The Role of a Wildlife Reservoir in the Epidemiology of Bovine Tuberculosis

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ABSTRACT

The objective of this project was to study the epidemiology of bovine tuberculosis in the presence of a wildlife reservoir species. Cross-sectional and longitudinal studies of possum populations with endemic bovine tuberculosis infection were analysed. The results were used to develop a computer simulation model of the dynamics of bovine tuberculosis infection in possum populations. A case-control study of breakdowns to tuberculosis infection in cattle herds in the Central North Island of New Zealand was conducted to identify risk factors other than exposure to tuberculosis in local possum populations.

The cross-sectional study was based on data gathered some years earlier in the Hauhungaroa Ranges from a number of traplines with a total length of 60km, hence it provided information about the epidemiology of possum tuberculosis on a large geographical scale with varying environmental conditions. The results from the study showed that disease occurrence was clustered in space with local prevalence reaching up to 20% while the overall prevalence was about 1.2%.

The longitudinal study was conducted using an area of 21 hectare of mixed pasture and bush on a sheep/beef farm. The study showed that incidence and prevalence of tuberculosis infection in possum populations has distinct spatial and temporal patterns. Environmental conditions were a major factor in determining the temporal pattern. Spatial and temporal analysis of the occurrence of different strains of *Mycobacterium bovis* allowed inferences to be made about the importance of particular transmission paths. Survival of possums depended on environmental conditions and tuberculosis disease status. Adverse weather conditions increased mortality and the incidence of clinical disease in possums. On average clinically tuberculous possums survived for about 2 to 3 months from the onset of clinical disease.

The simulation model uses a Monte-Carlo modelling approach and incorporates geographical features. Biological mechanisms which are considered important for population and infection dynamics were implemented in the model. These include mating, density-dependent and -independent mortality, pseudo-vertical transmission, transmission through spatial or temporal proximity, and transmission during mating contact. Each possum's movements and behaviour are simulated on a day-by-day basis. Simulations are conducted using a geography and possum population based on data from the longitudinal field study. For preliminary validation, model output was compared with field data from the longitudinal study. Sensitivity analyses and some initial simulation experiments were conducted to identify areas in the model structure which require the collection of additional field data. Use of the model for simulation of a possum population occupying a 400ha area in the Central North Island of New Zealand is demonstrated.

The case-control study of breakdowns to tuberculosis infection in cattle herds showed that in the Waikato area of New Zealand exposure to tuberculosis infection in local possum

populations was not the dominant cause of breakdowns when the study was conducted in 1989/90, at a time when tuberculous possums were first discovered in the region. Farmers who had breakdowns tended to follow cattle purchase and management practices which traditionally have been considered to put farms at risk of introducing tuberculosis. The results of the study indicate that there was a lack understanding among farmers about the epidemiology of tuberculosis.

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CHAPTER 1

INTRODUCTION

For a brief period after the advent of such advances in infectious disease control as antibiotics and modern vaccines, it was possible for people to envision a future with total control over most infectious human and animal diseases. This view changed dramatically with the advent of a growing range of apparently new diseases such as acquired immunodeficiency disease (AIDS) and the re-emergence of 'old' diseases such as human and bovine tuberculosis. It became recognized that traditional purely laboratory-based scientific approaches have difficulty in providing adequate solutions for complex disease problems. In keeping with a similar development in physics, this positivistic and reductionistic epistemology has had to be replaced by a more comprehensive scientific method which allows for uncertainty and offers a more holistic view of the nature of problems (van Gigch 1991).

With regard to tuberculosis infection, after a period in which the disease was seen to have declined to negligible proportions, quite pessimistic visions of the future have been drawn more recently both for human disease in most countries and for animal disease in countries where wildlife reservoirs exists. In the case of human tuberculosis, an alarming increase in tuberculosis incidence in humans has been seen in the United States (Glassroth 1992) and elsewhere. Discussing the rise of drug-resistance in *Mycobacterium tuberculosis,* Bloom points out that we may be working our way back to a frightening future (Bloom 1992). Recently, reports have come from the United States about deaths in HIV-infected prisoners caused by multi-drug resistant strains of *Mycobacterium tuberculosis*, which would pose a very major health hazard if they become established in the wider community. Stanford *et al* (1991) suggest that we are facing one of the greatest public-health disasters since the bubonic plague.

Although bovine tuberculosis does not represent a significant human health hazard now in the way it did in the nineteenth century, and the disease in animals has responded very favourably to classical test and slaughter control methods in the absence of a wildlife reservoir, such reservoirs have emerged in various countries over recent decades, and have undermined control efforts under these circumstances. Although various wild and feral species can become infected with *Mycobacterium bovis* (Allen 1991), the most important reservoir hosts so far discovered are the European badger (*Meles meles*), the Australian brushtail possum (*Trichosurus vulpecula* Kerr) and various species of deer.

