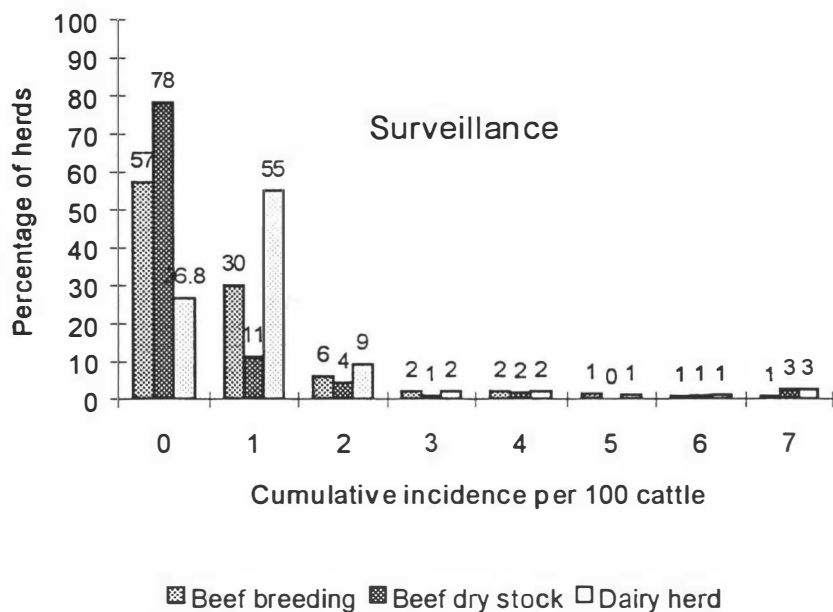


Figure 4.35. The distribution of levels of cumulative incidence from 0 to 7.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Surveillance areas

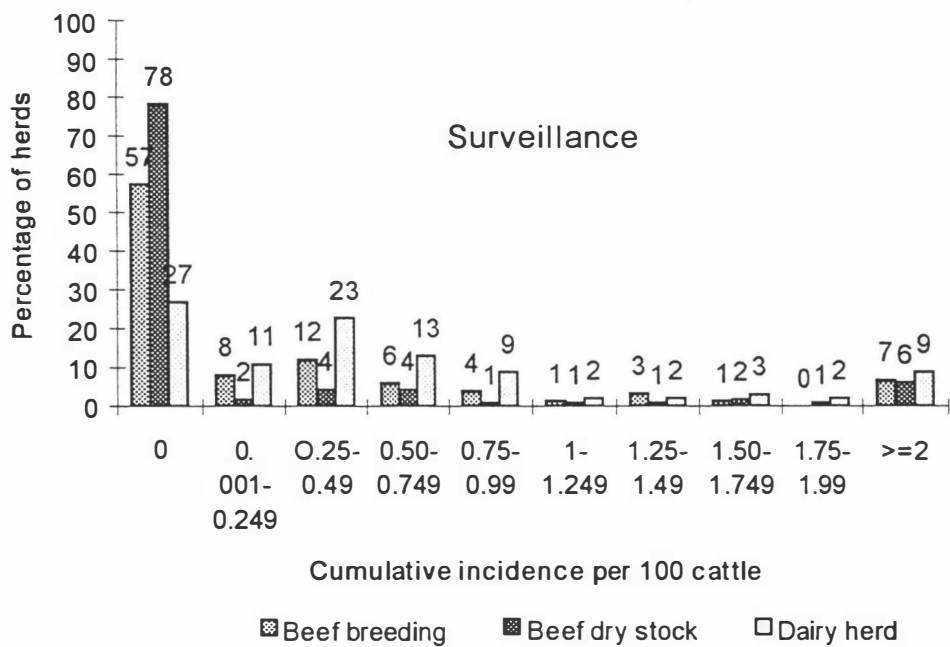


Figure 4.36. The distribution of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in beef breeding, beef dry stock and dairy herds in the Surveillance areas

Distribution of incidence density according to herd type: Surveillance areas

As illustrated in Figure 4.37, the rate of new infections was low in Surveillance areas with new infections more common in dairy herds, for the most part at low rates of infection as shown in Figure 4.38.

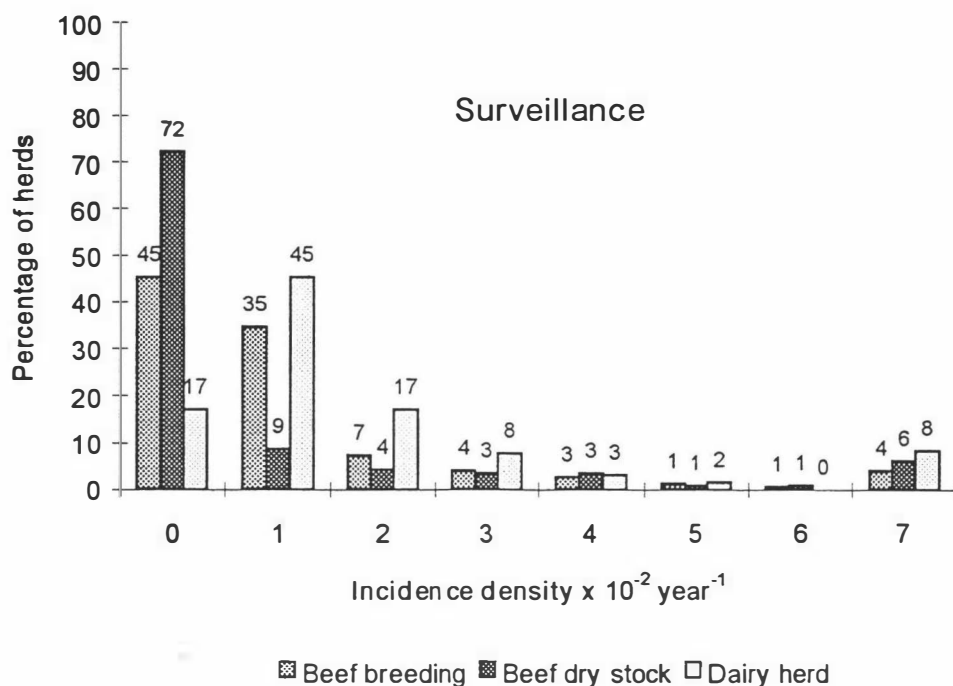


Figure 4.37. The distribution of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Surveillance areas

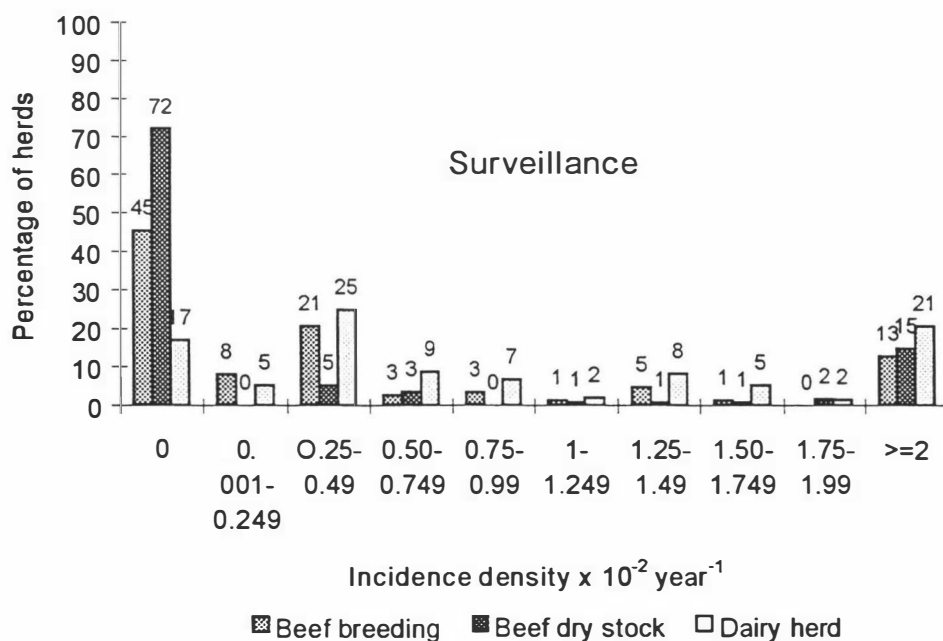


Figure 4.38. The distribution of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy herds in the Surveillance areas

**Cumulative incidence of lesioned reactors in six categories of herds classified by herd size:
Taumarunui district**

The distributions of cumulative incidence of lesioned reactors (i.e. reactors in which lesions were found post mortem) per 100 cattle in six categories of herds classified by herd size are shown for the Taumarunui district in Figure 4.39 and Figure 4.40. The pattern resembles that shown for reactors, (i.e. all animals which reacted positively to the ID test) in Figures 4.1 and 4.2 but as would be expected, the risk of the disease being detected post mortem was lower than by the intradermal test. The smallest herd size of 1-100 cattle had the highest cumulative incidences of lesioned reactors at all levels above 1.0 per 100 cattle and this was particularly evident at ≥ 6.0 where 15% of herds of that size were located.

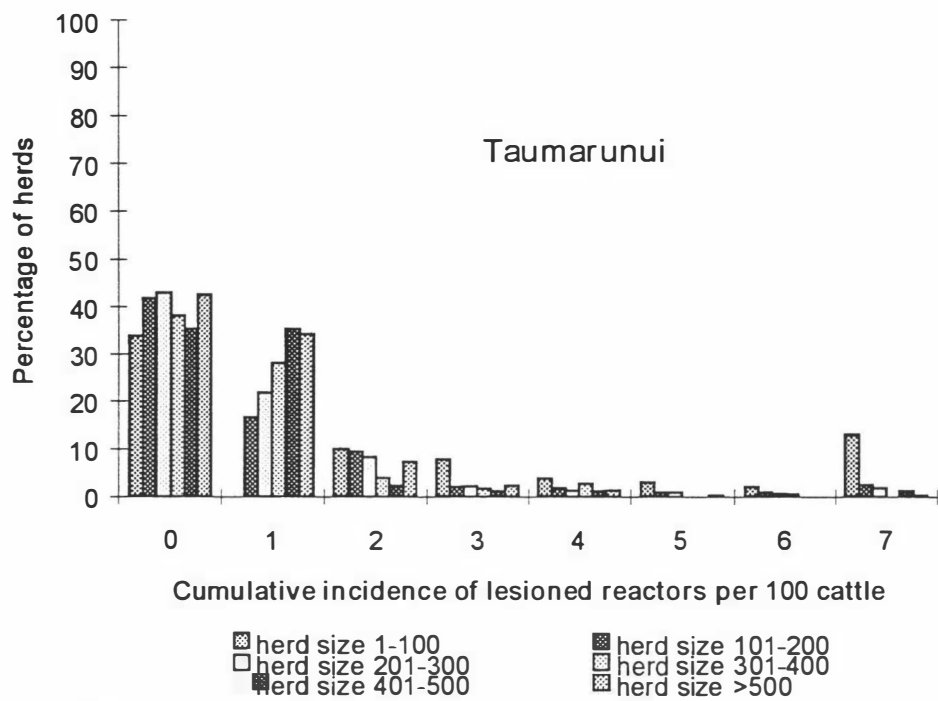


Figure 4.39. Taumarunui district: The distribution of cumulative incidence of lesioned reactors from 0 to 7.0 per 100 cattle in six categories of herds classified by size of herd

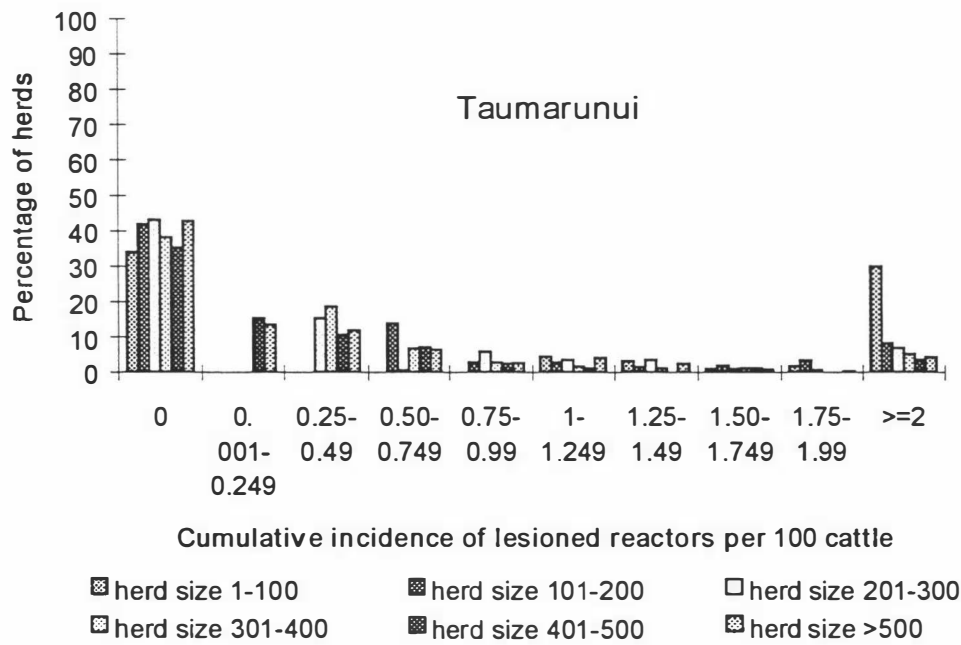


Figure 4.40. Taumarunui district. The distributions of cumulative incidence of lesioned reactors from 0 to ≥ 2.00 per 100 cattle in six categories of herds classified by size of herd

**Incidence densities of lesioned reactors in six categories of herds classified by herd size:
Taumarunui district**

The distributions of incidence densities of lesioned reactors for all levels up to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size are shown for the Taumarunui district in Figure 4.41. The pattern is very similar to that for cumulative incidence of lesioned reactors per 100 cattle. As the incidence density level increased, the percentage of herds affected at those levels steadily decreased.

The similarity between cumulative incidence and incidence density is again evident in Figure 4.42, which illustrates the distributions of percentages of herds affected with incidence densities ranging from no new cases to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$. Small herds with 1-100 cattle again were relatively strongly represented by about 25% of herds at the $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ level.

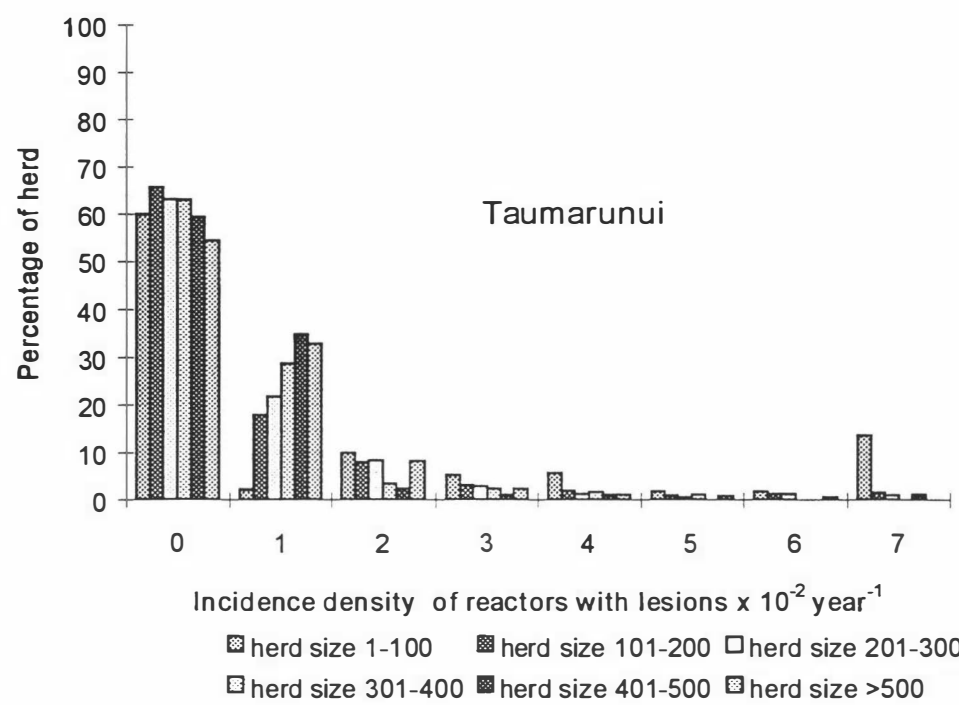


Figure 4.41. Taumarunui district: Distributions of incidence density of lesioned reactors from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size

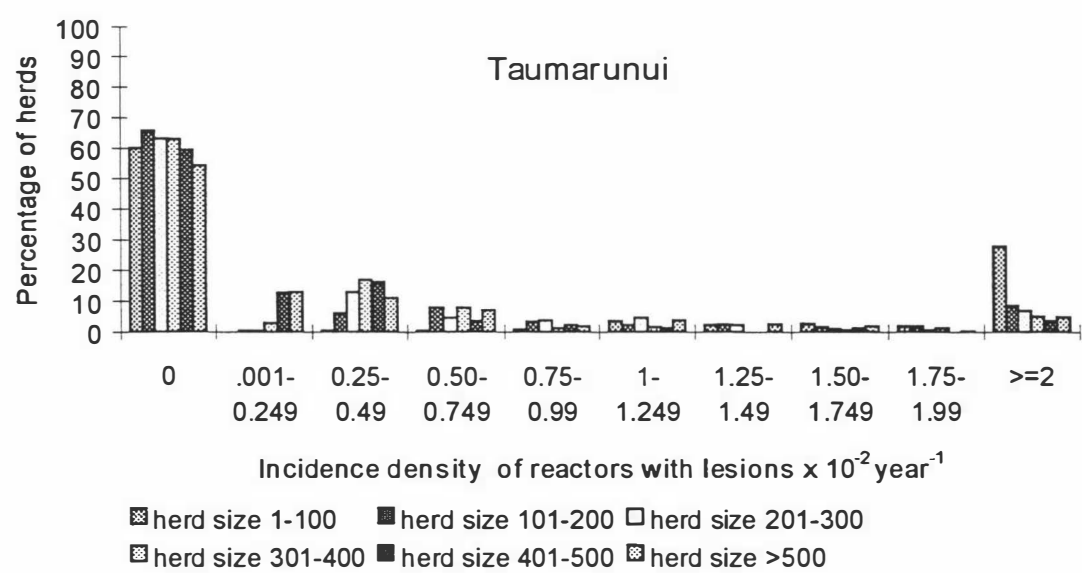


Figure 4.42. Taumarunui district: Distributions of incidence density of lesioned reactors from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size

**Cumulative incidence of lesioned reactors in six categories of herds classified by herd size:
Masterton district**

The distributions of cumulative incidences of lesioned reactors per 100 cattle in six categories of herds classified by herd size are shown for the Masterton district in Figure 4.43 and Figure 4.44. The distributions differed from those from Taumarunui where there were fewer herds of all sizes with no reactors with lesions. The highest levels of risk, evident at ≥ 2.0 per 100 cattle and in particular at 7.0 per 100 cattle, were again found in small herds of less than 100 cattle. This pattern was also seen in the Taumarunui data, although there were more Taumarunui herds of 1-100 cattle affected at both those levels.

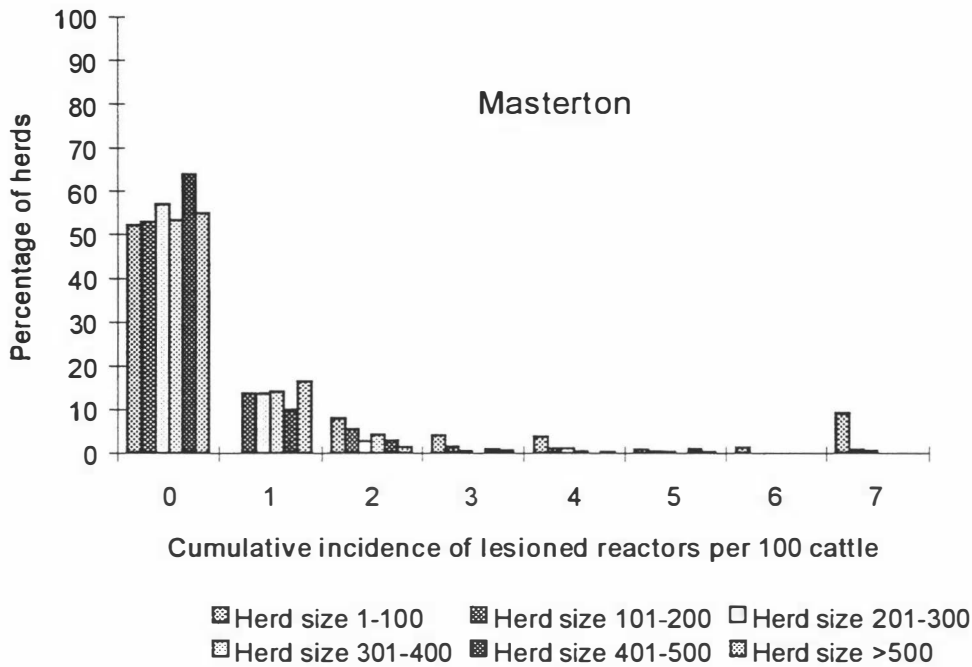


Figure 4.43. Masterton district. Distributions of cumulative incidence of lesioned reactors from 0 to 7.0 per 100 cattle in six categories of herds classified by herd size

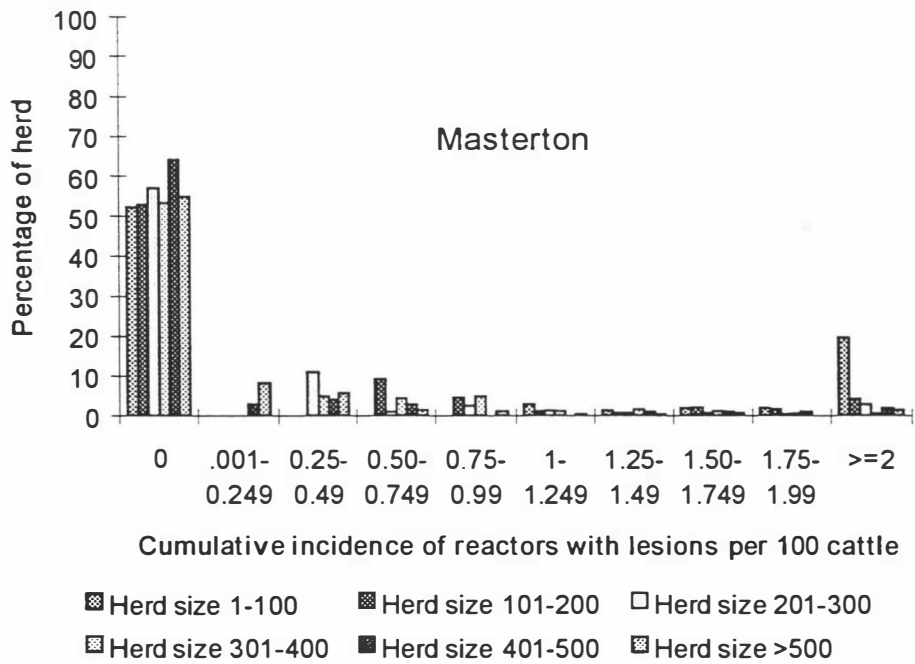


Figure 4.44. Masterton district. Distributions of cumulative incidence of lesioned reactors from 0 to ≥ 2.0 per 100 cattle in six categories of herds classified by herd size

Incidence densities of lesioned reactors in six categories of herds classified by herd size: Masterton district

The distributions of incidence densities of lesioned reactors for all levels up to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size are shown for the Masterton district in Figure 4.45, and for levels between 0 and $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in Figure 4.46. The histograms show similar patterns to those for cumulative incidence.

The major differences between herds in the Masterton and Taumarunui districts were that more Masterton herds had no new incident cases, and there were correspondingly fewer herds with more than 200 cattle at the $1.0 \times 10^{-2} \text{ year}^{-1}$ level (representing incidences of new cases up to 1%). A comparison with reactor incidences as shown in Figure 4.6, suggests different proportions of reactors with lesions to reactors over the range of herd sizes of more than 100 cattle in the Masterton district. No such differences were evident in the Taumarunui data.

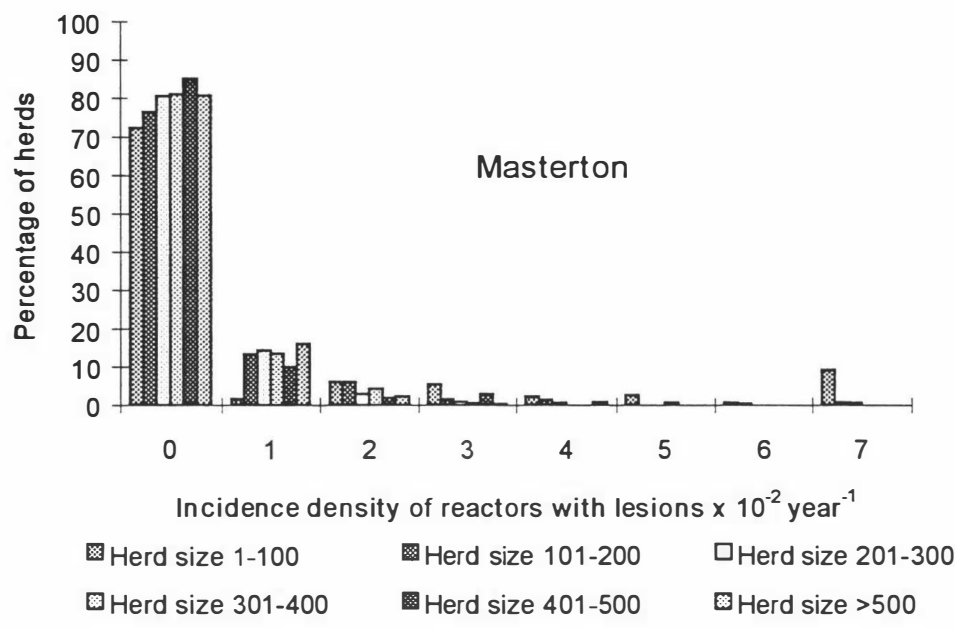


Figure 4.45. Masterton district. Distributions of incidence density of lesioned reactors from 0 to 7.0 x 10⁻² year⁻¹ in six categories of herds classified by herd size

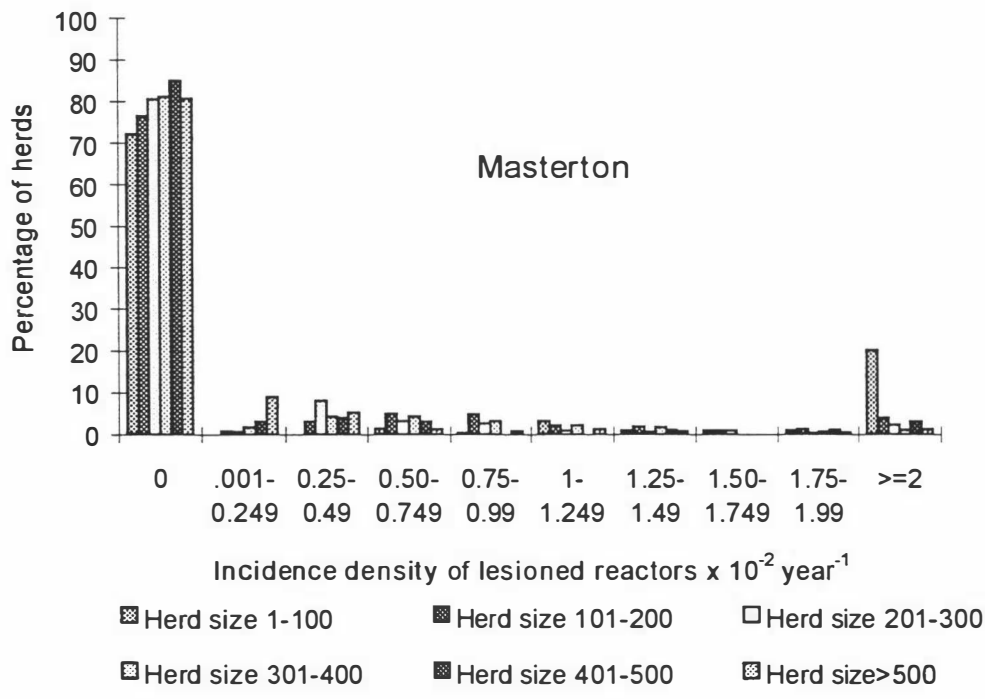


Figure 4.46. Masterton district. Distributions of cumulative incidence of lesioned reactors from 0 to ≥2.0 x 10⁻² year⁻¹ in six categories of herds classified by herd size

**Cumulative incidence of lesioned reactors in six categories of herds classified by herd size:
Surveillance areas**

The distributions of cumulative incidences of lesioned reactors per 100 cattle in six categories of herds classified by herd size are shown for the Surveillance areas in Figures 4.47 and 4.48. Apart from herds with 1-100 cattle, most herds of other sizes were either not at risk of having lesioned reactors or had low cumulative incidences. Seventeen percent of herds with 1-100 cattle had cumulative incidences of ≥ 2.0 per 100 cattle and more than half of these herds were at ≥ 6.0 per 100 cattle.

In surveillance areas, herds with more than 500 cattle had the lowest risk of having lesioned reactors identified. No such herd size difference was evident for the Masterton or Taumarunui districts.

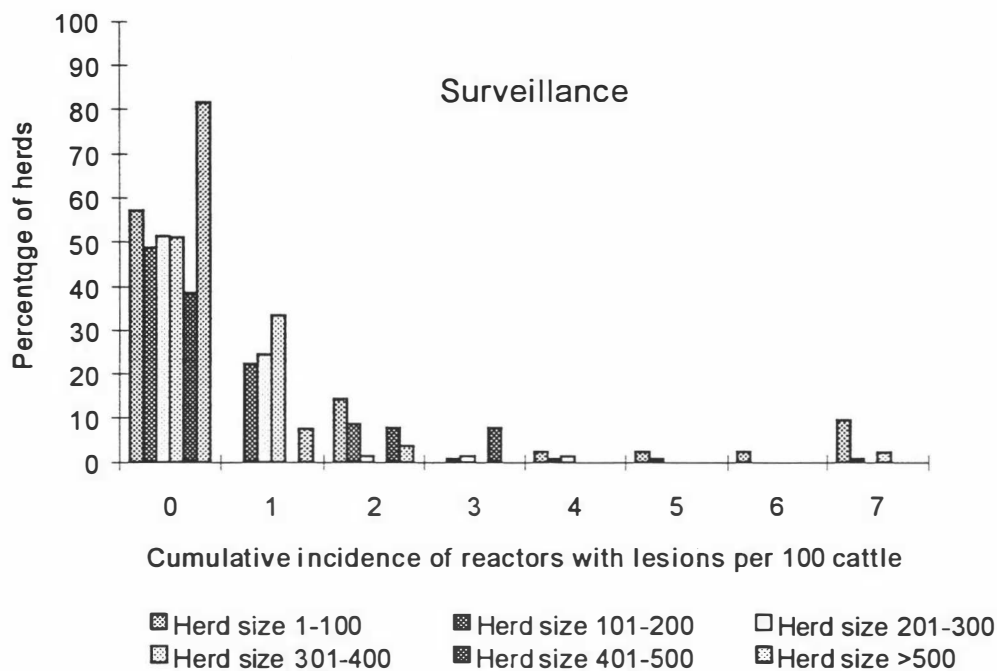


Figure 4.47. Surveillance areas. Distributions of cumulative incidence of lesioned reactors from 0 to 7.0 per 100 cattle in six categories of herds classified by herd size

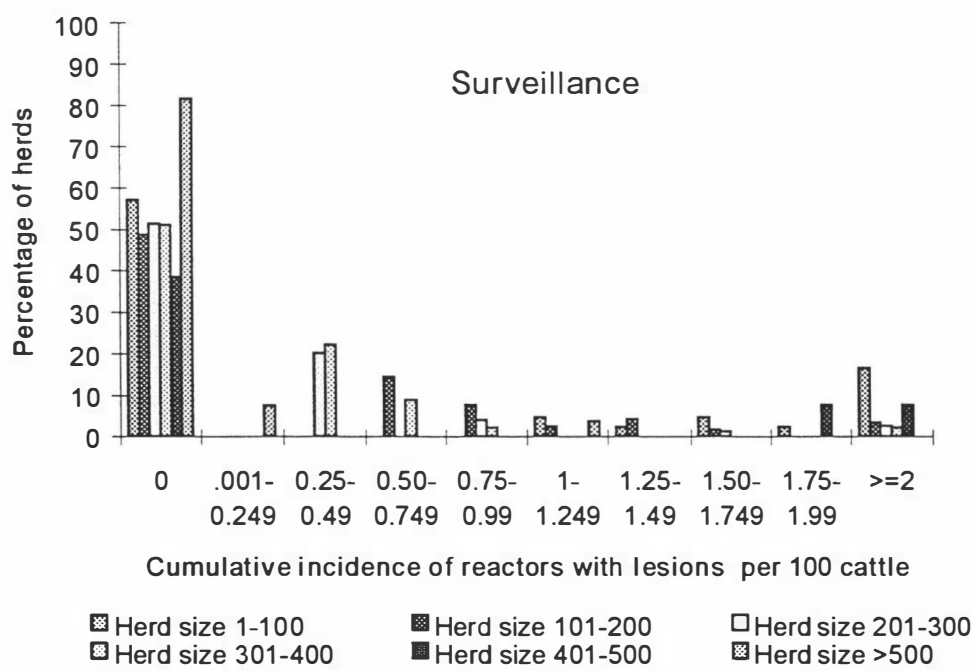


Figure 4.48. Surveillance areas. Distributions of cumulative incidence of lesioned reactors from 0 to ≥ 2.0 per 100 cattle in six categories of herds classified by herd size

**Incidence densities of lesioned reactors in six categories of herds classified by herd size:
Surveillance areas**

The distributions of incidence densities of lesioned reactors for all levels up to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size are shown for the Masterton district in Figure 4.49, and for levels between 0 and $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in Figure 4.50. All herd sizes were represented at the $1.0 \times 10^{-2} \text{ year}^{-1}$ level, whereas the statistics for cumulative incidence illustrated in Figure 4.44 indicate that herds with 1-100 cattle or 401-500 cattle were not at risk at that level of cumulative incidence.

Few herds in surveillance areas had incident cases of reactors which were subsequently shown to have lesions.

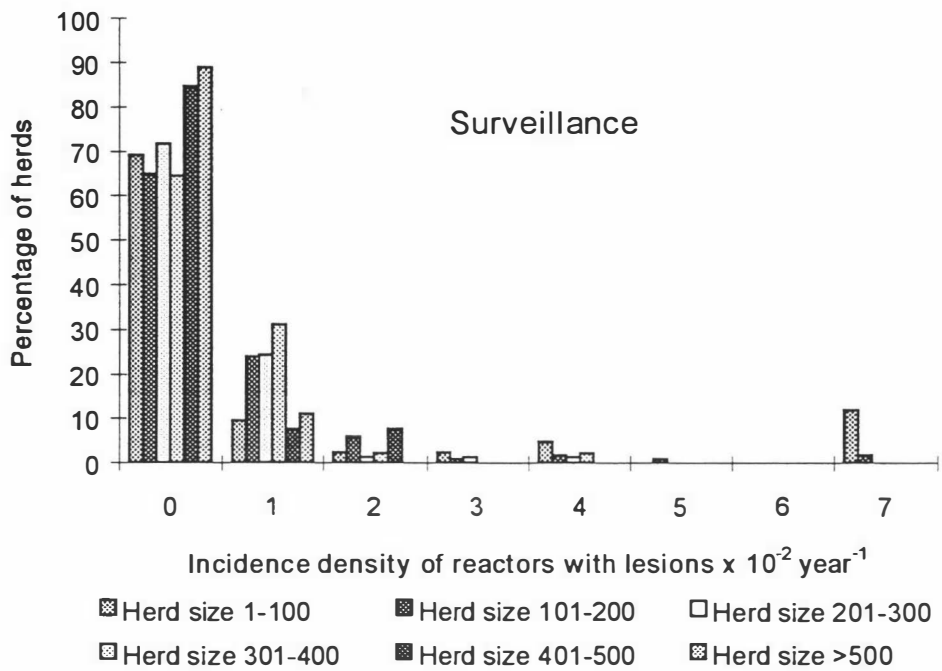


Figure 4.49. Surveillance areas. Distributions of incidence density of lesioned reactors from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size

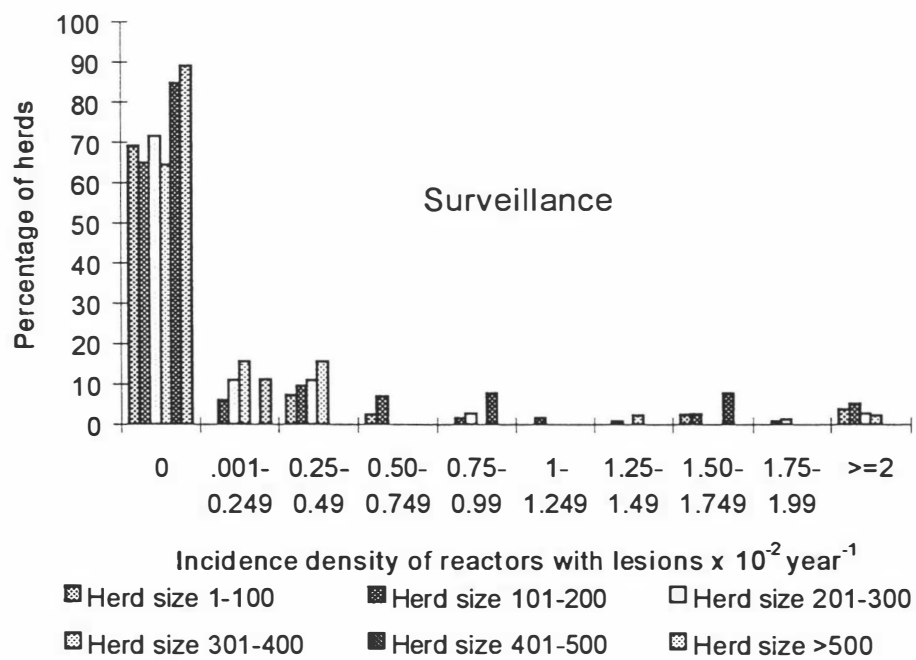


Figure 4.50. Surveillance areas. Distributions of incidence density of lesioned reactors from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in six categories of herds classified by herd size

Herd incidence densities of movement control herds at and following initial breakdowns:**Taumarunui district**

The average incidence densities $\times 10^{-2} \text{ year}^{-1}$ for movement control herds in the Taumarunui district for each year after initial breakdowns are shown in Figure 4.51. The highest incidence density ($2.2 \times 10^{-2} \text{ year}^{-1}$) was in the first year of breakdown, after which the rate declined before stabilising at about $1.75 \times 10^{-2} \text{ year}^{-1}$ in the 4th, 5th and 6th years.

First year after initial breakdown

The distributions of incidence densities from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ for all herd size categories are shown in Figure 4.52 for the first year after the initial breakdown. For most of the herd size classes the overall patterns were similar, with the greatest proportion having incidence densities $\leq 1.6 \times 10^{-2} \text{ year}^{-1}$. Small herds with up to 100 cattle were the exception with 66% of herds of that size having incidence densities $> 1.6 \times 10^{-2} \text{ year}^{-1}$.

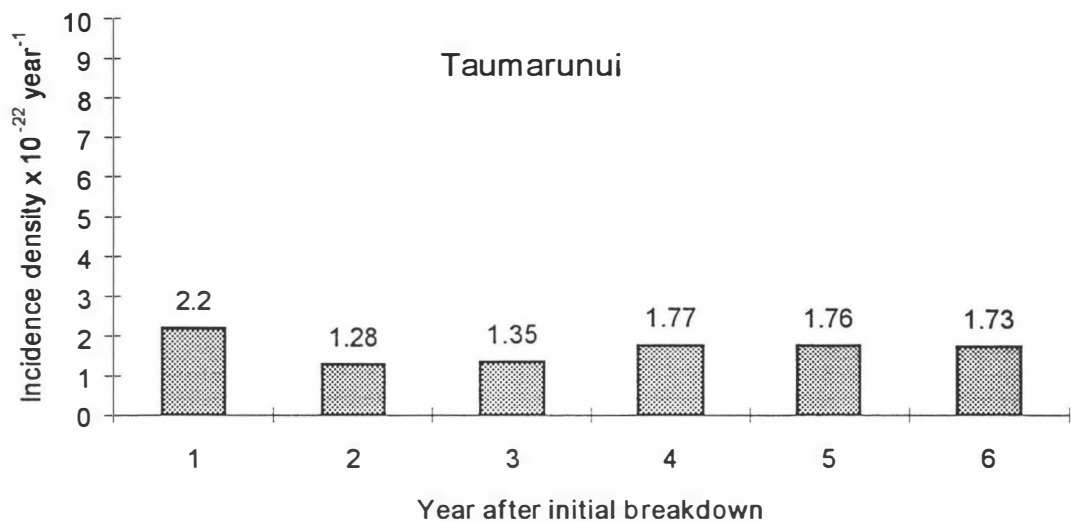


Figure 4.51. Average incidence densities x 10⁻² year⁻¹ for movement control herds in the Taumarunui district in each year after initial breakdowns

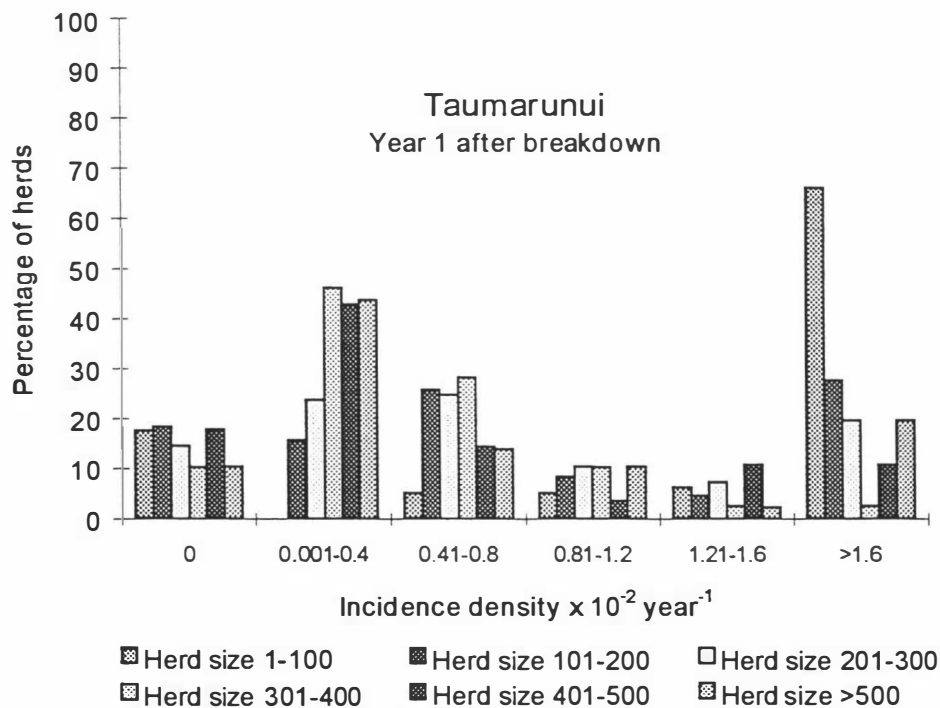


Figure 4.52. The relative frequencies of levels of incidence density from 0 to 1.6 x 10⁻² year⁻¹ in herds in the Taumarunui district in the first year after initial breakdowns

Second year after initial breakdowns

The distributions of incidence density from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ for all herd sizes in the second year following initial breakdowns are shown in Figure 4.53. As was found in year one after initial breakdowns, a large proportion of herds had incidence densities less than $1.6 \times 10^{-2} \text{ year}^{-1}$. The smallest herd size category of 1 to 100 cattle again had a different pattern, with 43% having incidence densities $>1.6 \times 10^{-2} \text{ year}^{-1}$.

Third year after initial breakdowns

The distributions of incidence density from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ for all herd sizes in the third year after their initial breakdowns are shown in Figure 4.54. Herds with more than 500 cattle had the fewest clear (incidence = 0) herds, although 69% percent of herds in this size category had had rates below $1.6 \times 10^{-2} \text{ year}^{-1}$. Small herds with fewer than 100 cattle were again represented well in the $>1.6 \times 10^{-2} \text{ year}^{-1}$ incidence density level by 36% of herds at those higher rates of infection.

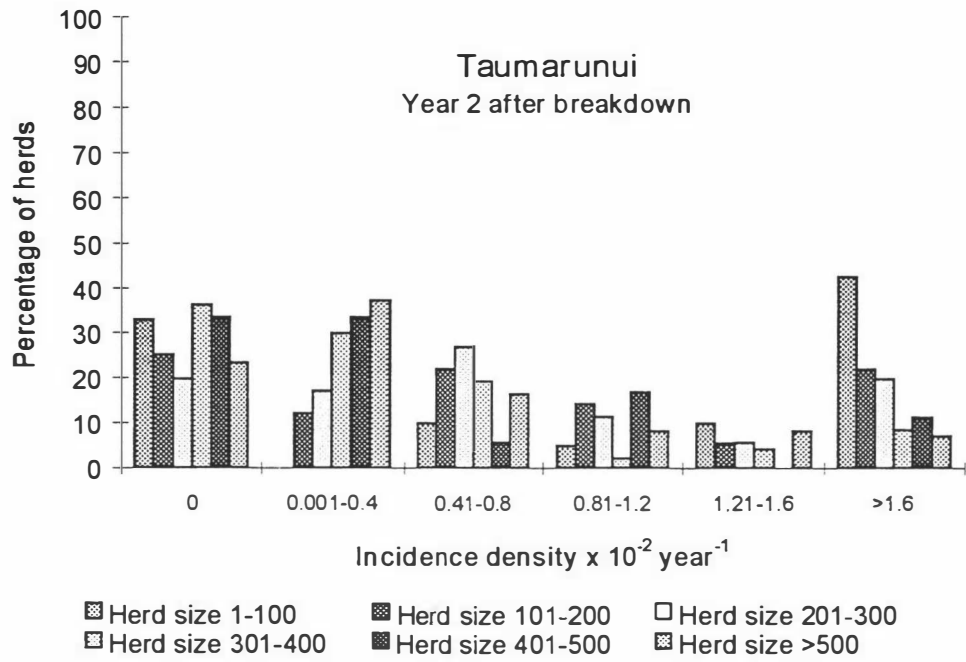


Figure 4.53. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in the Taumarunui district in the second year after initial breakdowns

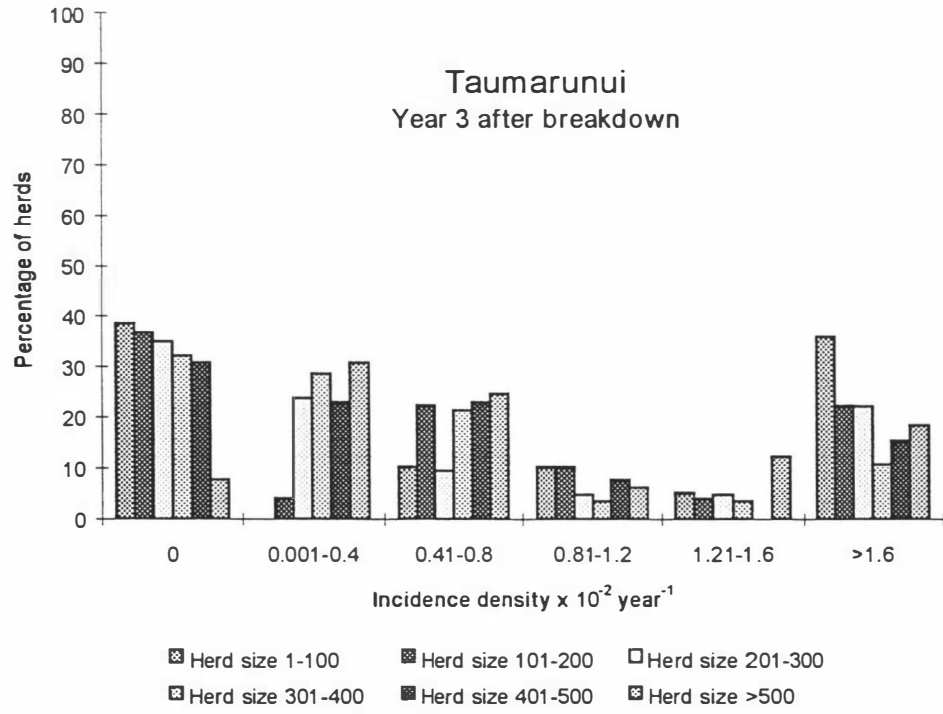


Figure 4.54. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in the Taumarunui district in the third year after initial breakdowns

Herd incidence densities of movement control herds at and following initial breakdowns:**Masterton district**

The average incidence densities for movement control herds in the Masterton district for each year after initial breakdowns are shown in Figure 4.55. The highest incidence density ($7.6 \times 10^{-2} \text{ year}^{-1}$) was in the second year after breakdown, after which the incidence declined and stabilised at about $1.75 \times 10^{-2} \text{ year}^{-1}$ in the 4th 5th and 6th years.