In New Zealand, Australian brushtail possums had been introduced to the country on multiple occasions between about 1840 and 1940, and wild populations grew and expanded from initial colonization sites to the point where they occur in most of the country and total numbers have been estimated at about 70 million. In 1967 the first tuberculous possum was found in an area of the West Coast of the South Island where it had proved difficult to eradicate bovine tuberculosis using methods which had been successful in other parts of the country. Over the following 25 years it has gradually become clear that possums are a major reservoir species for *Mycobacterium bovis* infection in New Zealand, although not in their native Australia. In the presence of this source of infection eradication of bovine tuberculosis

from the cattle population seems unlikely to succeed. Traditional methods of disease control such as test and slaughter of cattle and culling of possums have not proved adequate to achieve effective control of tuberculosis in either the cattle or the possums.

It was concluded from an examination of the situation that it was essential to better define the epidemiology of the disease in possums in New Zealand. Using an epidemiological approach, it would become possible to adopt a structured systems approach towards identifying and implementing effective control of tuberculosis infection in domestic cattle populations which are exposed to tuberculous possums. This study forms the first stage of a long term research program to achieve these goals.

CHAPTER 2

BACKGROUND TO THE STUDY

Since the start of the tuberculosis eradication campaign in 1945, tuberculosis incidence in New Zealand cattle has been successfully reduced from about 8.6% to 0.11% in dairy cattle and from 0.8 % to 0.29% in beef cattle, using figures derived for the testing year 1990/91 (Hennessy 1986, O'Hara 1992). In 1991 2.7% of herds in the national population (n=47000) were classified as infected or under movement control restrictions. However, despite continuing "test and slaughter" efforts, in 18 areas (in 1991) of the country reactor incidence remained significantly higher than in the rest of the country. These 'endemic areas' are now considered to be continuously infected with tuberculosis because of the existence of a reservoir of infection in possums and possibly in other wild animals. Some have expressed the view that eradication of the disease in cattle may be impossible (O'Hara 1992). After the first possum with tuberculosis infection was discovered in 1967, it took a few years before the first surveys for non-bovine sources of tuberculosis infection were conducted. It was then found that in most 'endemic' areas tuberculosis was present in the possum population. Introduction of extensive possum control operations resulted in a prolonged reduction in cattle reactor rates (Hennessy 1986).

An experiment to determine the risk from a tuberculosis - infected possum population to cattle grazing adjacent areas was conducted on the West Coast of the South Island in 1970/71. Twenty nine tuberculosis-free calves were introduced into an area where a tuberculosis prevalence of 12% had been found previously in the possum population. After 6 months 26 animals were tuberculin positive, and lesions were found at slaughter in 16 (Davidson 1976).

In a summary of experiences with *Mycobacterium bovis* infection in cattle on the West Coast Stockdale (1975) states that the likelihood of recurrence of cattle tuberculosis on individual farms appeared to be linked to the prevalence of tuberculosis in possums. Properties with a persistent problem seemed to have in common a topography and vegetation which made it difficult for possum control to be successful. Tuberculosis "break downs" in cattle herds usually coincided with the presence of only one tuberculous possum, which was found during subsequent investigations on the property. Often, possum tuberculosis was found in local clusters and it was then possible to demonstrate that only animals which had grazed the associated areas were infected. Other potential wildlife reservoirs of tuberculosis infection including deer, pigs, ferrets and hedgehogs were considered to be a minor risk of infection for cattle. Stockdale concluded that there was ample circumstantial evidence that *Mycobacterium bovis* infection cycling in possum populations was the major factor contributing to persistent tuberculosis problems in cattle in New Zealand.

In the Wairarapa the first tuberculous possum was detected in 1969. From then until 1981 tuberculous possums were found on 114 farms all over the Masterton district. Most of these farms, and very often their neighbouring properties, had a persistent tuberculosis problem in cattle (Shortridge 1981).

A number of reports have found that in problem areas cattle reactor rates decreased significantly over the years following a reduction in the possum population density as a result

of a control operation (Hennessy 1986). After recognizing that the disease situation did not respond to normal testing procedures a 3 monthly whole herd testing scheme was introduced in Buller County. This resulted in a reduction of reactor incidence from 12.5% of 4306 cattle tested in January 1970 to an average incidence of 4.97% (s.d. 1.02) in April 1972. Extensive aerial poisoning operations to kill possums were conducted in June-July 1972 and were followed by ground control work over the following years. Cattle reactor incidence dropped to an average of 1.36% (s.d. 0.46) over the period from January 1973 to July 1975 (Stockdale 1975). In the Wairarapa, cattle tuberculosis incidence averaged 2 - 2.3% per year over four years before possum population control was implemented. After two years reactor incidence reached a minimum of 0.3 and started to increase steadily over the following 4 years to about 0.7 (Hennessy 1986). Recurrence of tuberculosis in cattle has been observed in several areas of the country a number of years after initial reduction following possum control operations (Batcheler and Cowan 1988).

In the Central North Island between 1975 and 1987 the size of the area with endemic tuberculosis infection in possums was increasing by a multiplication factor of 1.104 every year. Assuming that spatial spread follows a model of logistic growth, tuberculosis infection would be present in all North Island possum populations by the year 2024 and in all South Island populations by 2031 (Batcheler and Cowan 1988).