First year after initial breakdown

The distributions of incidence density from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ are shown in Figure 4.56 for the first year after the initial breakdown. The overall pattern resembled that for the Taumarunui district, and as in that district, small herds of 1-100 cattle were strongly represented at the $> 1.6 \times 10^{-2} \text{ year}^{-1}$ incidence density level by about 60% of herds.



Figure 4.55. Average incidence densities for movement control herds in the Masterton district in each year after initial breakdowns

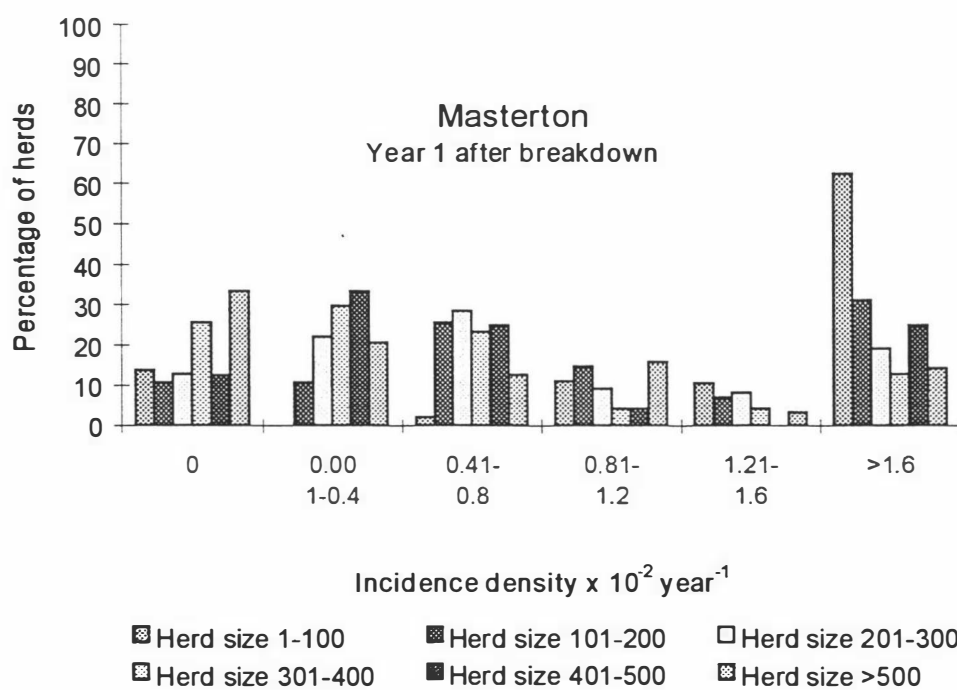


Figure 4.56. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in the Masterton district in the first year after initial breakdowns

Second year after breakdown

The overall pattern shown in Figure 4.57 followed that for the first year after breakdown and was similar to the pattern for year 2 in the Taumarunui district. About 60% of herds with 1-100 cattle had incidence densities $> 1.6 \times 10^{-2} \text{ year}^{-1}$.

Third year after breakdown

The pattern shown in Figure 4.58 was similar to that for the third year in the Taumarunui district but Masterton large herds were equally represented with other herd sizes in the zero reactor level. About 50% of herds with 1-100 cattle had incidence densities $> 1.6 \times 10^{-2} \text{ year}^{-1}$.



Figure 4.57. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in the Masterton district in the second year after initial breakdowns



Figure 4.58. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in the Masterton district in the third year after initial breakdowns

Distribution of incidence densities after herd breakdowns: Surveillance area

The average incidence densities for movement control herds in the Surveillance area for each year after initial breakdowns are shown in Figure 4.59 with the values for the Taumarunui and Masterton districts added for comparison. The highest incidence density ($2.0 \times 10^{-2} \text{ year}^{-1}$) was in the first year after breakdown, after which the rate declined over the next 2 years to be at zero for the 4th, 5th and 6th years.

First year after initial breakdown

The distributions of incidence density from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ are shown in Figure 4.60 for the first year after the initial breakdown. Few herds had incidence rate $>1.6 \times 10^{-2} \text{ year}^{-1}$. Apart from the smallest herd size category, 50-60% of all herds of other sizes had incidence densities $<1.6 \times 10^{-2} \text{ year}^{-1}$. About 20% of herds with 1-100 cattle had an annual incidence $>1.6 \times 10^{-2} \text{ year}^{-1}$.

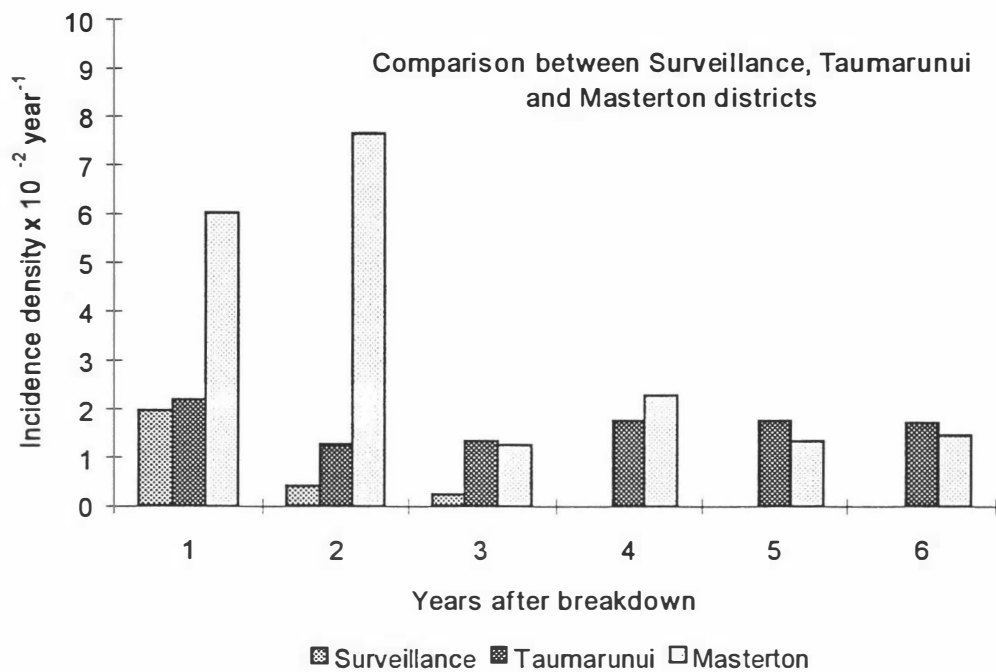


Figure 4.59. Average incidence densities for movement control herds in the Surveillance areas and the Taumarunui and Masterton districts in the years after an initial breakdown

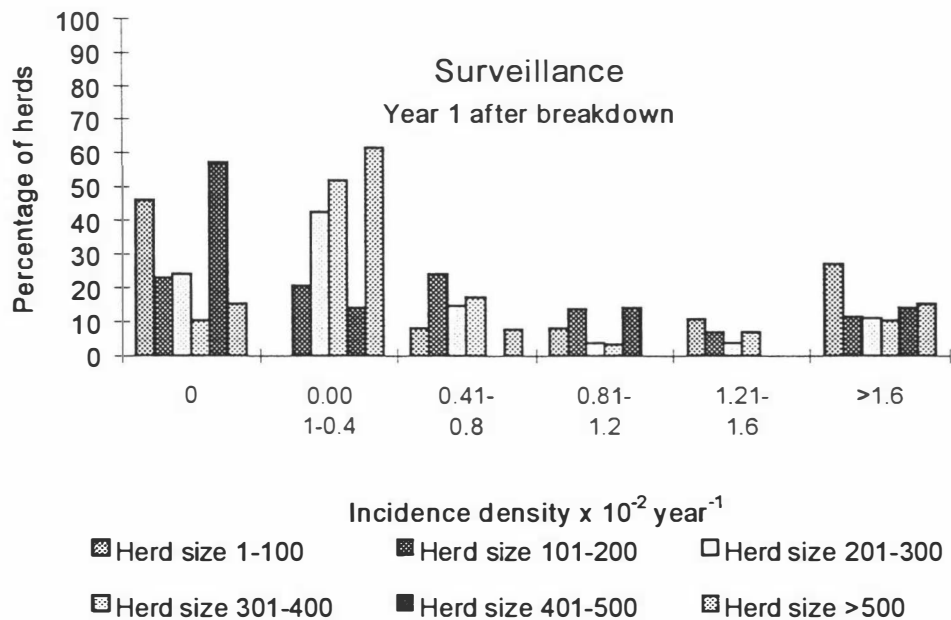


Figure 4.60. The relative frequencies of levels of incidence density from 0 to 1.6 x 10⁻² year⁻¹ in herds in Surveillance areas in the first year after initial breakdowns

Second and third years after breakdown

The distributions of incidence density from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in surveillance areas are shown in Figure 4.61 and Fig 4.62 for the second and third years after the initial breakdown. The distributions are patchy when compared with the endemic districts, a consequence of the greater number of herds in Surveillance areas which had no breakdowns.

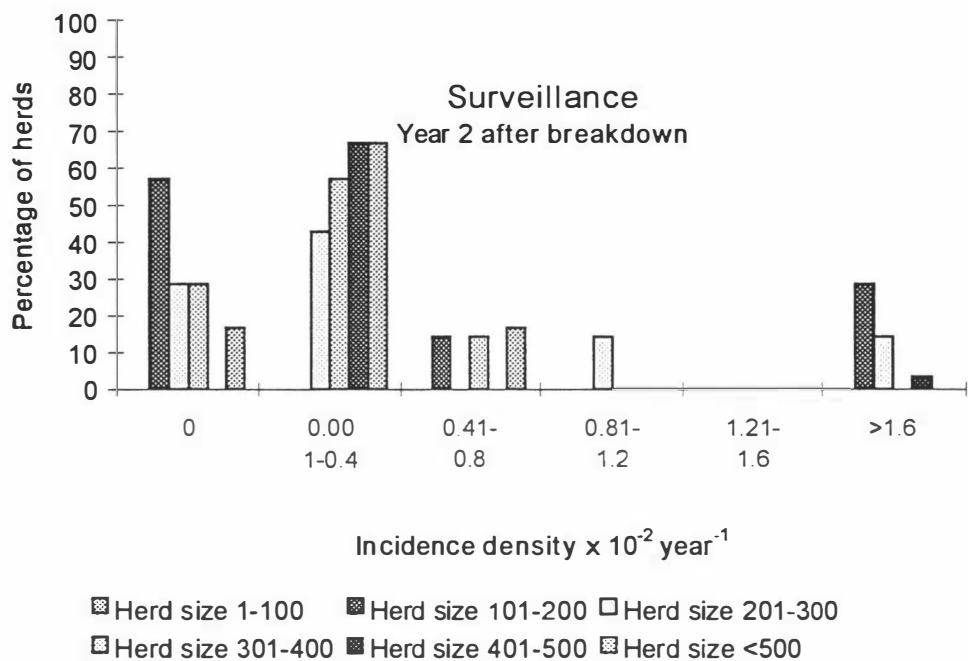


Figure 4.61. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in Surveillance areas in the second year after initial breakdowns

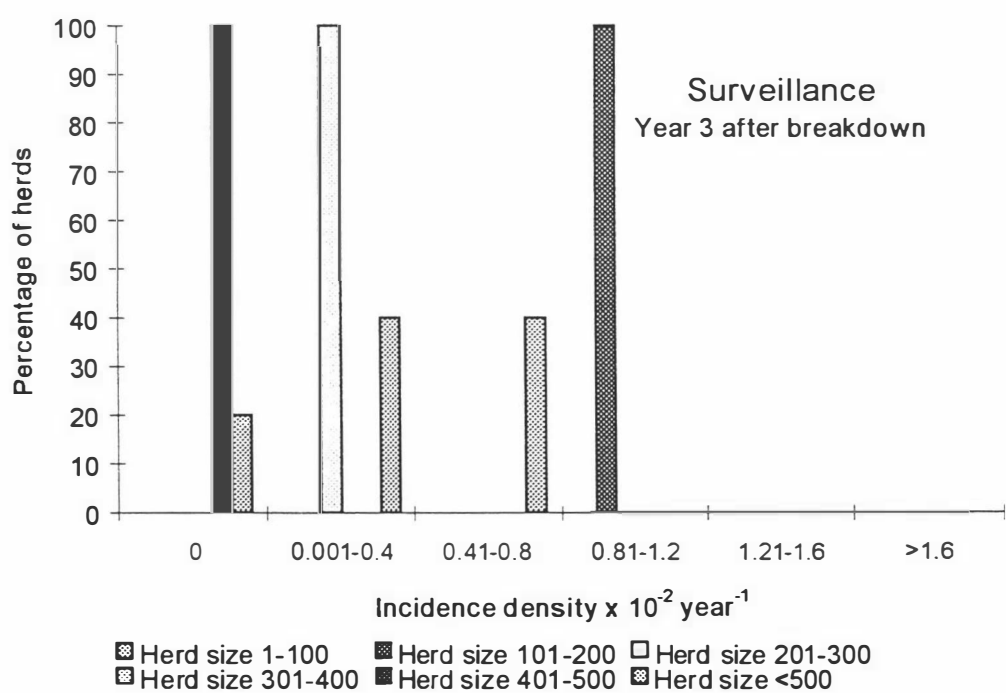


Figure 4.62. The relative frequencies of levels of incidence density from 0 to $1.6 \times 10^{-2} \text{ year}^{-1}$ in herds in Surveillance areas in the third year after initial breakdowns

Rates of reactors with lesions: Taumarunui district

The average incidence densities of reactors with lesions in movement control herds in the Taumarunui district in each year after initial breakdown are shown in Figure 4.63. The highest incidence density ($2.2 \times 10^{-2} \text{ year}^{-1}$) was in the first year of breakdown and the overall pattern closely follows that for all reactors shown in Figure 4.51.

The distribution of levels of incidence from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ for reactors with lesions are shown in Figure 4.64 for the first year after the initial breakdown. The pattern resembles that for all reactors seen in Figure 4.52 but in the case of reactors with lesions there is a general left shift in the percentages of herds affected towards zero levels.

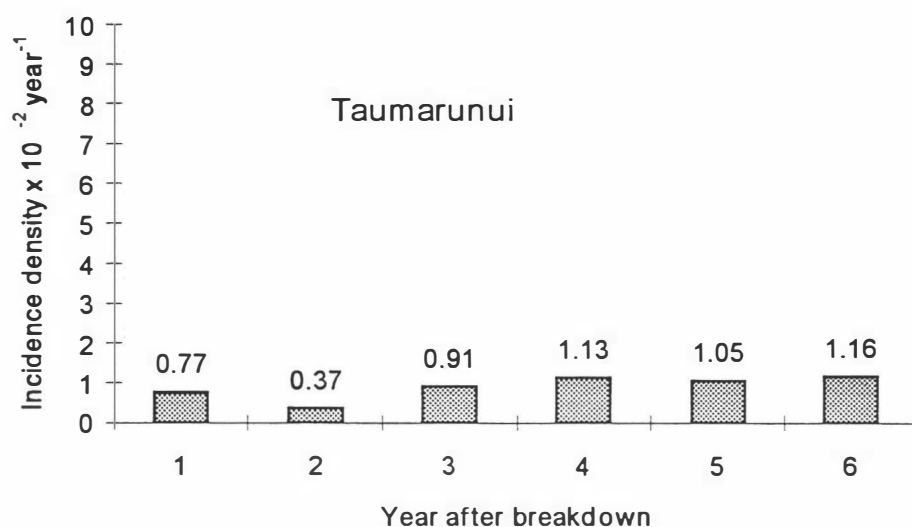


Figure 4.63 Average incidence densities of reactors with lesions in movement control herds in the Taumarunui district in each year after initial breakdown

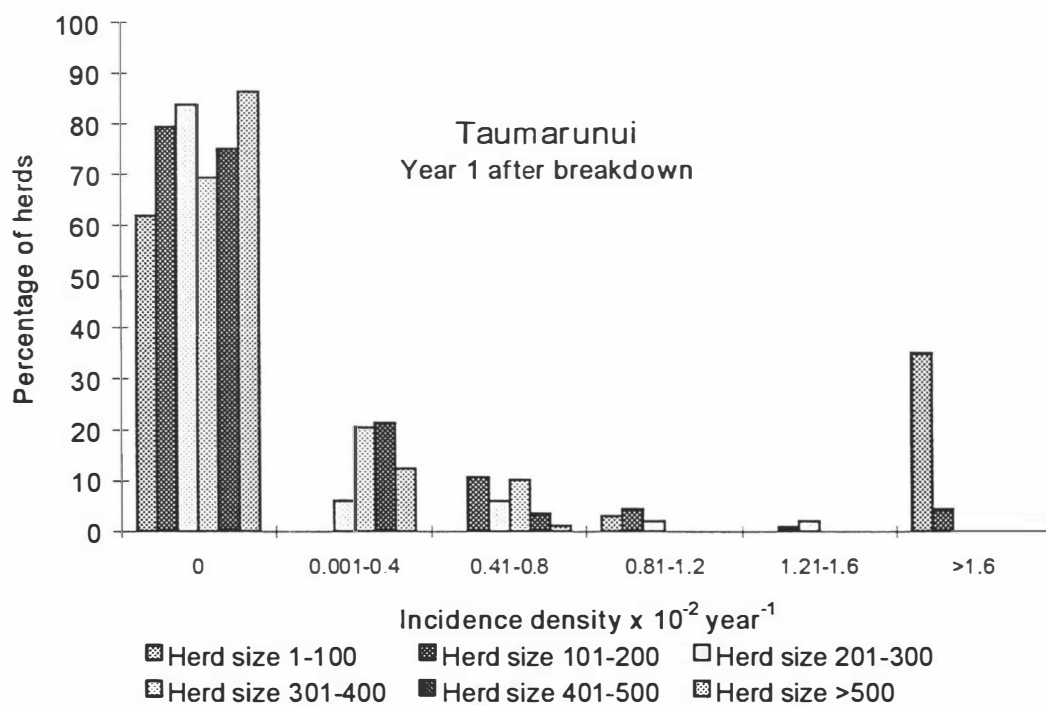


Figure 4.64. Percentages of different sized herds in the Taumarunui district at levels of incidence density of reactors with lesions from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ in the first year after the initial breakdown

Rates of reactors with lesions: Masterton district

The average incidence densities of reactors with lesions in movement control herds in the Masterton district in each year after initial breakdown are shown in Figure 4.65. The levels of incidence were similar for all years but the overall pattern varies considerably from that for all reactors shown in Figure 4.55, particularly in the first two years.

The distribution of levels of incidence from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ for reactors with lesions in Masterton district herds are shown in Figure 4.66 for the first year after the initial breakdown. The pattern resembles that for reactors in Figure 4.56, but the rates for reactors with lesions are lower than those of reactors.

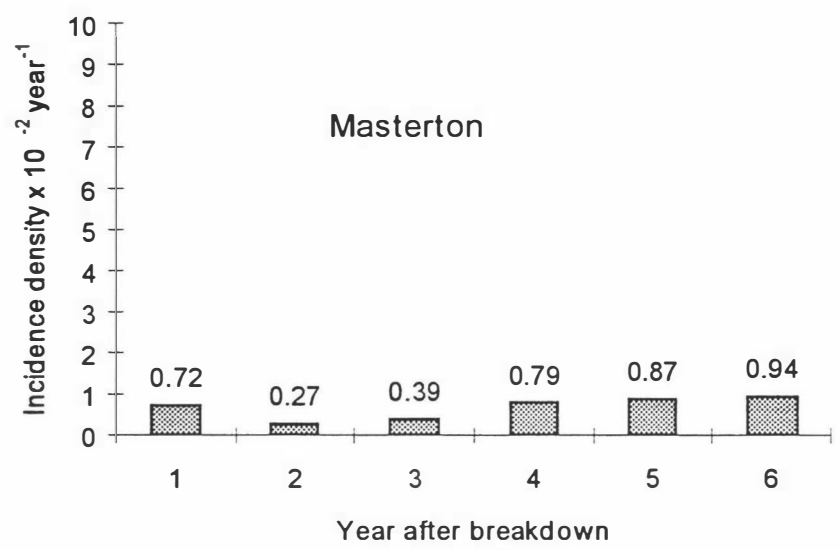


Figure 4.65. Average incidence densities of reactors with lesions in movement control herds in the Masterton district in each year after initial breakdown

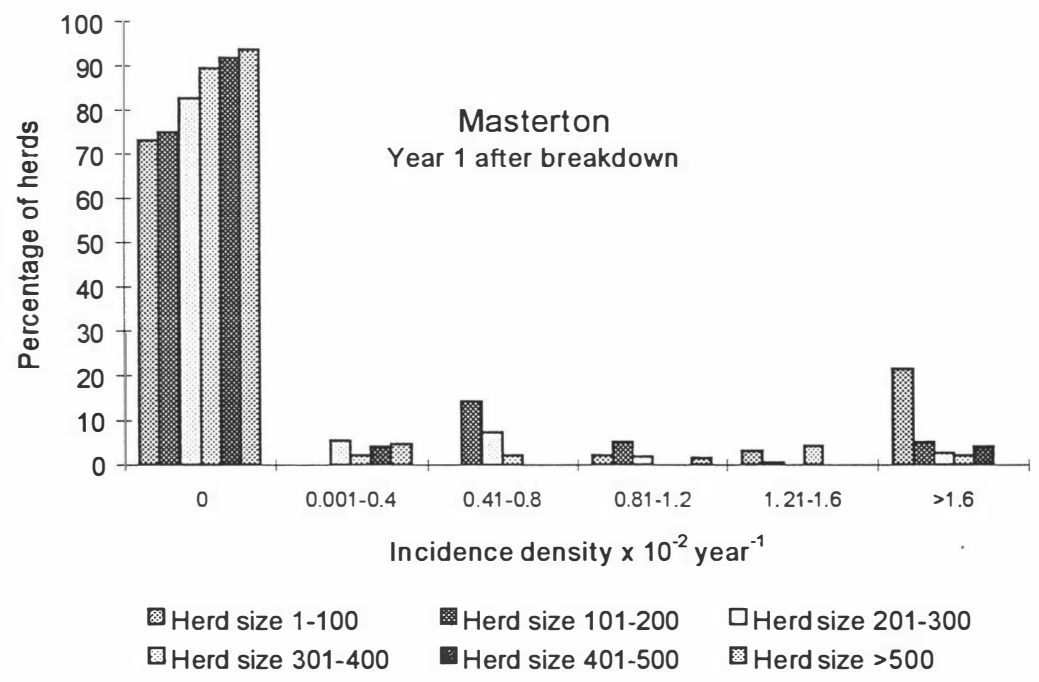


Figure 4.66. Percentages of different sized herds in the Masterton district at levels of incidence of reactors with lesions from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ in the first year after the initial breakdown

Rates of reactors with lesions: Surveillance areas

The average incidence densities of reactors with lesions in movement control herds in surveillance areas in each year after initial breakdown are shown in Figure 4.67. The pattern closely follows that shown for all reactors in Surveillance area herds in Figure 4.59.

The distribution of incidence densities for herds classified by size of herd in the Surveillance areas at rates of 0 to >1.6 reactors with lesions $\times 10^{-2} \text{ year}^{-1}$ are shown in Figure 4.68 for the first year after the initial breakdown.

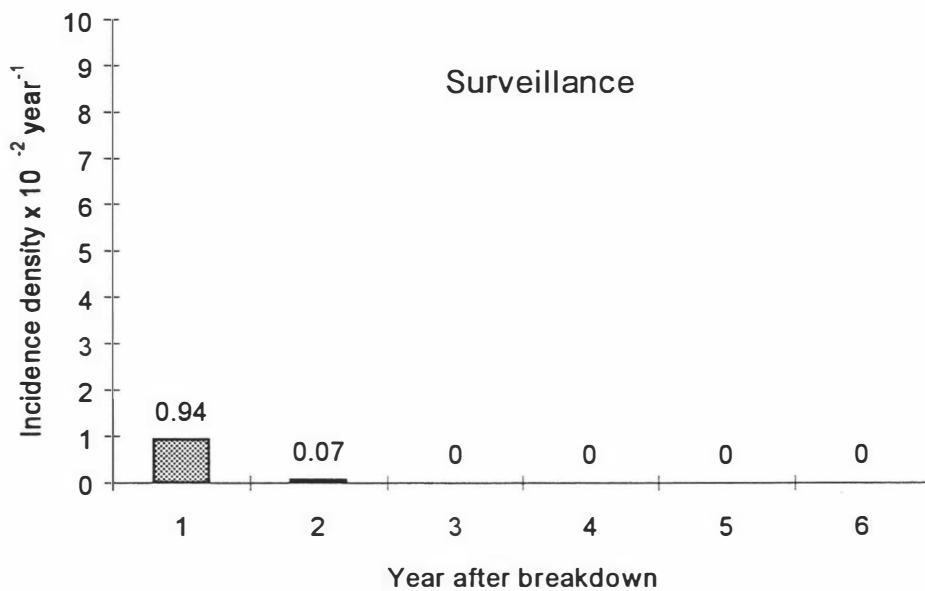


Figure 4.67. Average incidence densities of reactors with lesions in movement control herds in Surveillance areas in each year after initial breakdown

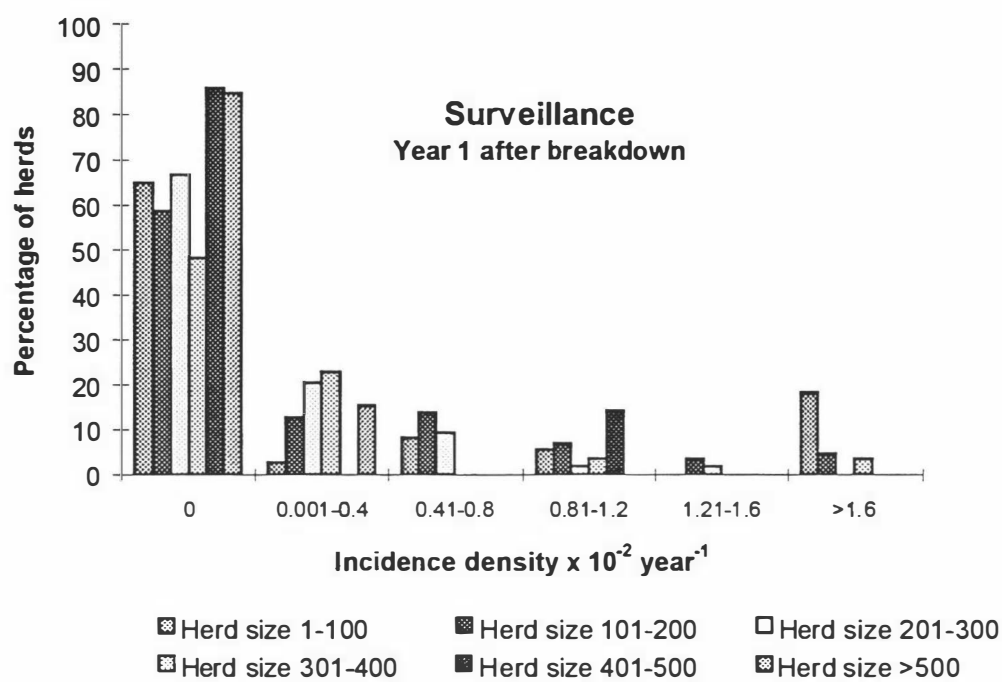


Figure 4.68. Percentages of different sized herds in Surveillance areas at levels of incidence of reactors with lesions from 0 to $>1.6 \times 10^{-2} \text{ year}^{-1}$ in the first year after the initial breakdown

Changes in herd status over time: Taumarunui district

The proportional changes in herd status over the six year period under consideration are shown in Table 4.1. Eighty-three percent of infected herds retained their movement control (infected) status and only 16 % became clear. Of herds which started with a clear status, 24 % reverted to infected status, 39 % remained clear and 36% reached free status. There was a reversion back to infected status for 25% of herds which were initially accredited.

Table 4.1. Matrix table showing changes in herd status over the period 1985-89 for herds in the Taumarunui district

	Infected	Clear	Free	Accredited
Infected	0.83	0.24	0.26	0.25
Clear	0.16	0.39	0.01	-
Free	0.01	0.36	0.56	0.01
Accredited	0	0.01	0.17	0.74

Changes in herd status over time: Masterton district

The proportional changes in herd status in the Masterton district over the six year period under consideration are shown in Table 4.2. Progress appeared to have been better in this district than in the Taumarunui district for herds which started as Infected, Clear and Free, but accredited herds fared slightly worse in the Masterton district with relatively more becoming infected.

Table 4.2. Matrix table showing changes in herd status over the period 1985-89 for herds in the Masterton district

	Infected	Clear	Free	Accredited
Infected	0.51	0.27	0.29	0.30
Clear	0.28	0.11	0.04	0.02
Free	0.16	0.32	0.27	0.01
Accredited	0.05	0.30	0.40	0.67

Changes in herd status over time: Surveillance area

The starting status for surveillance area herds was better than in the other regions and progress was satisfactory although 52% of herds which were accredited became re-infected.

Table 4.3. Matrix table showing changes in herd status over the period 1985-89 for herds in Surveillance areas

	Infected	Clear	Free	Accredited
Infected	0.41	0.05	0.16	0.52
Clear	0.40	0.21	-	0.02
Free	0.18	0.68	0.42	0.02
Accredited	0.01	0.06	0.42	0.44

RELATIONSHIPS BETWEEN INCIDENCE MEASURES

The relationships between cumulative incidence, corrected cumulative incidence and incidence density are shown in Table 4.4 as correlation coefficients and in Table 4.5 as rank coefficients. Spearman rank coefficients (r_s) are the most reliable measure when frequency distributions of data show non-normal distributions, as was the case for the data under consideration which showed marked positive skewness

Table 4.4 Correlations between cumulative incidence, corrected cumulative incidence and incidence density measured by the Pearson product moment correlation

	CI	Corrected CI	ID
Corrected CI	0.84(3001)		0.33(2947)
CI		0.84(3001)	0.28(3321)

() = (sample size)

Table 4.5 Correlations between cumulative incidence, corrected cumulative incidence and incidence density measured by the Spearman rank correlation (sample size)

	CI	Corrected CI	ID
Corrected CI	0.94(3001)		0.94(2947)
CI		0.94(3001)	0.96(3321)

() = (sample size)

Correlations between herd cumulative incidence and cumulative incidence calculated separately for adult cows and yearling and 2 year-old bulls and heifers

The correlations between herd cumulative incidence and cumulative incidence in adult cows, yearling males and heifers are shown in Tables 4.6. Comparable data are shown in Tables 4.7 and 4.8 for correlations between corrected cumulative incidence and incidence density measures. There was little variation in the Spearman rank coefficients measures of the relationships between the separate incidence measures, although there was considerable variation between subclasses of cattle. The risk of disease for any animal in a herd was most closely related to the risk for individual adult cows. There was a poor relationship between the risks for any individual animal in the herd and yearlings, and a fair relationship between herd members and 2 year-olds.

Table 4.6 Pearson correlations (r) and Spearman rank correlations (r_s) between herd cumulative incidence and cumulative incidence stratified by age and sex

Cumulative incidence	r	r_s
cows	0.61 (2098)	0.68 (2098)
yearling males	0.42 (1543)	0.34 (1543)
yearling heifers	0.51 (1577)	0.33 (1577)
2 year-old males	0.33 (1365)	0.49 (1365)
2 year-old heifers	0.43 (1627)	0.50 (1627)

() = (sample size)

Table 4.7 Pearson and Spearman rank correlations between herd corrected cumulative incidence and cumulative incidence stratified by age and sex

Cumulative incidence	r	r_s
cows	0.55 (1992)	0.68 (1992)
yearling males	0.32 (1480)	0.38 (1480)
yearling heifers	0.38 (1507)	0.35 (1507)
2 year-old males	0.35 (1341)	0.50 (1341)
2 year-old heifers	0.45 (1555)	0.51 (1555)

() = (sample size)

Table 4.8 Pearson and Spearman rank correlations between herd incidence density and cumulative incidence stratified by age and sex

Cumulative incidence	<i>r</i>	<i>r_s</i>
cows	0.27 (2087)	0.66 (2087)
yearling males	0.40 (1536)	0.36 (1536)
yearling heifers	0.52 (1571)	0.34 (1571)
2 year-old males	0.16 (1355)	0.49 (1355)
2 year-old heifers	0.45 (1615)	0.50 (1615)

() = (sample size)

Comparisons of distributions of mean cumulative incidences calculated for whole districts and Surveillance areas, zones designated by status, and different types of herds

The mean cumulative incidences for herds in whole districts and surveillance areas, in zones designated by status, and for herds of different types are shown in Table 4.9. The mean cumulative incidences were highest in herds in surveillance areas (2.91 per 100 cattle) and lowest in those in the Masterton district (2.36 per 100 cattle) with a statistically significant difference between their distributions in Taumarunui and Masterton.

Herds in the endemic zones had the highest average cumulative incidence (2.46 per 100 cattle) and herds in surveillance zones had the lowest (0.34 per 100 cattle) and there were statistically significant differences between the distributions of incidence in endemic and fringe and endemic and surveillance zones.

Beef dry stock herds had the highest cumulative incidences of the three most prevalent herd types and there were statistically significant differences between distributions of incidence in beef breeding and beef dry stock herds and beef dry stock and dairy herds. The miscellaneous types of herds had the highest levels of risk but it was not possible to make sound comparisons between them and other types of herds because of small sample sizes.

Table 4.9 Kruskal-Wallis test of comparisons of distributions of mean cumulative incidence calculated for whole districts and Surveillance areas, zones designated by status, and herds of different types

Categories	Mean C I per 100 cattle	N	K-W comparison	P- value
<u>District</u>				
Taumarunui (T)	2.41	1520	T-M	0.001
Masterton (M)	2.36	1615		
Surveillance (S)	2.91	317		
<u>Zone</u>				
Endemic (E)	2.46	3004	E-F,E-S	0.000

Categories	Mean C I per 100 cattle	N	K-W comparison	P- value
Fringe (F)	0.77	114		
Surveillance	0.34	13		
Non-endemic (N)	1.25	4		
Herd type				
Beef breed (BB)	1.93	2335	BB-BD	0.000
Beef dry stock (BD)	4.28	486	BD-DH	
Dairy herd (DH)	1.75	555		
Miscellaneous dry stock ^a	11.12	11		
Miscellaneous Surveillance ^a	12.76	50		
Non eligible ^a	4.76	15		

^a inadequate sample sizes for comparisons

Comparisons of distributions of means of corrected cumulative incidence calculated for whole districts and Surveillance areas, zones designated by status, and herds of different types

The mean corrected cumulative incidences for herds from whole districts and surveillance areas, zones designated by disease status, and for herds of different types are shown in Table 4.10. The mean corrected cumulative incidence was higher in the Taumarunui district (2.47 per 100 cattle) than in the Masterton district (2.07 per 100 cattle) with a statistically significant level of difference between the distributions.

Herds in the endemic zones had the highest average corrected cumulative incidences (2.33 per 100 cattle) and those in surveillance zones had the lowest (0.13 per 100 cattle) and there were statistically significant differences between the distributions of the corrected cumulative incidences in endemic and fringe and endemic and surveillance zones.

Beef dry stock herds had the highest corrected cumulative incidences of the three most prevalent herd types and there were statistically significant differences between distributions of incidence in beef breeding and dairy herds and dry stock herds and between beef breeding and dairy herds. As in the cumulative incidence statistics, the miscellaneous types of herds again had the highest levels of risk and comparisons were hindered by low sample sizes.

Table 4.10 Kruskal-Wallis test of comparisons of distributions of mean corrected cumulative incidences calculated for whole districts and Surveillance areas, zones designated by status, and herds of different types

Categories	Mean corrected CI per 100 cattle	N	K-W comparison	P-value
<u>District</u>				
Taumarunui (T)	2.47	1436	T-M	0.000
Masterton (M)	2.07	1565		
<u>Zone</u>				
Endemic (E)	2.33	2882	E-F,E-S	0.000
Fringe (F)	0.58	105		
Surveillance (S)	0.13	11		
Non-endemic	0.63	3		
<u>Herd type</u>				
Beef breeding (BB)	2.11	2335	BB-BD, BB-DH BD-DH	0.000
Beef dry stock (BD)	3.34	413		
Dairy herd (DH)	1.12	372		
Miscellaneous dry stock	12.14	7		
Miscellaneous Surveillance	8.57	31		
Non eligible	6.30	9		

Comparisons of distributions of means of incidence density calculated for whole districts and Surveillance areas, zones designated by status, and herds of different types

The mean incidence densities for herds from whole districts and Surveillance areas, zones designated by status, and for herds of different types are shown in Table 4.11. The mean incidence density was highest in the Surveillance areas ($0.11 \times 10^{-2} \text{ year}^{-1}$) and lowest in the Masterton district ($0.038 \times 10^{-2} \text{ year}^{-1}$) with statistically significant levels of difference between the distributions for Taumarunui and Masterton and Taumarunui and Surveillance areas.

Herds in the endemic zones had the highest average incidence densities and there were statistically significant differences between distributions of the incidence densities in endemic and fringe and endemic and Surveillance zones.

Beef dry stock herds had the highest average incidence density ($0.092 \times 10^{-2} \text{ year}^{-1}$) of the three most prevalent herd types and there were statistically significant differences between distributions of incidence in beef breeding and dairy herds and dry stock herds and dairy herds. As in the cumulative incidence statistics, the miscellaneous types of herds had the highest rates of infection and comparisons were again hindered by low sample sizes.

Table 4.11 Kruskal-Wallis test of comparisons of distributions of herd incidence densities calculated for whole districts and Surveillance areas, zones designated by status, and herds of different types

Categories	Mean ID ^a x 10 ⁻² year ⁻¹	N	K-W comparison	P- value
<u>District</u>				
Taumarunui (T)	0.027	1494	T-M T-S	0.001
Masterton (M)	0.038	1539		
Surveillance (S)	0.11	342		
<u>Zone</u>				
Endemic (E)	0.034	2904		0.000
Fringe (F)	0.0073	112		
Surveillance	0.0017	13		
Non-endemic	0.0082	4		
<u>Herd type</u>				
Beef breed	0.025	2283		
Beef dry stock	0.092	473		
Dairy herd	0.032	550		
Miscellaneous dry stock	0.10	12		
Miscellaneous Surveillance	0.41	43		
Non eligible	0.05	14		

ID^a = incidence density

Comparison between cumulative and corrected cumulative incidences calculated for Calendar, Financial and Test years

The Wilcoxon signed ranks test demonstrated statistically significant differences ($P = <0.0001$) between the cumulative and corrected cumulative incidences calculated for Calendar, Financial and Test years. Both cumulative incidence measures were highest when calculated on the basis of Test years.

REGRESSION MODELS

Logistic regression analysis

The relationship between cumulative incidence per 100 cattle (dependent variable) and the independent variables herd type, area status, district, the number of animals in tested herds and the year of test were examined using logistic regression (Table 4.12).

Variables with multiple categories were represented by a set of indicator or dummy variables. Only indicator variables which had a statistically significant regression coefficient were included in the final model and those variables which had no effect represent the base line risk.

The risk of tuberculosis was higher in beef dry stock and miscellaneous type herds than in dairy herds. The odds of cattle testing positive in herds in endemic areas was about five times as high as in herds in surveillance and fringe areas, where the risks of tuberculosis were about the same. The likelihood of reacting to the tuberculin test was considerably lower for animals in the Masterton and Taumarunui districts than for animals from movement control herds in surveillance areas outside those districts. The overall risk of infection increased slightly from 1985 to 1990.

Table 4.12 Final Logistic regression model for relationship between predictor variables and cumulative

Variable	Category	Coefficient	Std Error	P-value	OR ()
<u>Constant</u>		-7.06	0.42	<0.0001	
<u>Herd type</u>	Dairy herd	Base line			
	Beef breed	-0.09	0.03	0.0003	0.91 (0.86-0.97)
	Beef dry stock	0.21	0.04	<0.0001	1.23 (1.14-1.33)
	Miscellaneous	1.27	0.09	<0.0001	3.56 (2.99-4.25)
	Others herd type	-0.52	0.17	0.0028	0.59 (0.43-0.83)
<u>Area status</u>	Surveillance	Base line			
	Fringe	Base line			
	Endemic	1.67	0.06	<0.0001	5.31 (4.72-5.98)
<u>District</u>	Surveillance area	Base line			
	Masterton	-1.89	0.07	<0.0001	0.15 (0.13-0.17)
	Taumarunui	-1.40	0.07	<0.0001	0.25 (0.21-0.28)
<u>Year</u>		0.03	0.01	<0.0001	1.03 (1.01-1.05)

Deviance = 37250, degrees of freedom = 6066, P-value = <0.01

OR () = Odds Ratio (approximate 95% confidence intervals)

Poisson regression

The relationship between the same independent variables and incidence density was examined using poisson regression (Table 4.13). There was an increased rate of infection in Beef breeding, Beef dry stock and other herd types compared to Dairy herds. Herd in endemic areas had rates of infection about seven times those of Fringe and Surveillance area herds, where the rates were about the same. The rate of infection in herds increased with increased herd size and the rate of infection was considerably less in the Masterton and Taumarunui districts than in movement control herds in the Surveillance areas. The logistic and poisson regression models show good

general agreement in the overall relationships between the predictor variables common to both models and their respective dependent variables.

Table 4.13. Final poisson regression model for effects of predictor variables on incidence density

Variable	Category	Coefficient	S E	P-value	RR ()
<u>Constant</u>		-3.32	0.27	0	
<u>Herd type</u>	Dairy herd	Base line			
	Beef breeding	0.5	0.27	0.0686	1.65 (.97-2.8)
	Beef dry stock	1.73	0.3	0	5.64 (3.13-10.16)
	Other herd types	2.18	0.33	0	8.85 (4.63-16.89)
<u>Area status</u>	Surveillance	Base line			
	Fringe	Base line			
	Endemic	1.94	0.97	0.0449	6.96 (1.04-46.58)
<u>District</u>	Surveillance area	Base line			
	Taumarunui	-3.17	0.98	0.0013	0.042 (0.01-0.29)
	Masterton	-2.81	0.98	0.0043	0.06 (0.01-0.41)
<u>Number tested</u>		-0.0015	0.01	0.0061	0.99 (0.15-6.82)

RR () = Relative risk (approximate 95% confidence intervals),
Deviance = 694.8, degrees of freedom = 6122, P-value = 0.00

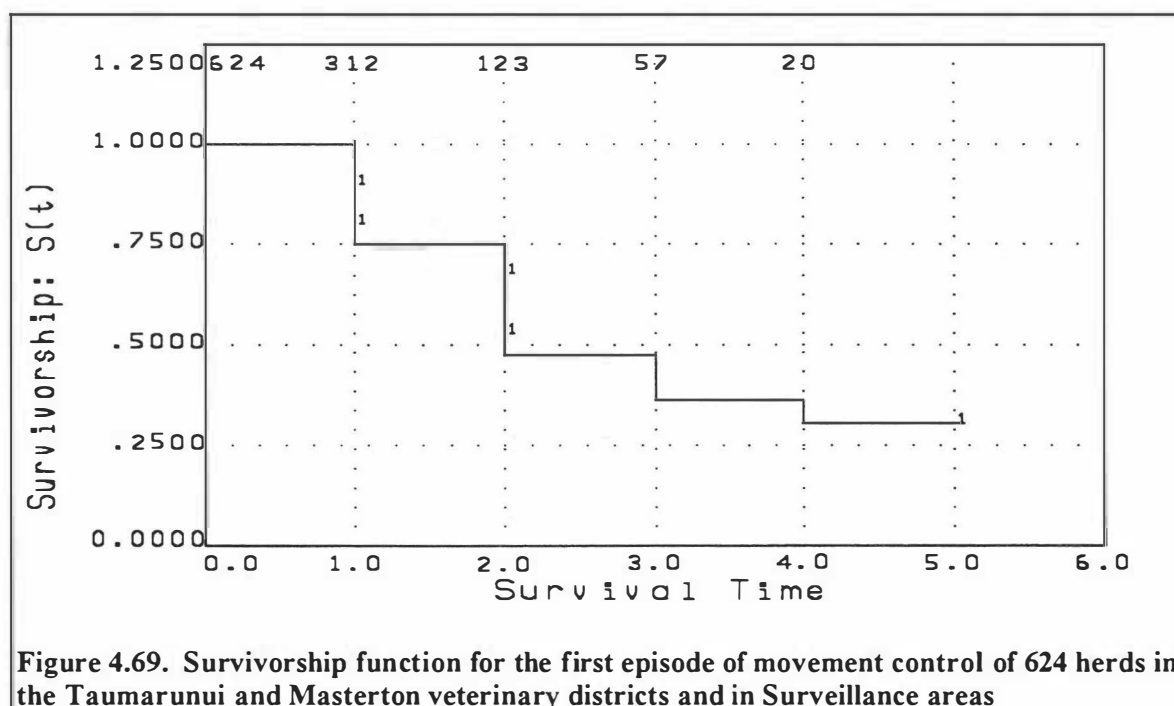
SURVIVAL ANALYSIS

Factors considered likely to influence the length of time herds stayed on movement control were investigated using survival analysis. The study was confined to the first episode of movement control in herds which had previously had no reactors and Figure 4.69 presents the survivorship function for time on movement control for the 624 herds which met that criterion. The survivorship function represents the probability that a randomly selected herd from the group on movement control would stay on movement control versus time. About 75% of herds were still on movement control after 12 months and about 50% after 2 years.

Herds in the Masterton veterinary district (N = 257) stayed on movement control longer than herds in the Taumarunui veterinary district and surveillance areas (combined total N = 367, shown as baseline in Figure 4.70). After 2 years of testing, about 60% of infected herds in the Taumarunui veterinary district and surveillance areas and 40% of infected herds in the Masterton veterinary district had come off movement control. A statistically significant difference was shown between the survival probabilities of herds in the Masterton district and herds in the

Taumarunui district and surveillance areas (Log-rank $\chi^2 = 18.75$, P-value=0.0023). The Log-rank test is a nonparametric procedure which compares the entire area under each curve and uses a χ^2 statistic based on the difference between observed and expected events.

The survivorship functions for movement control herds in zones classified according to tuberculosis status are shown in Figure 4.71. The survivorship probability of infected herds in fringe, surveillance and non-endemic zones was different to that for infected herds in endemic zones. There was a statistically significant difference between survivorship in the two groups of herds (Log rank $\chi^2 = 13.28$, P-value = 0.0004). The estimated median time on movement control was 3 years for herds from endemic areas and 2 years for herds (shown as baseline in Figure 4.71) in the fringe, surveillance and non-endemic zones. The median time identifies the mid-point on the survivorship function, and indicates how much time passes before half of the group have experienced the event of interest, viz. becoming free from infection.



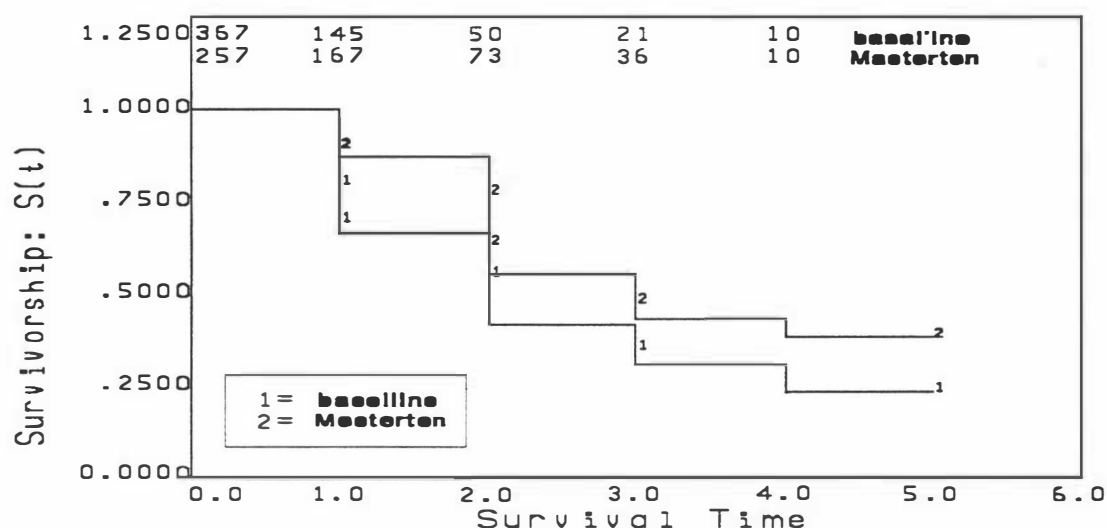


Figure 4.70. Comparison of survivorship functions between first episode movement control herds in the Masterton veterinary district (2) and first episode movement control herds from the Taumarunui veterinary district and Surveillance areas (1)

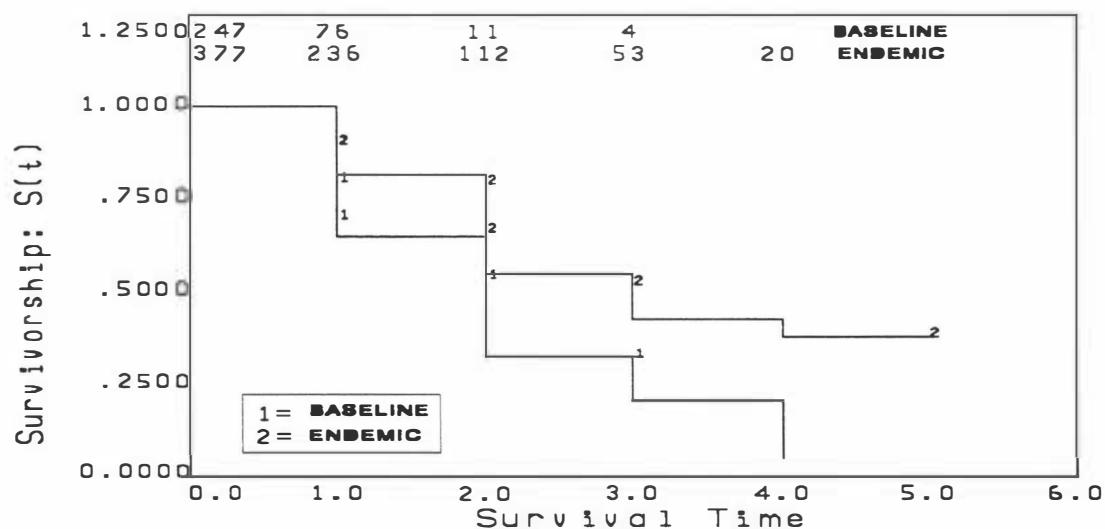


Figure 4.71. Comparison of survivorship functions between first episode movement control herds in endemic zones and in Surveillance and fringe zones

Proportional Hazards Regression Model

Cox's proportional hazard regression was applied in a stepwise fashion to identify and rank the importance of factors significantly associated with the probability of becoming clear from infection. The final regression model selected is set out in Table 4.14 and confirms the effects of

location illustrated in Figures 4.70 and 4.71. The calculated risk ratios indicate that herds located in endemic area were more likely to stay on movement control for longer periods than herds in fringe and surveillance zones, as were herds in the Masterton veterinary district compared to those from the Taumarunui veterinary district or surveillance areas. The risk of coming off movement control decreased as herd size increased although more detailed analyses would be required to determine the linear nature of the association between herd size and time. Herds with high levels of cumulative incidence were more likely to stay on movement control for longer periods than those with lower levels of incidence.

Table 4.14. Final Cox's proportional hazards regression model

Variable	Category	Coefficient	Std. error	P-value	Risk Ratio
Tb area class	Surveillance	Base line			
	Fringe Endemic	Base line -0.49	0.14	0.0005	0.61 (0.47-0.81)
TB district	Surveillance area	Base line			
	Taumarunui Masterton	Base line -0.37	0.14	0.01	0.69 (0.52-0.91)
No. tested		-1.77	0.04	<0.0001	0.17 (0.13-0.22)
CI		-0.044	0.01	<0.0001	0.96 (0.94-0.98)

Risk Ratio () = Exponential of the coefficient (95% confidence limits): $\chi^2 = 63.13$; P-value = <0.0001.

CI = Cumulative Incidence per 100 cattle

DISCUSSION

Routinely collected data were used in this study to describe the epidemiology of bovine tuberculosis in the Masterton and Taumarunui veterinary districts and surveillance regions throughout New Zealand. The Masterton district has a long history of endemic tuberculosis, whereas endemic status was conferred upon the Taumarunui district relatively recently after tuberculosis was discovered there in wildlife populations.

Epidemiological measures of cumulative incidence and incidence density were calculated and displayed graphically for reactors and reactors with visible gross lesions at slaughter. A variety of statistical techniques were then used to examine relationships between those measures and to

statistical techniques were then used to examine relationships between those measures and to identify herd and location factors influencing the incidence of the disease.

All analyses were constrained to some degree by the range of data available since they were based on data routinely collected as part of surveillance and control operations rather than specifically to investigate the nature of the disease. The purpose of the study reported here, however, was to closely examine the available data and evaluate its usefulness for providing a better understanding of the disease and its causality.

Simple tests of comparisons of incidence measures between districts, zones and herd types gave a somewhat confused overall picture of disease occurrence that was difficult to interpret. Multivariable analyses using logistic and poisson regression provided a better explanation:

- Both models showed higher risk and rates of disease in beef enterprises than in dairy herds;
- Higher risk and rates in endemic than in fringe and surveillance zones
- An increased rate of infection with increasing herd size
- A higher risk and rate in movement control herds in surveillance areas than in either the Masterton or Taumarunui veterinary districts.

Survival analysis gave a clearer insight into the length of time that herds stayed on movement control than did descriptive techniques such as matrix tables. Herds came off movement control sooner in the Taumarunui district than in the Masterton district and this may have reflected a more firmly and generally established endemic state of tuberculosis in wildlife in the Masterton district. This explanation might also explain a similar difference in the behaviour of the disease in herds in endemic and surveillance areas over time. Further investigation to determine factors responsible for the difference would be worthwhile and might identify important causal factors for disease persistence and occurrence.

CHAPTER 5

**An analysis of bovine tuberculosis case
data from a case-control study**

MATERIALS AND METHODS

Data used in this investigation was extracted from case herd data and data from control herds matched on management practices from a case-control study (Pfeiffer, 1995). Cases were defined as herds which were free of infection prior to being placed on movement control within the period 1986-1989, following identification of tuberculous cattle through test and/or other routine surveillance procedures. There were 95 case farms, of which 91 were used for analysis in this study, and they were located in the Waikato region in the central North Island of New Zealand. The questionnaire used in the case control study comprised 134 questions, of which 118 were answered by the person in charge of cattle management on the farm. Interviews were conducted by field personnel of the Ministry of Agriculture and Fisheries between 01/12/88 and 30/05/1990. The questionnaire covered general herd and farm management procedures plus general personal information about the interviewee.

Case herds

This data set, hereafter referred to as the case study data set, included data from 91 case herds. Of these 16 (17.58%) herds were beef breeding, 23 (25.27%) were beef dry stock, 51 (56.05%) were dairy herds and 1 (1.10%) was miscellaneous general purpose.

These distribution of these case herds by tuberculosis area class was 43 (47.25%) in surveillance areas, 43 (47.25%) in non-endemic areas, 3 (3.30%) in endemic areas and 2 (2.20%) in fringe areas.

RESULTS

DESCRIPTIVE STATISTICS

Cumulative incidence in case herds classified by herd size

The distributions of cumulative incidence per 100 cattle for all sizes of case herds are shown in Figure 5.1. Herds containing less than 100 cattle and between 101 to 200 cattle showed a cumulative incidence pattern which was distinctly different to those of other size herds. In 66% of herds with less than 100 cattle, the level of risk was at the 2.0 per 100 cattle level. When this finding was examined in more detail in Figure 5.2, it could be seen that about 55% of herds of that size were at the 1.0 to 1.249 level and 33% at ≥ 2.0 per 100 cattle level. In 39% of herds containing 101 to 200 cattle the risk was ≥ 2.0 per 100 cattle. The larger herds generally had low levels of cumulative incidence.

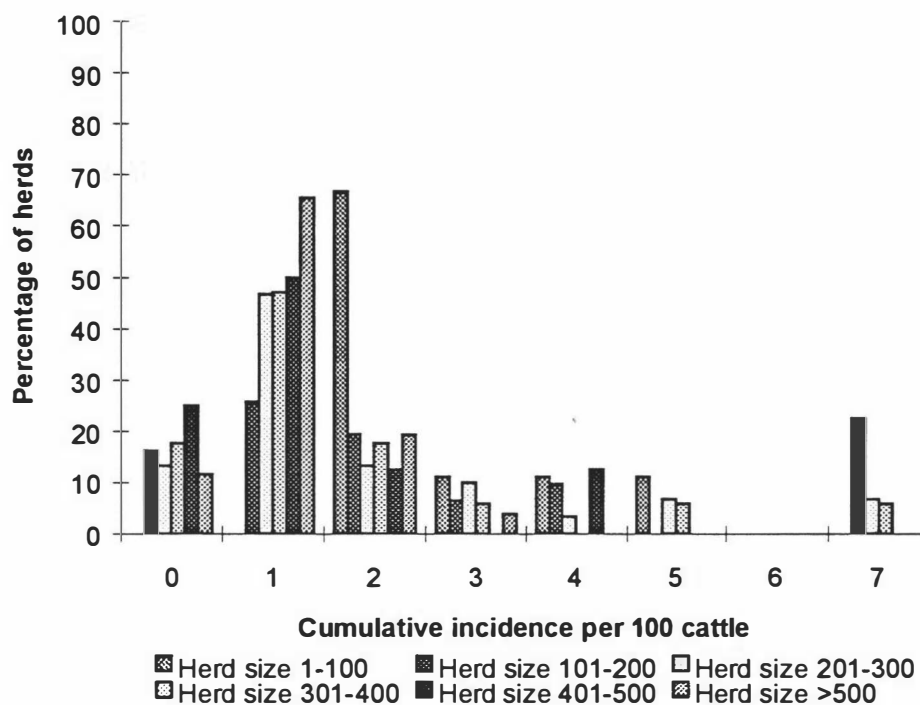


Figure 5.1. The relative frequencies of all levels of cumulative incidence from 0 to 7 per 100 cattle in six categories of case herds, classified by size of herd

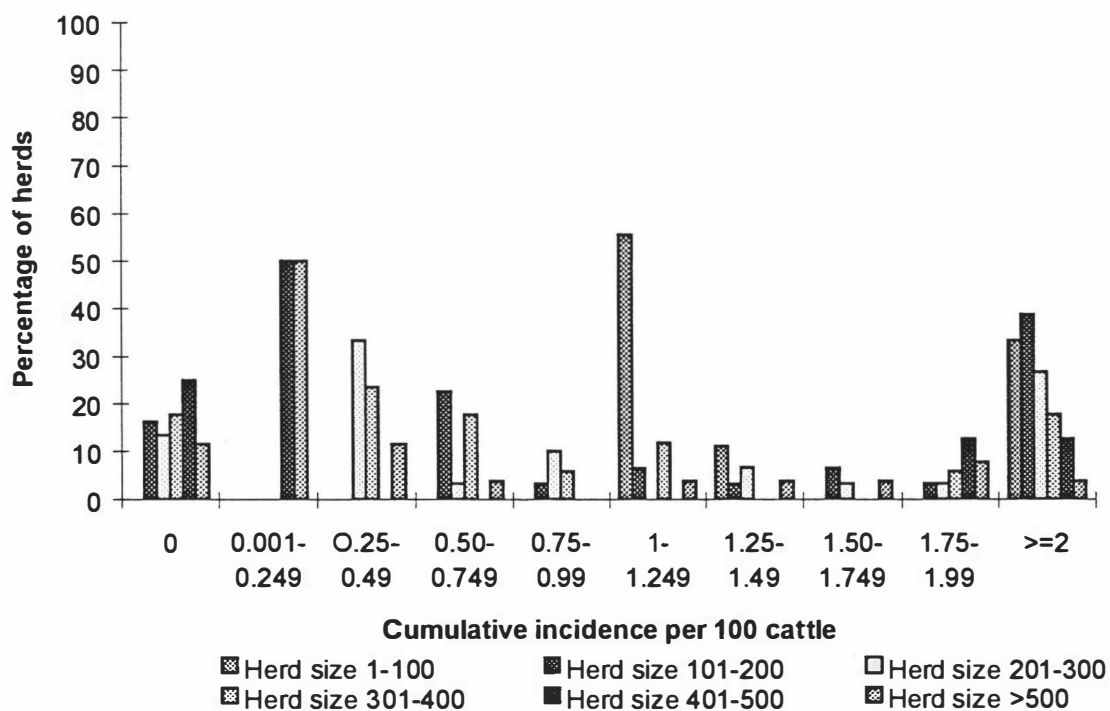


Figure 5.2. The relative frequencies of all levels of cumulative incidence from 0 to ≥ 2 per 100 cattle in six categories of case herds, classified by size of herd

Distributions of incidence density in case herds classified by herd size

The distributions of incidence density in the six different size categories of herds are shown in Figure 5.3. The pattern is similar to that for cumulative incidence. With the exception of the smallest herd size category of 1 to 100 cattle, 50% to 60% of larger herds had incidence densities less than $1.0 \times 10^{-2} \text{ year}^{-1}$. All of the herds with between 101 and 200 cattle had incidence rates between ≥ 1.0 and $\leq 2.0 \times 10^{-2} \text{ year}^{-1}$.

The distribution of incidence densities from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ for the range of herd sizes are shown in Figure 5.4. Most of the herds containing 1 to 100 cattle had incidence densities $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$. In most of the other size herds incidence densities were less than $1.0 \times 10^{-2} \text{ year}^{-1}$.

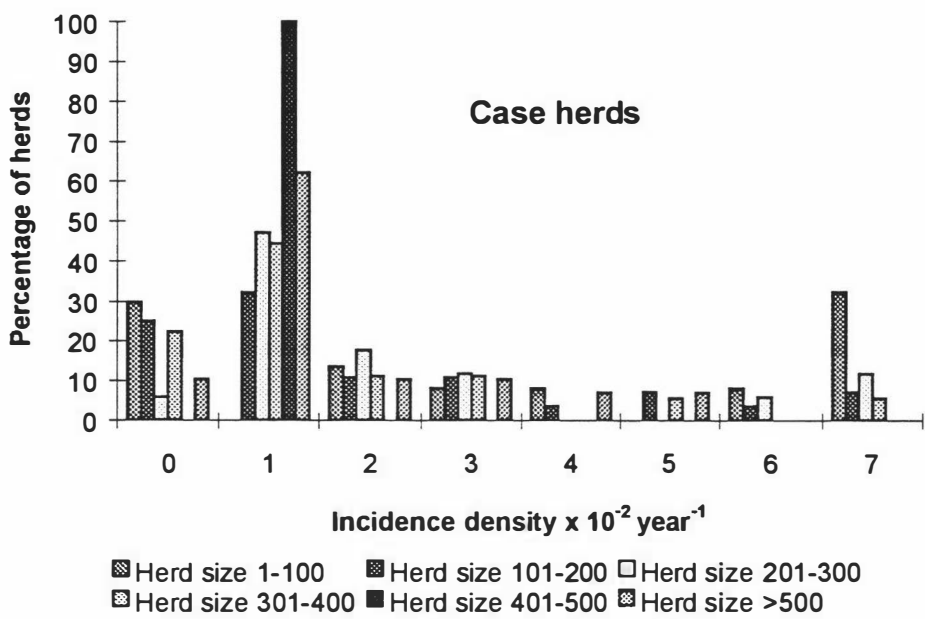


Figure 5.3. The relative frequencies of all levels of incidence density from 0 to $7 \times 10^{-2} \text{ year}^{-1}$ in six categories of case herds, classified by size of herd

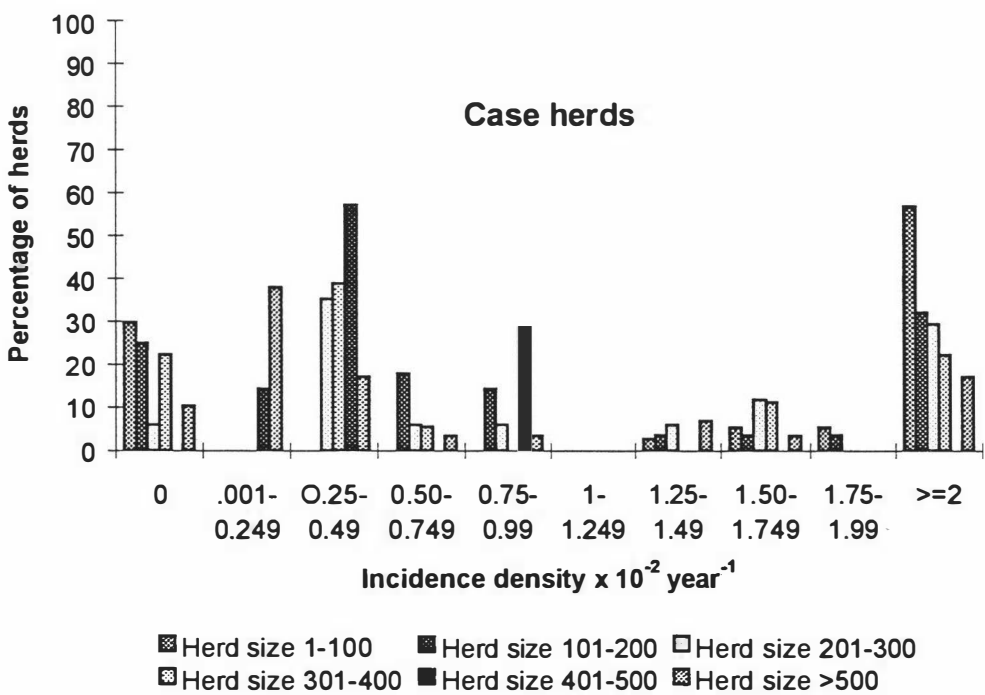


Figure 5.4. The relative frequencies of all levels of incidence densities from 0 to $\geq 2 \times 10^{-2} \text{ year}^{-1}$ cattle in six categories of case herds, classified by size of herd

Distribution of cumulative incidence in case herds in endemic, non-endemic and surveillance areas

The distributions of cumulative incidence in herds in the endemic non-endemic and surveillance zones in the Taumarunui district are shown in Figures 5.5 and 5.6. There were slightly more herds at a risk level of 1.0 per 100 cattle in non-endemic zones than in endemic and surveillance areas, and the overall tendency was for herds located in endemic zones to have lower levels of risk than herds in non-endemic and surveillance areas. Thirty-six percent of surveillance herds, 17% of non-endemic herds and 7% of endemic herds had annual cumulative incidences ≥ 2.0 per 100 cattle.

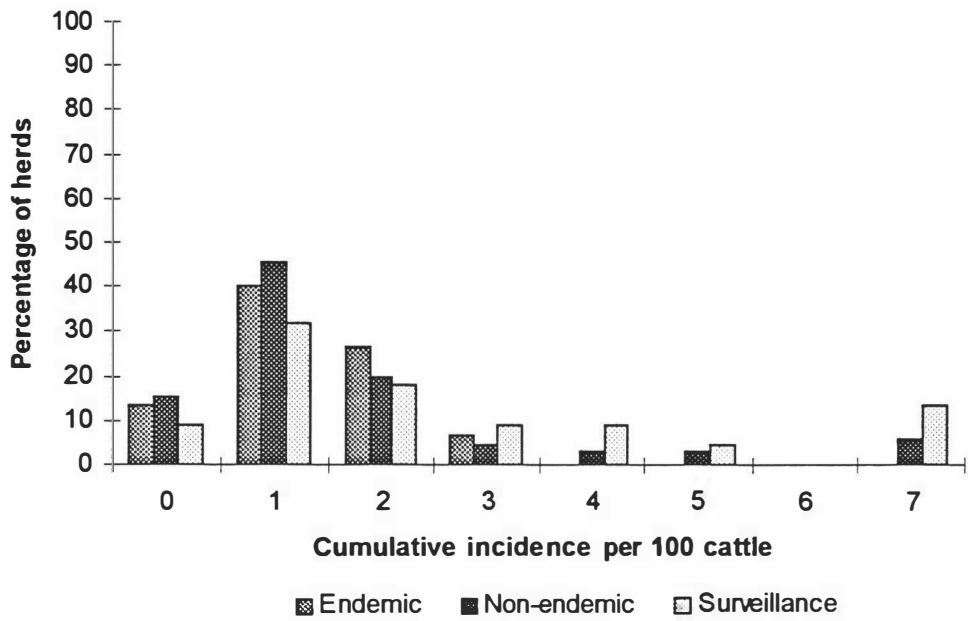


Figure 5.5. Frequency distributions of levels of cumulative incidence from 0 to 7.0 per 100 cattle in case herds in endemic, non-endemic and surveillance areas

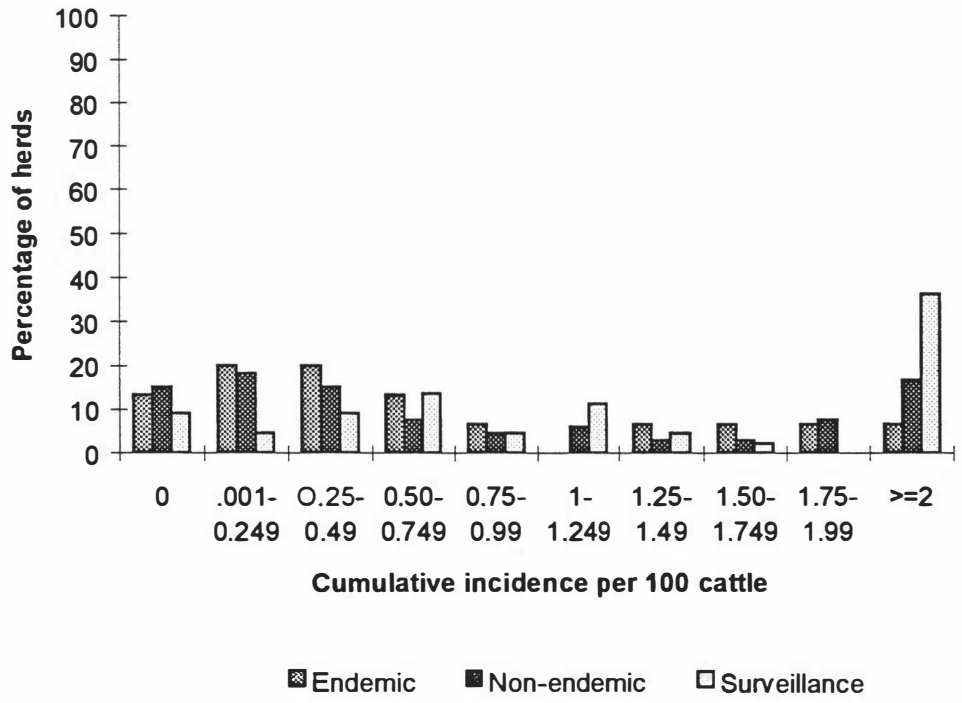


Figure 5.6. Frequency distributions of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in case herds in endemic, non-endemic and surveillance areas

Distribution of incidence density in case herds in endemic, non-endemic and surveillance areas

The distributions of incidence density in the three separate zones are shown in Figures 5.7 and 5.8. The rates of infection tended to be lower in herds in endemic and highest in surveillance zones. The general pattern of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ was similar to the pattern of cumulative incidence shown in Figure 5.6, except that about equal proportions of herds from all locations had rates of infection $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$.

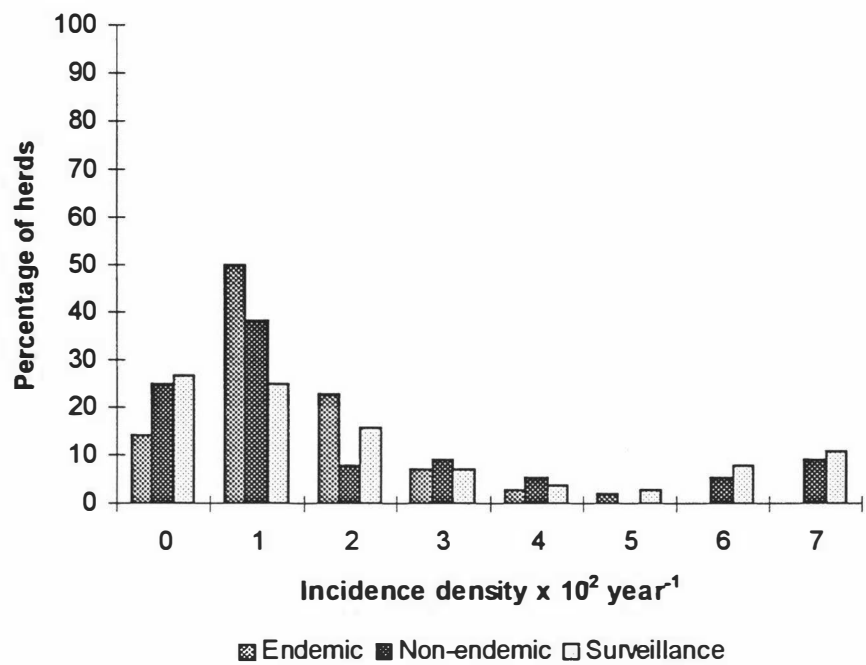


Figure 5.7. Frequency distributions of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in case herds in endemic, non-endemic and surveillance areas

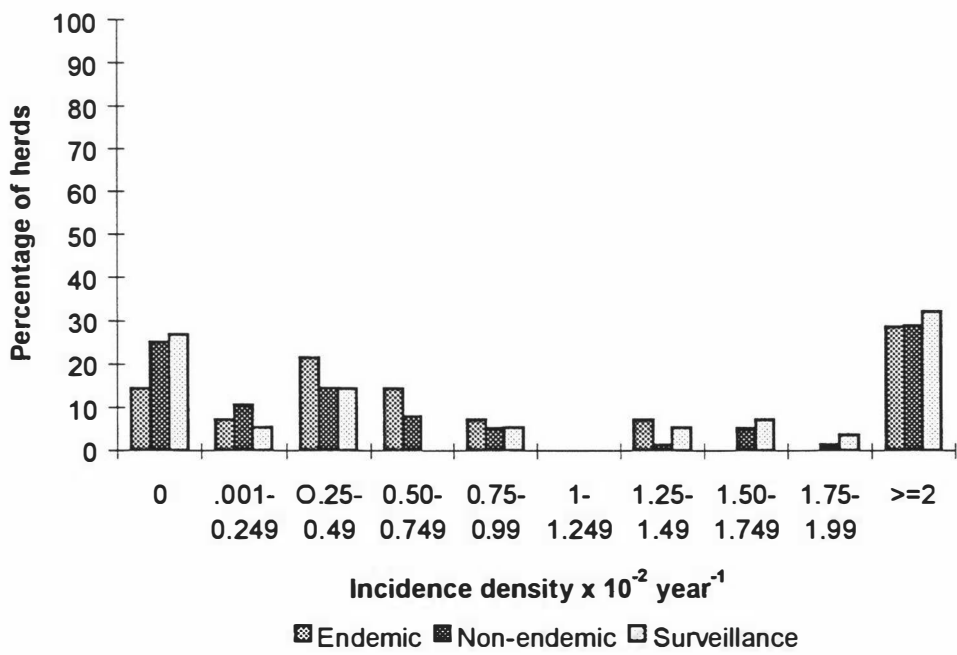


Figure 5.8. Frequency distributions of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in case herds in endemic, non-endemic and surveillance areas

Distributions of cumulative incidence in case herds according to type of herd

No regular patterns are evident in the distributions of cumulative incidence shown in Figures 5.9 and 5.10 for the three herd types. Some differences among the levels of risk for each herd types occur at the 1.0 and 2.0 per 100 cattle levels, but they are small and almost cancel out if those two levels are combined. The respective cumulative incidence levels at ≥ 2.0 and 7.0 per 100 cattle show that beef breeding herds generally tended to have lower levels of risk than other types of herds.

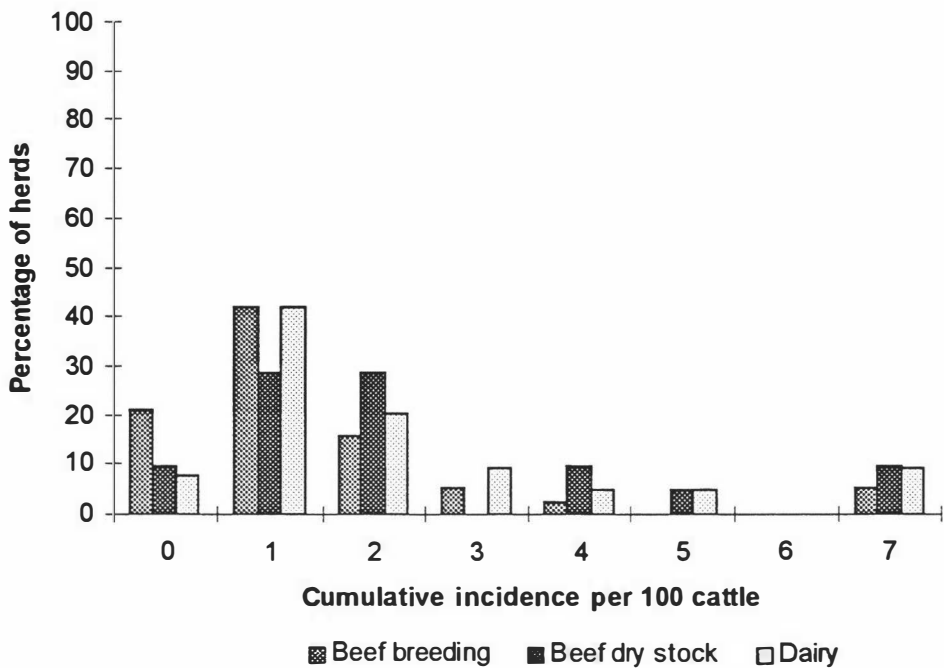


Figure 5.9. Frequency distributions of levels of cumulative incidence from 0 to 7.0 per 100 cattle in beef breeding, beef dry stock and dairy case herds

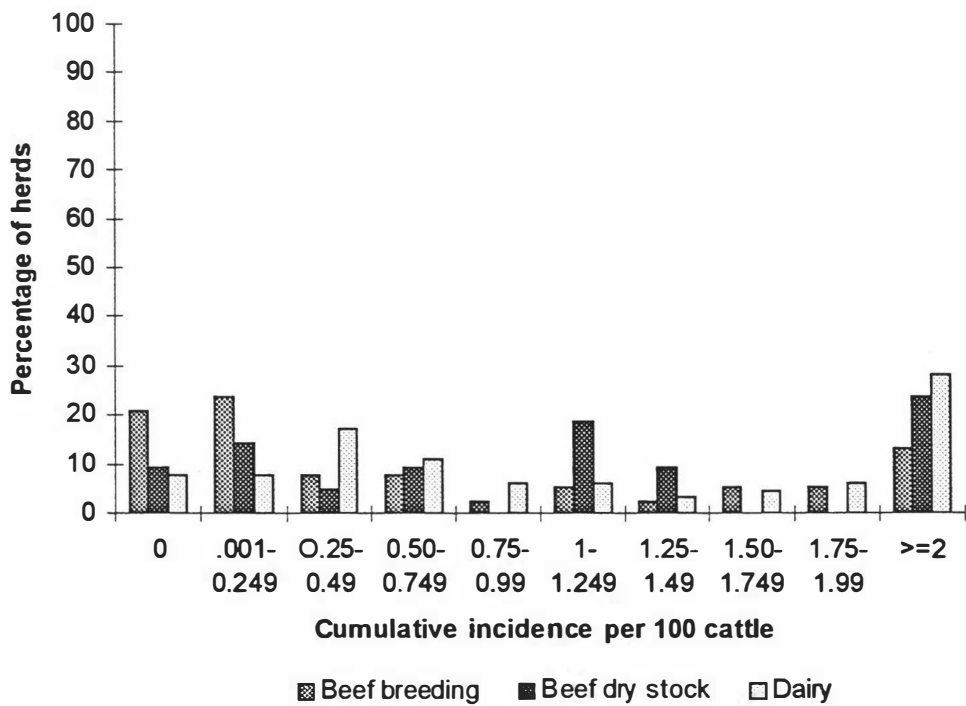


Figure 5.10. Frequency distributions of levels of cumulative incidence from 0 to ≥ 2.0 per 100 cattle in beef breeding, beef dry stock and dairy case herds

Distributions of incidence density according to herd type

The distributions of incidence density for beef breeding, beef dry stock and dairy herds are shown in Figures 5.11 and 5.12. Relatively few dairy herds had no incident cases and the highest rates of infection at levels of ≥ 2.0 , 6.0 and $7.0 \times 10^{-2} \text{ year}^{-1}$ were found in beef dry stock herds.

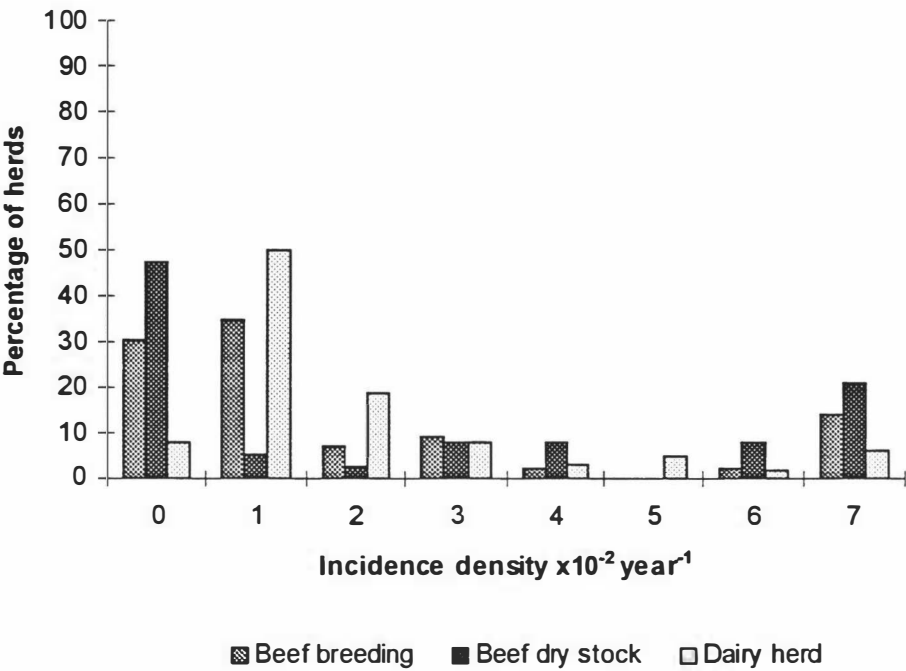


Figure 5.11. Frequency distributions of levels of incidence density from 0 to $7.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy case herds

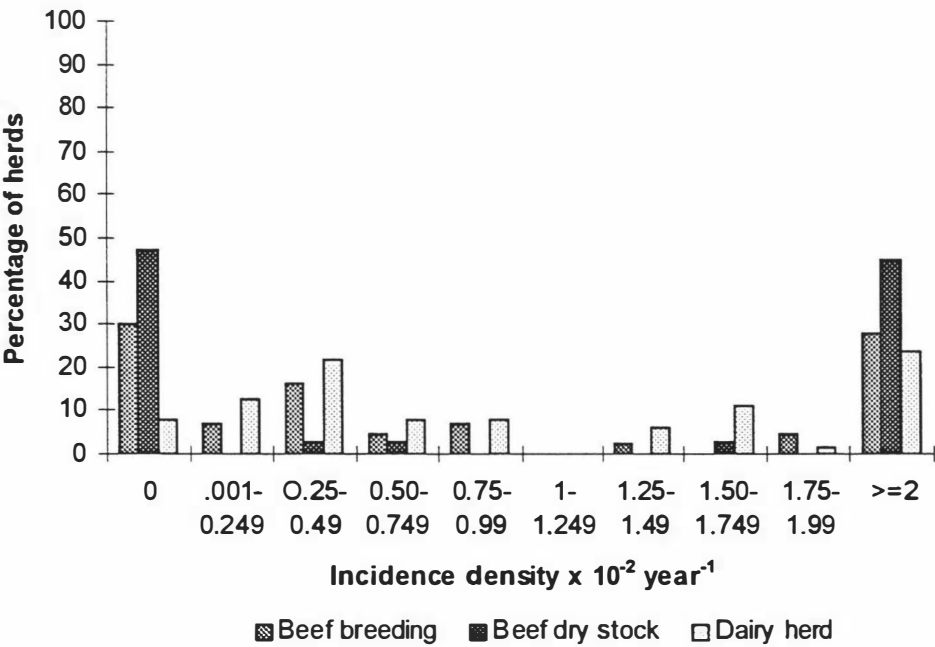


Figure 5.12. Frequency distributions of levels of incidence density from 0 to $\geq 2.0 \times 10^{-2} \text{ year}^{-1}$ in beef breeding, beef dry stock and dairy case herds

Length of time between consecutive herd tests

The distribution of the inter-test intervals, measured in days, for case herds is shown in Figure 5.13. Although most tests (61%) were carried out between 1 and 100 days after previous tests, 12% were conducted between 101-200 days, and 11% were done at more than 600 days after a previous test.

The distribution of the inter-test interval for control herds matched on management practices is shown in Figure 5.14. For those herds, 32% of tests were conducted at more than 600 days and 31% at between 301 and 400 days. The distributions shown in Figures 5.13 and 5.14 show that the pattern of testing over time for the matched control herds was considerably different to that for case herds.

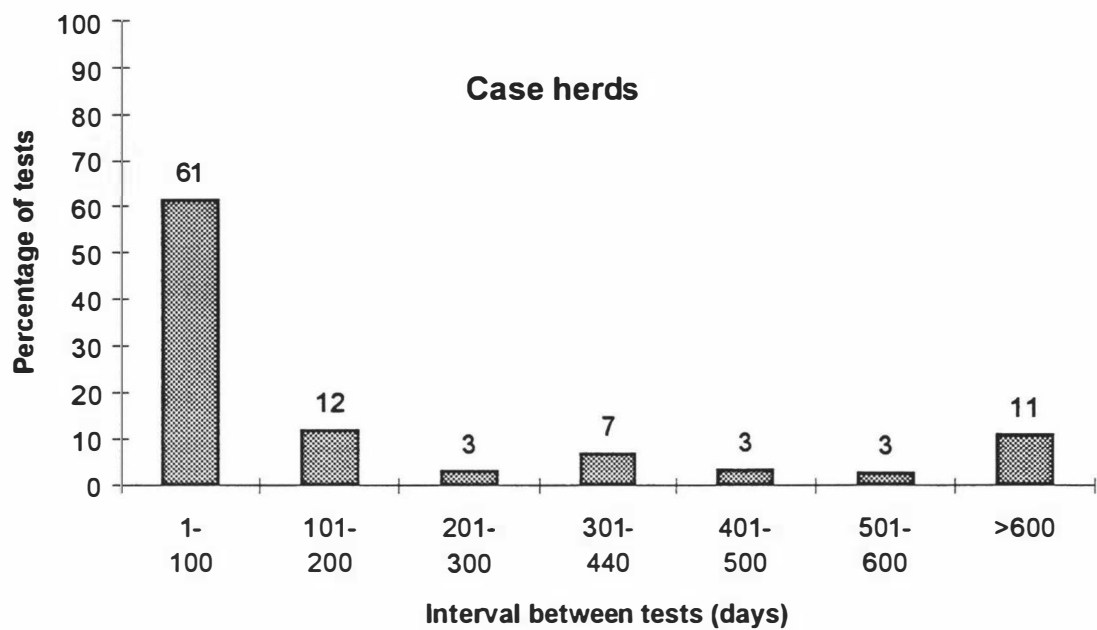


Figure 5.13. Distribution of periods of time between consecutive herd tests of case herds

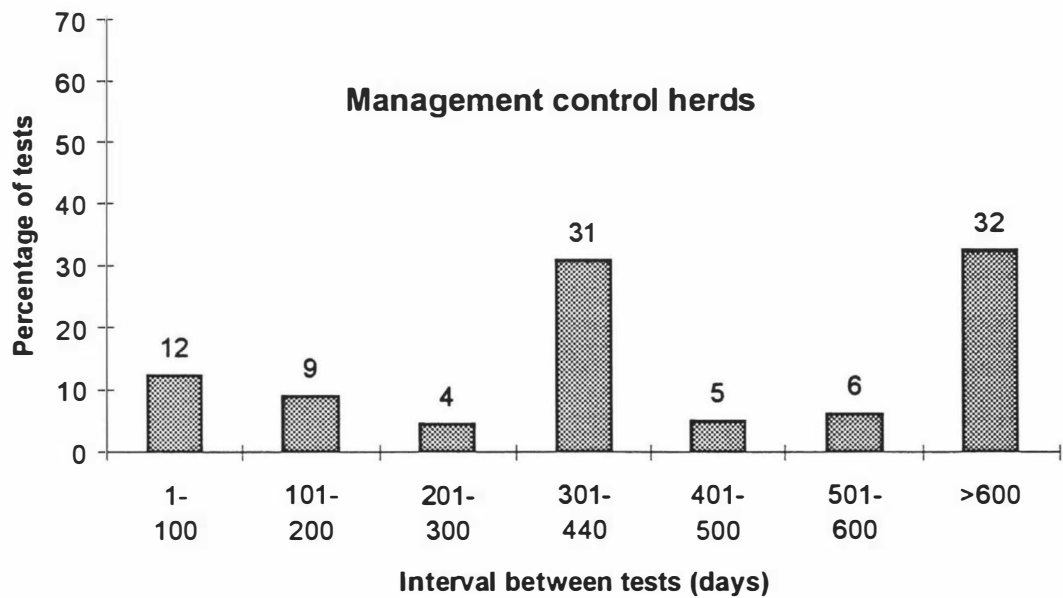


Figure 5.14. Distribution of periods of time between consecutive herd tests of control herds matched on management practices

Distribution of time of tuberculin testing by month of year

The relative monthly patterns of tuberculin testing of herds over the period under consideration for case and management control herds are shown in Figure 5.15. Most herd tests were done in May (22% of management control and 17% of case herds) and fewest tests in September (1% of management control and 2.6% of case herds). Relatively few tests were carried out during winter and early spring, although during that period proportionately more case herds were tested than management control herds.

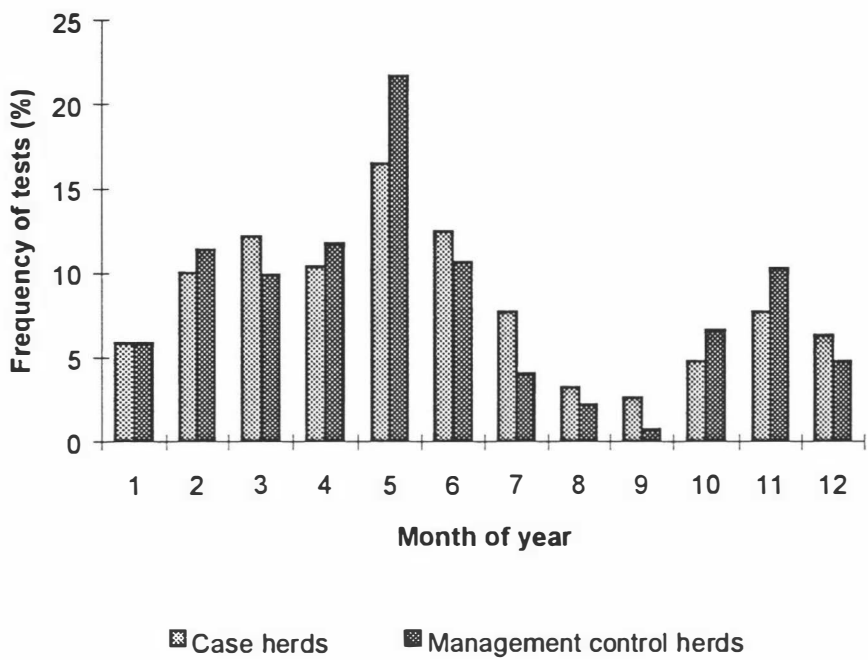


Figure 5.15. Monthly patterns of herd tests for tuberculosis for case and matched control herds

Average rates of infection in case herds over three year periods following herd breakdowns

The average rates of infection in case herds over the three year period following herd breakdowns are shown in Figure 5.16. The average rate was greatest at $1.4 \times 10^{-2} \text{ year}^{-1}$ in the year following herd breakdowns and gradually decreased over the following two years.

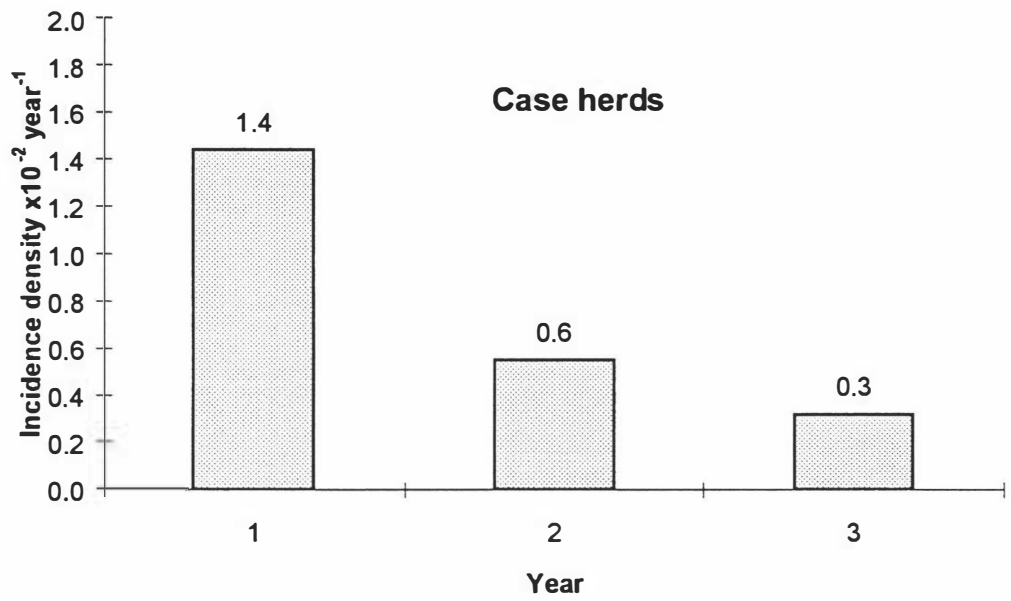


Figure 5.16. Average rates of infection in case herds over the three years after herd breakdowns

Distributions of incidence densities after herd breakdowns in six classes of herds categorised by herd size

The relative distributions of rates of infection for six the categories of herds in the year after a breakdown are shown in Figure 5.17. The rates tended to be lower for large herds containing more than 400 cattle, and no herds of those sizes had incidence densities $>0.8 \times 10^{-2} \text{ year}^{-1}$.

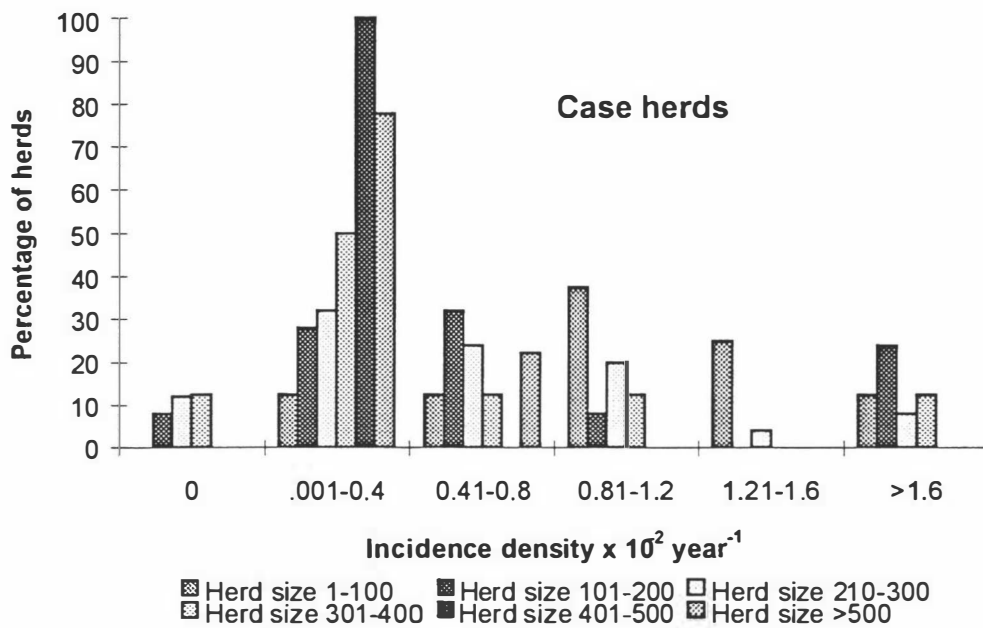


Figure 5.17. Relative distributions of incidence densities in the year after herd breakdowns in six classes of herds categorised by herd size

Distributions of incidence densities in year two and three after herd breakdowns in six classes of herds categorised by herd size

The relative rates of infection for each class of herd in year two after breakdowns are shown in Figure 5.18. Relatively more herds with fewer than 100 cattle had rates $\geq 1.6 \times 10^{-2} \text{ year}^{-1}$ and larger herd with more than 300 cattle tended to have low rates of infection.

The relative distributions of incidence densities for all categories of herds in year three after breakdowns are shown in Figure 5.19. Herds with less than 100 cattle and with 301 to 400 cattle are not represented in the figure because they had no new cases in the third year after breakdowns.

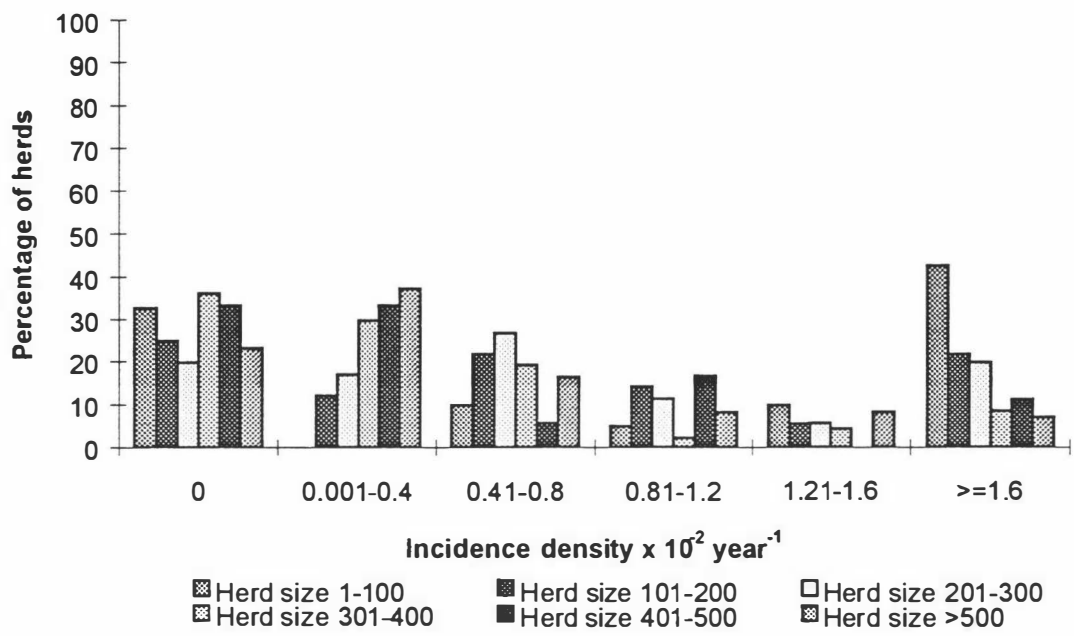


Figure 5.18. Relative distributions of incidence densities in the second year after herd breakdowns in six classes of herds categorised by herd size

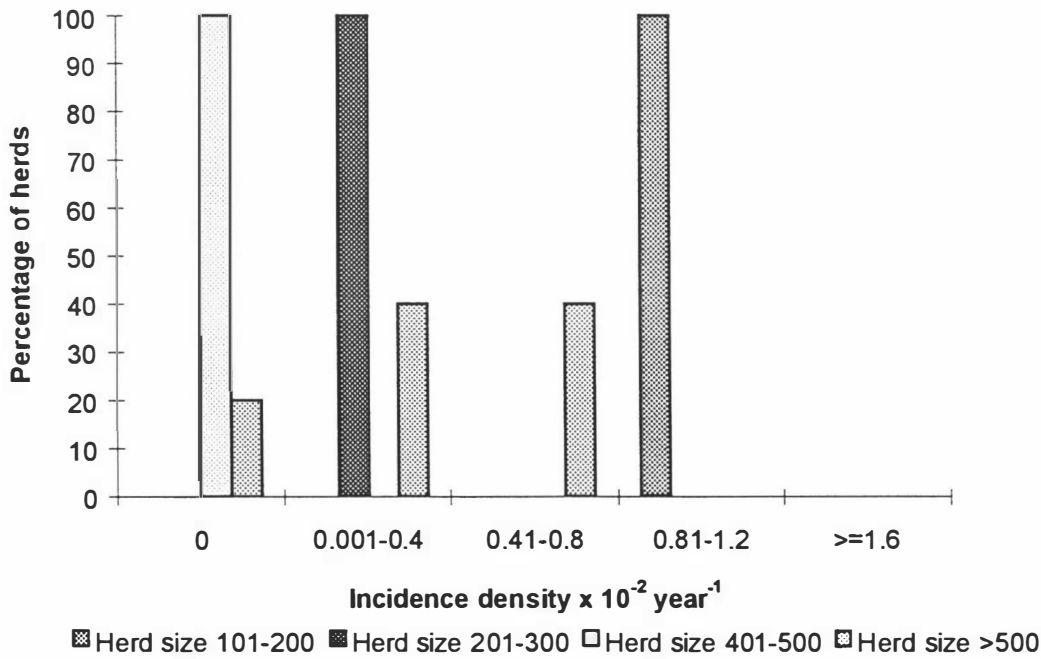


Figure 5.19. Relative distributions of incidence densities in the third year after herd breakdowns in herds with 101 to 200, 201 to 300, 401 to 500 and more than 500 cattle

UNIVARIATE ANALYSES

Examination of linear relationships between putative risk factors and risk of breakdowns and rates of disease using simple linear regression

Prediction of cumulative incidence

Simple linear regression was used to examine the linear relationship between each of the following independent variables, distance from the nearest case herd with tuberculosis, distance from the Rangitoto buffer, distance from the nearest case in year one after breakdown, total number of cattle purchased, and cumulative incidence(dependent variable). The results are shown in Table 5.1 and the P-values indicate that predictive values of all variables tested on cumulative incidence were poor.

Table 5.1 Summary results for simple linear regression for prediction of cumulative incidence

Independent variable	Coefficient (s.e.)	N	P-value
Distance from nearest case	2.38 (0.25)	122	0.84
Distance from Rangitoto buffer	2.69 (0.06)	111	0.91
Total no. of cattle purchased	2.67 (0.02)	127	0.72

Prediction of incidence density

The predictive value of each of those same variables on incidence density was examined using simple linear regression. The results are shown in Table 5.2 and indicate poor relationships between the independent variables tested and incidence density.

Table 5.2 Summary results for simple linear regression for prediction of incidence density

Independent variable	Coefficient (s.e.)	N	P-value
Distance from nearest case	1.16 (0.09)	112	0.92
Distance from Rangitoto buffer	1.03 (0.03)	110	0.79
Total no. of cattle purchased	1.12 (0.02)	120	0.42

MULTIVARIATE ANALYSES

In these analyses the independent variables were derived from a questionnaire of 134 items based on interviews conducted by field personnel of the Ministry of Agriculture and Fisheries (Pfeiffer *et al.*, 1991).

Cumulative incidence during first year on movement control

The association between cumulative incidence and potential risk factors during the first year after a breakdown was examined using stepwise logistic regression. The final model selected is set out in Table 5.3 and indicates that the level of risk of tuberculosis could be expected to vary according to the stocking rate employed, with the levels of risk decreasing in herds with higher stocking rates per hectare (LIVESDEN). The model also predicted that herds containing higher proportions of adult cattle (ADULTCAT) could be expected to have lower levels of risk than herds with lower proportions of adult cattle.

Risk levels were lower on farms where the main activity was dairying (MAINOP) than on farms which were mainly beef operations. Cumulative incidence decreased with increasing farm size (FARMSIZE), but increased as the pasture area was increased (PASTURE).

Farmers who had other work commitments besides managing their farm (OTHEMPL) were generally at lower levels of risk than farmers who did not have other employment. Personal traits of farmers which were associated with lower levels of risk included proneness to giving in easily (GIVINGIN), good record keeping habits (RECORDS), and a liking of new ideas (NEWIDEAS). Risk levels in herds managed by farmers who enjoyed working hard (HARDWORK) were likely to be higher than those in herds run by farmers who were less inclined to work hard.

Herds managed by farmers who were ranked as having a better knowledge about reservoir species for tuberculosis (SPECIES), and risky farming practices for disease introduction (INTRODUC), or who had confidence in the efficiency of MAF disease controls (CONTEFFE) could be expected to be at lower levels of risk than those run by farmers who were more poorly informed about technical aspects of tuberculosis or had lower levels of confidence in the ability of MAF to control the disease. Farmers who had a better knowledge about transmission of tuberculosis from cattle to human (CHSPREAD) were likely to have higher levels of cumulative incidence in their herds than farmers who had poorer understanding of that aspect of risk to humans.

Table 5.3 Final logistic regression model of cumulative incidence in case herds during the first year following an initial breakdown.

Variable group	Variable	Coefficient	Std Error	P-value	OR ()
<u>Constant</u>		-2.6546	0.5970	0.0001	
<u>Herd characteristic</u>	LIVESDEN	-0.0334	0.0084	0.0001	0.97 (0.95-0.98)
	ADULTCAT	-0.9822	0.4144	0.0178	0.37 (0.17-0.84)
<u>Farm characteristic</u>	MAINOP	-0.4871	0.2047	0.0174	0.61 (0.41-0.92)
	FARMSIZE	-0.0044	0.0009	0.0001	0.99 (0.99-1.00)
	PASTURE	0.00563	0.0013	0.0001	1.006 (1.00-1.01)
	GIVINGIN	-0.1838	0.0889	0.04	0.83 (0.70-0.99)
	HARDWORK	0.1306	0.0820	0.1111	1.14 (0.97-1.34)
	RECORDS	-0.1192	0.0479	0.0128	0.89 (0.81-0.98)
	NEWIDEAS	0.3237	0.0691	0.0001	1.38 (1.21-1.58)
<u>Epidemiological understanding</u>	SPECIES	-0.1332	0.0696	0.0557	0.88(0.76-1.00)
	CHSPREAD	0.4763	0.0853	0.0001	1.61 (1.36-1.90)
	INTRODUC	-0.2893	0.0796	0.0003	0.75 (0.64-0.88)
	CONTEFFE	-0.2890	0.1054	0.0061	0.75 (0.61-0.92)

Deviance = 236.764; Degrees of freedom = 14; P-value = 0.0001

OR () = Odds ratio (95% confidence limits); Std error = standard error

Cumulative incidence for the full period for which case herds were on movement control

The associations between putative risk factors and cumulative incidence calculated for the full period for which herds were on movement control were examined using stepwise logistic regression. The final model selected is set out in Table 5.4.

The model indicated that higher levels of risk could be expected from increasing the proportion of beef cattle in herds (BEEFCATT) and predicted lesser risk in herds farmed at higher stocking rates (LIVESDEN).

Farms devoted mainly to beef production and farms who had bush access (BUSHACCE) generally had proportionately fewer reactors over the whole time for which they were on movement control. Farmers who bought replacement stock (BUYREPLA) had herds at higher levels of risk than farmers who bred their own replacements.

An overall trend shown by the model was for farmers who considered themselves modest (MODEST) to have lower herd incidences, while farmers who preferred to work with livestock (LIVESPREF) and farmers who liked new ideas (NEWIDEAS) could be expected to have higher cumulative incidences in their herds over the period.

Farmers who were well informed about the importance of reservoir species in the epidemiology

of bovine tuberculosis (SPECIES), and about possible mechanisms for disease introduction (INTRODUC) into cattle herds could be expected to experience relatively lower cumulative incidence levels

Table 5.4 Final logistic regression model of cumulative incidence in case herds during the whole period for which they were on movement control

Variable group	Variable	Coefficient	Std error	P-value	OR ()
<u>Constant</u>		-1.9275	0.7212	0.0075	
<u>Herd characteristics</u>	BEEFLSU	-0.00035	0.000147	0.0165	0.99
	LIVESDEN	-0.0643	0.0127	0.0001	0.94 (0.91-0.96)
	BEEFCATT	1.6499	0.4745	0.0005	5.20 (2.05-13.2)
<u>Farm characteristics</u>	MAINOP	-1.3154	0.3716	0.0004	0.27 (0.13-0.56)
	OTHERCAT	0.9038	0.1637	0.0001	2.46 (1.79-3.40)
	BUSHACCE	-0.6991	0.1759	0.0001	0.50 (0.35-0.70)
	FARMSIZE	-0.00323	0.00101	0.0013	1.00 (0.99-1.00)
	PASTURE	0.00511	0.00161	0.0015	1.02
<u>Purchasing behaviour</u>	BUYREPLA	0.5653	0.2123	0.0077	1.76 (1.16-2.67)
<u>Farmer characteristics</u>	MODEST	-0.1530	0.0637	0.0164	0.86 (0.76-0.97)
	LIVEPREF	0.1209	0.0555	0.03	1.13 (1.01-1.26)
	NEWIDEAS	0.5201	0.0744	0.0001	1.68 (1.60-1.76)
<u>Epidemiological understanding</u>	SPECIES	-0.2609	0.0606	0.0001	0.77 (0.68-0.87)
	CHSPREAD	0.4519	0.0838	0.0001	1.57 (1.33-1.85)
	INTRODUC	-0.3295	0.0813	0.0001	0.72 (0.61-0.84)

Deviance = 312.79; Degrees of freedom = 18; P-value = 0.0001

Incidence density in case herds in their first year on movement control

The associations between putative risk factors and incidence density in herds during their first year after a breakdown were examined using stepwise Poisson regression (Table 5.5). The model predicted higher rates of infection in herds in which replacement stock was bought in (BUYREPLA) than in herds with a policy of breeding their own and the estimated Risk Ratio of 3.5 (2.40-5.11) indicated that this practice was very risky.

Farmers who considered themselves to be sociable (SOCIABLE) and farmers who liked new concepts (NEWIDEAS) were likely to experience higher rates of infection in their herds. Good record keeping habits was a personal quality which appeared to be sparing.

As was found in the analyses of cumulative incidence, farmers who were well informed about the importance of reservoir species in the epidemiology of bovine tuberculosis (SPECIES), and were

aware of possible mechanisms for disease introduction (INTRODUC) into cattle herds could be expected to have relatively lower rates of infection in their herds. Rates were likely to be higher in herds managed by farmers who had a good knowledge of cattle to human transmission of the disease (CHSPREAD).

Table 5.5 Final Poisson regression model of incidence density in case herds in their first year after breakdown

Variable group	Variable	Coefficient	Std error	P-value	RR ()
<u>Constant</u>		-4.2649	0.41	0.0001	
<u>Herd characteristics</u>	CATLLSU	-0.00009	0.000067	0.1660	1.00 (1.00-1.00)
	LIVESDEN	-0.0262	0.00636	0.0001	0.97 (0.96-0.99)
<u>Purchasing practices</u>	BUYREPLA	1.2531	0.1932	0.0001	3.50 (2.40-5.11)
	CATTPURC	0.000223	0.0009	0.0133	1.000 (1.00-1.00)
<u>Farmer characteristics</u>	SOCIABLE	0.2662	0.0592	0.0001	1.30 (1.16-1.47)
	RECORDS	-0.2502	0.0532	0.0001	0.78(0.70-0.86)
	NEWIDEAS	0.4315	0.0696	0.0001	1.56 (1.35-1.80)
<u>Epidemiological understanding</u>	SPECIES	-0.2823	0.0682	0.0001	0.75 (0.66-0.86)
	CHSPREAD	0.4441	0.0733	0.0001	1.56 (1.35-1.80)
	INTRODUC	-0.1387	0.0784	0.0770	0.87 (0.75-1.02)

Deviance = 211.536; degrees of freedom = 11; P-value = 0.0001

Poisson regression model for disease rates for the whole period for which case herds were on movement control

The final investigation in which stepwise Poisson regression was used was the association between putative risk factors and incidence density over the whole period for which herds were under movement control (Table 5.6). The model predicted that the overall rate of infection would be expected to be lower in herds with higher stocking rates(LIVESDEN) and higher on farms which ran agisted cattle (OTHERCAT). Farms in which cattle had greater access to bush tended to have lower rates of infection than farms with lesser amounts of bush access. The model predicted that as the proportion of weaners in the total cattle livestock unit increased so did the rate of infection for the whole period under consideration.

The predictions from farmer personal characteristics were in general agreement with predictions from the other models reported here (Tables 5.3, 5.4 and 5.5) with the exception of SPECIES for which this particular model suggested a positive effect on incidence density.

Table 5.6 Final Poisson regression model for incidence density for case herds for the whole time they were on movement control

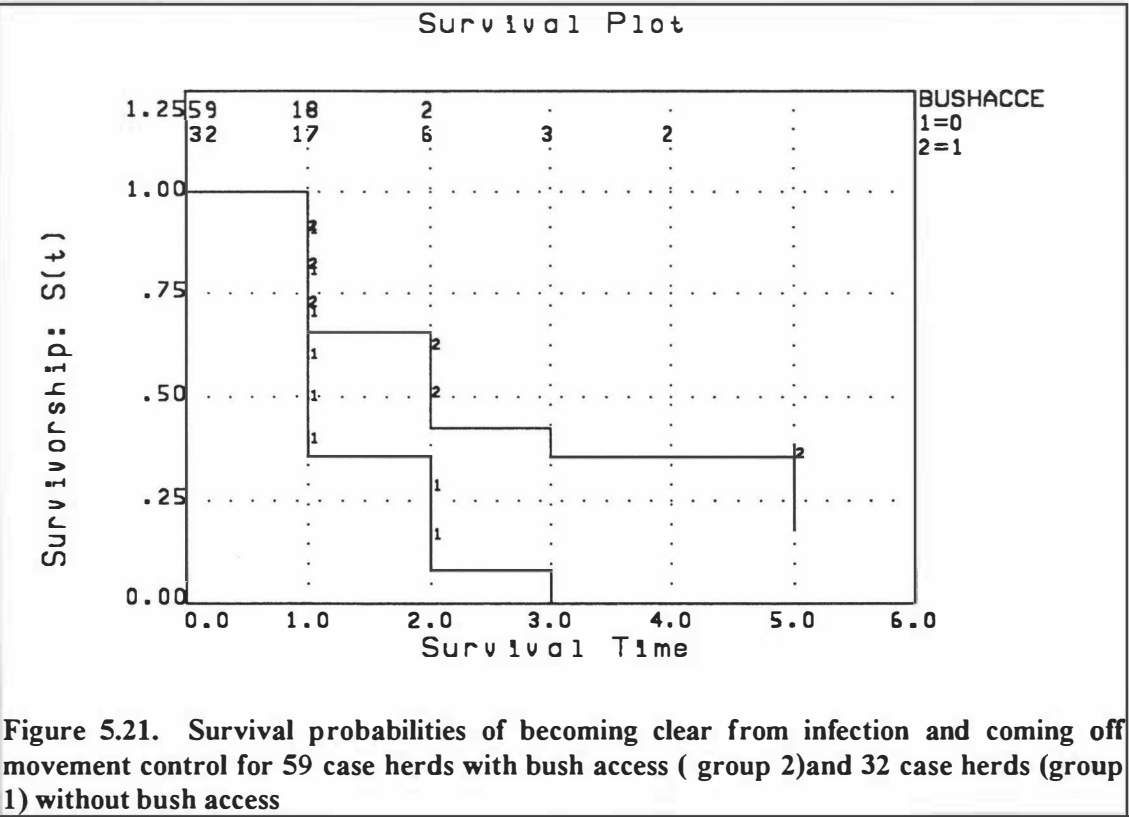
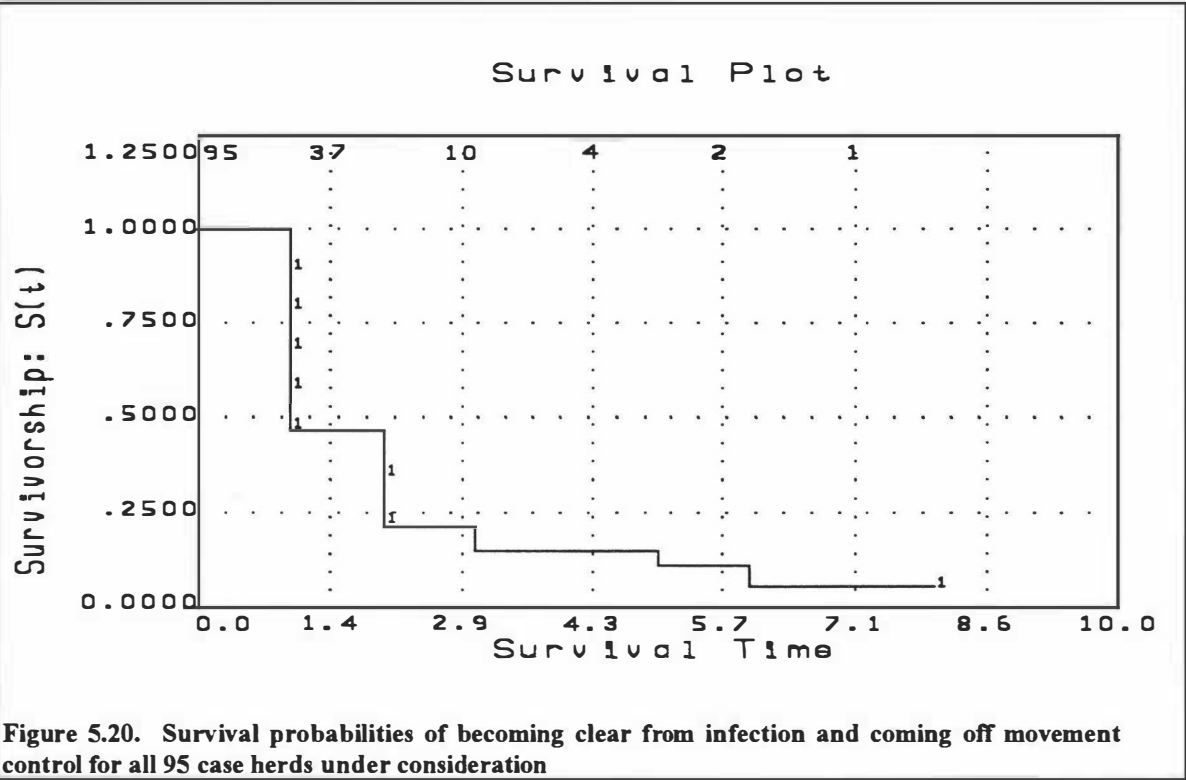
Variable group	Variable	Coefficient	Std. error	P-value	RR ()
Constant		-3.4305	0.4883	0.0001	
Herd characteristics	CATTDENS	0.0483	0.0179	0.0070	1.05 (1.01-1.09)
	LIVESDEN	-0.1095	0.0223	0.0001	0.90 (0.86-0.94)
Farm characteristics	OTHERCAT	0.8372	0.1469	0.0001	2.30 (1.73-3.08)
	BUSHACCE	-0.3795	0.1529	0.0130	0.68 (0.51-0.92)
Purchasing characteristics	WEATOTPU	1.1775	0.3441	0.0006	3.25 (1.65-6.37)
Farmer characteristics	SOCIABLE	0.1097	0.0563	0.0513	1.12 (1.00-1.25)
	GIVINGIN	-0.2330	0.0717	0.0012	0.79 (0.69-0.91)
	RECORDS	-0.2102	0.0430	0.0001	0.81 (0.74-0.88)
	NEWIDEAS	0.5348	0.0652	0.0001	1.71 (1.50-1.94)
Epidemiological understanding	SPECIES	0.4049	0.0611	0.0001	1.50 (1.33-1.69)
	CHSPREAD	0.3954	0.0731	0.0001	1.48 (1.29-1.71)

Deviance = 293.864; degrees of freedom = 13; P-value = 0.0001

Survival analysis

Survival analysis was used to investigate factors likely to influence the length of time herds stayed on movement control. Figure 5.20 presents the survivorship function for duration on movement control for all 95 case herds. After one year on movement control about 50% of infected herds had come off movement control and after 3 years about 20% of infected herds were still on movement control.

The survivorship functions for the duration on movement control for 59 case farms with access to bush and 32 farms without access are presented in Figure 5.21. The median time on movement control was 1 year for farms without access to bush and over 2 years for farms with access to bush. After 3 years, all case farms without bush access had clear status, whereas it took about 5 years for farms with bush access to become clear of tuberculosis. The difference in survivorship between herds with and herds without access to bush was statistically significant (Log-rank $\chi^2 = 11.20$, P-value=0.0001).



Cox's proportional hazard regression model

Stepwise Cox's proportional hazard regression was used to identify factors with statistically significant associations with the probability of case farms becoming clear from tuberculosis. The final regression model chosen is set out in Table 5.7. Both independent variables in the final model, (total livestock units on the farm (TOTALLSU) and farm access to bush (BUSHACCE), had negative correlation coefficients, and were therefore inversely related to the probability of coming off movement control. The probability of getting off movement control decreased with increased herd size. Bush access had a sparing effect against becoming free from infection, or expressed more simply, case farms with bush access could be expected to stay on movement control longer than farms without bush access. The model supports the survivorship function analysis shown graphically in Figure 5.21.

Table 5.7. Final Cox's proportional hazard regression model

Variable	Coefficient	Std error	P-value	RR ()
<u>Bush access</u>	-0.74	0.27	0.0073	0.47 (0.28-0.80)
<u>Total livestock units</u>	-0.00016	0.000063	0.0096	0.99

RR () = approximate Relative Risk (95% confidence limits); Sample size = 91 (18 censored and 73 completed); $\chi^2=9.3$; P-value = 0.0023

DISCUSSION

This study provided a good opportunity to compare the findings from a case control study (Pfeiffer, 1995) with an epidemiological analysis of the disease on case farms in that study. A case series study is a valid method for identifying causal relationships where the cases represent a census as in this study, although results cannot be extrapolated to a wider population with the same degree of confidence that attends a case control study. It was reassuring to find reasonably close agreement with the linear regression and multivariate models in this study and the results of Pfeiffer's (1995) case control study.

A higher risk of disease was associated with beef than with dairy enterprises and the risk increased as the proportion of beef cattle in herds under consideration increased. This probably reflected the way in which beef cattle were managed on farms with their particular grazing patterns putting them at greater risk of encountering tuberculous possums.

A finding of higher rates of infection associated with buying-in of cattle was also in close agreement with a conclusion from the case control study.

Survival analyses provided additional insight into the nature of the disease and clearly showed that herds with access to bush could be expected to stay on movement control for longer periods than farms with no bush access. The median time on movement control was 1 year for farms without bush access and slightly more than 2 years for farms with access to bush. Movement control farms without bush access could be expected to be free of infection after 3 years whereas farms with bush access took more than 5 years.

This particular finding suggests that more consideration should be given to the nature of individual farms with respect to bush access when formulating testing and control regimes tailored to individual farm circumstances.

CHAPTER 6

Database storage of animal disease control data in New Zealand

DATABASE STORAGE OF ANIMAL DISEASE CONTROL DATA IN NEW ZEALAND

Basic requirements

Roe (1979) identified eight important features for consideration when designing an Australian animal health recording system for national disease control programs (Australian National Disease Information System, ANADIS). They were:

- Decentralised and district autonomy of database operations
- Field staff have direct access to database
- Database designed to assist field staff
- Direct interactive entry of data from field reports
- Flexibility to meet requirements of individual district schemes
- Emphasis on the dynamic nature of disease problems
- Scope for continuous modification and adaptation
- Able to assist with forward planning of the disease control programme

The National Disease Control System (NDCIS) operating in New Zealand for the control of tuberculosis in cattle and deer and bovine brucellosis consists of a set of 21 independent personal computer databases located in each of the 21 disease control districts. This system was initially developed because of dissatisfaction with the previous centralised system, the "Tuberculosis Information System" (TIS), principally by district staff who considered it to be a clumsy administrative tool mainly designed for head office decision making. While the TIS had a poor rating in terms of the criteria laid down by Roe, the NDCIS was designed with all of those issues in mind.

A central issue in the design of national disease recording systems is the identification of the end users of the information which is stored and generated by the database (Ryan and Wilson, 1991). The principal end users in New Zealand for tuberculosis control information are the Animal Industry groups and the Animal Health Board, the Ministry of Agriculture, field operational groups such as veterinarians and pest control operators, and researchers in universities and government research establishments.

Problems have been encountered with the operation of the NDCIS and it was intended that areas of concern would be addressed wherever possible when the information system was to be

given an extensive overhaul. Ryan and Wilson (1991) identified problem areas in the NDCIS related to database design and operation pertinent to that time:

- Access to and amalgamation of data from district databases caused by the independent nature of 21 database sites throughout the country
- Administrative problems associated with training and supplying experienced staff in 21 separate sites
- Costs associated with maintenance of computing hardware
- Research requirements for data storage which need the data to be available in a suitable form for retrospective analyses
- Linkage with other database systems for the benefits of other disease control programmes

Upgrading the current system

Retrieval of information is essential for disease control program to provide field staff easy and rapid access to the information that they require. Confidentiality should be protected and should not be available to outside bodies unless approved by the appropriate authority or owner of the data.

Programming support is necessary for centralised maintenance of the integrity of the information and for monitoring day to day operational problems within the national administrative and delivery network. Programmers working in consultation with veterinary epidemiologists are able to provide constant development and modification to computer programs to meet specific local or national needs.

Currently the New Zealand Ministry of Agriculture and Fisheries has under final development a new national livestock database (NLDB) to replace the one currently being used for bovine tuberculosis and brucellosis control (Ryan and Yates, 1994). Current management and technical problems such as those broadly described above have been addressed, as were additional challenges associated with providing interfaces for proposed and existing new management support systems.

It is envisaged that the new database will integrate all current animal surveillance and disease control databases, and be networked to provide for easy collation of data from all districts. Because of its additional complexities and requirements for greater technical and management support, its operational costs are expected to be higher.

Key issues which were addressed included:

- The need for information to be based on farm management units, with herds or flocks contained therein (in contrast to the current herd based system). Currently, a range of disease control programmes operate as totally independent systems and there was a real need to have identification based on unique farm identifiers to enable linkages with other databases and expert systems. For diseases where geographical ecological and other environmental factors are important issues in disease transmission, an integrated Geographical Information System (GIS) allows quantitative investigation of those factors for improved control and decision making processes.
- Compatibility with both the EPIMAN decision support system developed at Massey University for emergency response to Foot and Mouth Disease and the AGRIBASE database which is linked to EPIMAN.
- Incorporation of features that will maintain and enhance support for district field staff, and also efficiently provide for both regional and national user needs. The ability to provide an efficient service to district staff and be able to conduct regional and national analyses at any time was given high priority.
- Although the immediate focus was to support the national tuberculosis control scheme, the opportunity was taken to design a database which will provide a common base for developing information systems for both infectious and non-infectious disorders, residue testing and production related problems.
- Being able to have effective control over reactor animals and being able to incorporate the results of new diagnostic procedures and tests.

Data entities and attributes

The database has been developed as a set of six interacting modules:

The people, farm, and herd or flock module has management units (farms) as the prime identifier with herds or flocks identified at a lower level within each farm. There will be a unique farm identifier to will enable the transfer of data between complementary systems such as EPIMAN and AGRIBASE. Herds and flocks will carry their own identifiers and descriptors, and movement between farms, as is common with dairy herds managed by sharemilkers, will be accommodated. This module will contain a file of people associated with the farm and/or herd with their role, e.g. farm owner, herd manager etc.. Geographic (e.g. local government), business (e.g. dairy factory), census (e.g. animal numbers) and regulatory (e.g. MAF veterinary district) items will be associated with each farm

A history of each herd will be accumulated in the **surveillance module** and will cover both "surveillance" and "disease testing" episodes. A quarantine table will contain a record of movement control and the imposition of other official restrictions such as a "Disease Control Place Notice".

As occurs with the current database system, disease policy (testing frequency, test types, test interpretation) will be recorded and testing instructions will be able to be generated for field operational staff and abattoir inspectors from the **testing control module**.

A **vector module** will allow farms to be classified and assessed according to an "area disease class", based on factors such as population densities of vectors, estimated disease prevalences for diseases such as tuberculosis in possums and presence of arboviruses.

A **special module** was incorporated to cater for future needs which are not apparent at present. In the tuberculosis control module a new facility was incorporated to record lesion sites and microbiology test results.

The review for changes to the current information system was done in steps in which user needs were identified and accommodated in the design of the database. Software and hardware options were explored and cost-benefit analyses guided the whole process.

Software

The database system "Oracle" was used to develop the database, a windows interface was used and the development tool was "Power Builder". The NLDB is to be installed on the national MAF network which is a network of personal computers with supporting servers. It is planned to establish one database in each of the three MAF regions and have another backup database. District offices will link up with their regional database and there will be daily transfer of data to the backup database.

A "Windows" environment was chosen because:

- Windows is rapidly becoming the universal interface.
- A Windows approach is expected to enhance staff performance. (Better data entry and report screens, in-built help functions covering not only the data base operations but tuberculosis control in general, ability to use graphics to enhance the interface).

- It was realised that a considerable programming effort would be required to develop a windows interface using a character based product. Further, the system would be difficult to maintain and enhance.
- A system developed with a Windows development tool can have all the required security and network features built into it with ease,. Data can be extracted without difficulty and transferred to other systems.

CONCLUSIONS

The NLDB will be a more useful epidemiological tool for control of tuberculosis and have the capacity for developing other disease control schemes and assisting trade and livestock management considerations.

CHAPTER 7

General Discussion

GENERAL DISCUSSION

The general expectation which accompanied the start of the national tuberculosis eradication program was that the disease would be eradicated or reduced to negligible levels within 20 to 25 years. While this rate of progress has been achieved by some of New Zealand's trading partners and notably in Australia, the presence of a wildlife vector in New Zealand has caused regulatory authorities to revise their original estimates and accept that at present there are no realistic methods available for complete control in regions of the country classified as endemic.

In this thesis, centrally stored data collected during routine operation of the national disease control program has been examined using a variety of standard statistical and epidemiological techniques. Descriptive epidemiology was used as a first step in the process to allow a thorough examination of the data, to highlight any obvious data entry errors and at the same time lead to a sound understanding of the nature of the data.

Standard epidemiological measures of disease occurrence were calculated and displayed graphically in this process. Descriptive epidemiology by itself does not allow deductions to be made about disease, but graphic displays of frequency distributions draw attention to aspects of the nature of the disease in question, and in turn help to develop a range of hypotheses which can be tested using more rigorous techniques. Histograms and graphs of the type frequently displayed in the early chapters of this thesis are relatively simple to construct and display provided the data has been first arranged in a manner which allows epidemiological measures to be easily calculated. The process should be attractive to regional controlling veterinarians as a management aid for charting progress and making comparisons between districts and between subgroups within districts. As an example, the descriptive examination of herd records highlighted a notably high incidence of tuberculosis in herds of less than 100 animals in both the Taumarunui and Wairarapa districts. The causes and detailed nature of these high incidences were not specifically sought in this thesis and did not become apparent even after more advanced analytical techniques were performed. A more intensive analysis of this phenomenon is probably warranted and could lead to the design of specifically tailored control programs for herds in that size category.

A comparison of incidence measures showed a strong positive correlation between cumulative, corrected and true incidence values. Cumulative and corrected cumulative incidence values calculated for calendar, financial and test years were compared. Some disparity was found

between annual cumulative incidences and annual corrected cumulative incidences calculated on the basis of calendar and test years, with measures calculated on the basis of test year having the highest values, although reassuringly not of any extent which would cause any confusion or unsound inferences to be made.

Regional comparisons showed that movement control herds in surveillance areas had higher incidences of tuberculosis than did herds in the Taumarunui and Masterton veterinary districts. Beef dry stock herds had higher incidences of tuberculosis than did dairy or beef breeding enterprises. Endemic areas had the highest incidence of all tuberculosis area classes. These findings were not unexpected and fall into line with the area and herd classifications based on risk of infection.

Simple regression analyses indicated that the risk of tuberculosis for any animal in a herd was more closely related to the level of infection in adult cows than any other age or sex group. Cumulative incidence in yearlings was a poor predictor of risk for individual animals in a herd but there was a stronger relationship for the level of infection in 2 year-old animals. Information of this nature is valuable when designing herd testing programmes and deciding which animals are given priority for examination in modified programmes wherein parts of herd are tested. It was probably influential in the design of the present scheme which takes those factors into consideration. It also fits well with the epidemiology of the disease which predicts higher incidences in older animals.

Stepwise logistic regression was used to explore and quantify associations between cumulative incidence and putative risk factors. The odds of cattle testing positive in herds in endemic areas was about five times as high as in herds in surveillance and fringe areas, where the risks of tuberculosis were about the same. The likelihood of reacting to the tuberculin test was considerably lower for animals in the Masterton and Taumarunui districts than for animals from movement control herds in surveillance areas outside those districts. The overall risk of infection increased slightly from 1985 to 1990.

Poisson regression was used to examine the relationships between incidence density and the same independent variables which were examined using logistic regression. The relative risks for infection were higher in beef breeding, beef dry stock and other herd types than in dairy herds. Herds in endemic areas had rates of infection about seven times those in Fringe and Surveillance area herds, where the rates were about the same. The rate of infection in herds

increased with increased herd size and was considerably less in the Masterton and Taumarunui districts than in movement control herds in the Surveillance areas. Thus there was good general agreement between the logistic and poisson regression models in the overall relationships between the predictor variables common to both models and their respective dependent variables.

Survival analysis showed that after going on to movement control for the first time, about 75% of herds could be expected to be still on movement control after 12 months and about 50% after 2 years. Herds in the Masterton veterinary district tended to stay on movement control longer than herds in the Taumarunui veterinary district and Surveillance areas (Risk ratio = 0.69). After 2 years of testing, about 60% of infected herds in the Taumarunui veterinary district and Surveillance areas had come off movement control, compared to 40% of infected herds in the Masterton veterinary district. Survival analysis is an extremely valuable tool for examining patterns of disease in subgroups and for making between district comparisons and complements regression procedures by identifying differences which are not made apparent by regression analysis. The outcome of interest in survival analysis is the time to a prescribed event; in this case, time to coming off movement control, and this outcome is considerably different to probability and extent of infection nominated as dependent variables in the logistic and poisson regression analyses.

The survivorship probability of infected herds in Fringe, surveillance and non-endemic zones for coming off movement control was lower than that for infected herds in endemic zones (Risk ratio = 0.61). The estimated median time on movement control was 3 years for herds from endemic areas and 2 years for herds in Fringe, Surveillance and non-endemic zones. The risk of coming off movement control decreased with increasing herd size. Herds with high levels of cumulative incidence were more likely to stay on movement control for longer periods than those with lower levels of incidence. This type of information is extremely useful to field controllers for deciding on optimal test programmes, particularly in relation to frequency of testing, because it focuses on the core of the target of the national control programme, viz. Removing herds from movement control. These analyses would also be very useful in an audit process where individual and district performances could be examined and compared with national averages. Survival analysis is relatively easy to perform and has the very desirable quality of being easily understood by lay persons when displayed graphically.

The results from an analysis of all case herds from a case-control study (Pfeiffer. 1995) provides an interesting comparison between the case series and case-control methods of analyses. The case series approach is a valid method for identifying causal relationships when the cases represent a census as they did in this particular study, although results cannot be extrapolated to a wider population with the same degree of confidence as accompanies a case-control study. In the first step of the case series analysis, simple linear regression indicated poor predictive values of distance from the nearest case herd with tuberculosis, distance from the Rangitoto buffer, distance from the nearest case in year one after breakdown and total number of cattle purchased on cumulative incidence were poor. In these regards and in the results from the multivariable analyses, there was reasonably good agreement with Pfeiffer's (1995) case-control results.

A multivariate logistic regression analysis of the association between cumulative incidence and putative risk factors during the first year after a breakdown indicated that risk was lower on farms where the main activity was dairying (MAINOP) and in herds in which the proportion of adult cattle (ADULTCAT) was high. Over the whole period for which herds were under movement control, risk levels remained lower for dairy farms and increased as the proportion of beef cattle was increased.

Increased rates of infection were associated with the practice of buying replacements (BUYREPLA) in the first year after breakdown and rates were higher for the whole period in herds which ran cattle on agistment (OTHERCAT). Rates were lower on farms where cattle had access to bush (BUSHACCE), but despite the higher rates, survival analysis clearly showed that herds with access to bush could be expected to stay on movement control for longer periods than farms with no bush access. The median time on movement control was 1 year for farms without access to the bush and slightly more than 2 years for farms with access to the bush. Movement control farms without bush access were clear from infection by 3 years, whereas farms with bush access took more than 5 years. Thus cattle access to bush was identified by survival analysis as an important constraint to herds coming off movement control, whereas this important feature was not identified with regression analysis.

Both the risk and rate of infection tended to be lower at higher stocking rates in the short and long term after first going onto movement control. The association between personal qualities of farmers and the risk and rate of infection were also examined using multivariable regression analyses and again there was good general agreement with Pfeiffer's analysis. The addition of

survival analysis to the range of analytical techniques gave a more complete and improved understanding of the expression of the disease in those particular herds of interest.

The prime objectives of this thesis were to provide the writer with a sound epidemiological understanding of bovine tuberculosis in New Zealand, to give experience in handling large data sets of the type used in national disease control campaigns and to give an insight into the complexity of those campaigns and the justification of program designs. A wide range of analytical techniques were performed as part of that process, and the important issues which require special attention in the design of databases for national animal disease control programmes were examined.

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