Following recognition of tuberculosis-infected possums as a significant source of persistent infection in cattle herds in the early seventies it became the policy of the Ministry of Agriculture and Fisheries to conduct possum population control operations in areas where there was a persistent cattle tuberculosis problem and tuberculous possums were found on or near the property. In the early eighties it was concluded that despite the huge financial investment incurred in reducing possum population density, total eradication of tuberculosis in the major areas with endemic tuberculosis infection in possums, was neither technically feasible nor cost-effective. The modified objective therefore became to achieve a break-even point between the costs of reactor compensation and possum population control (Anon. 1984). In 1985 the objectives of the tuberculosis control scheme were redefined and included the cost-effective prevention of spread of tuberculosis infection from endemic areas (Anon. 1985). According to the Chief Veterinary Officer's annual reports for the period it was decided in 1986 in consultation with ecologists to establish low possum density buffers of about 3-5 km width on the edge of a major endemic area in Central North Island to contain the spread of tuberculosis infection in possum populations (Anon. 1986, Livingstone 1988). This policy was revised in 1989 when tuberculous possums were found outside the buffer zone. The current objective is to restrict the spread of tuberculous possums from the major endemic areas and to reduce cattle tuberculosis incidence by localised cost-effective possum population control within these areas. Based on a results from a deterministic simulation model, management plans for eradication of tuberculosis from possum populations were developed for a number of small- to medium-sized endemic areas (Anon. 1989).

At the end of the tuberculosis testing year 1991/92 there were 20 areas in New Zealand where tuberculosis infection was present in possum populations. In 1991/92 about 83% of cattle reactors to the tuberculin test and herds under movement control restrictions were found in these areas (TB endemic and tuberculosis investigation areas; O'Hara 1993). The predicted cost of the New Zealand bovine tuberculosis control programme for the financial year 1992/93 was NZ$ 21.82 million. Of this sum a total of NZ$ 5.1 million was to be spent on possum population control (Anon. 1992).

CHAPTER 3

REVIEW OF THE LITERATURE

In this review special emphasis will be given to the epidemiology of bovine tuberculosis in species of potential importance as disease reservoirs. A section on human tuberculosis is included to take advantage of the wealth of historical information available on the epidemiology of tuberculosis in humans.

EPIDEMIOLOGY OF TUBERCULOSIS IN HUMANS

Tuberculosis infection occurs throughout the world. A recent report by the World Health Organization (Anon. 1992) stated that about a third of the world population is harbouring the pathogen. Tuberculosis in man is caused primarily by *Mycobacterium tuberculosis*, but *Mycobacterium bovis* remains a contributor to the total disease, even though it is now much less important than it was in the nineteenth century before control of transmission from cattle. The World Health Organization reports that in humans there are about 20 million active cases of tuberculosis in the world, who probably infect 50 to 100 million people (mainly children) annually. It is estimated that about 3 million die due to the disease every year, at least 80% in developing countries (Stead and Dutt 1988). As species identification is not carried out routinely, it is difficult to estimate the present contribution of *Mycobacterium bovis* to total tuberculosis morbidity and mortality in humans (Pritchard 1988).

Since the beginning of this century the epidemiology of human tuberculosis has been studied extensively. This review looks at parallels between mycobacterial infection in humans and animals. Rich (1951) has reviewed reports of the occurrence of tuberculosis caused by *Mycobacterium bovis* in humans. He writes that after Koch's insistence that this bacillus would not be pathogenic in man, it took a number of years before it was generally accepted that *Mycobacterium bovis* can cause all of the forms of tuberculosis which the human-type bacillus is able to produce. Milk turned out to be the most important source of *Mycobacterium bovis* infection for humans. Hence, the incidence of infection in any area where the bacillus was prevalent in cattle was determined by the extent to which raw milk and milk products were consumed, which in turn was determined by local habits. Rich quotes a number of studies from the beginning of this century where up to 60% of cases with bone and joint tuberculosis and 90% of cases with tuberculous cervical adenitis in Scottish children were found to be caused by infection with *Mycobacterium bovis*. At that time human tuberculosis of bovine origin was proportionately more common in the British Isles than in any other industrialised country in the world. It was estimated that around the beginning of this century at least 2000 children died annually from bovine tuberculosis in Great Britain, because powerful dairy interests had prevented the introduction of legislation requiring the pasteurization of milk sold to the public. Rich writes that infection and progressive disease caused by *Mycobacterium bovis* is more likely to occur in children than in adults. He attributes this to a combination of factors. These include, that children are more susceptible, that they are more likely to be exposed and that infection through the alimentary tract (which is the main portal of entry for the bovine bacillus) is easier to achieve. Rich extensively discusses the relative importance of the alimentary and respiratory tract as portals of entry

with particular reference to *Mycobacterium tuberculosis* infection in humans. He concludes that the respiratory tract is the most common portal of entry, and that to achieve infection via the alimentary tract far greater quantities of bacteria are needed. Rich writes that intrauterine infection occurs infrequently. He also mentions that infection can be induced by dropping tubercle bacilli into the conjunctival sac, which has led to cervical adenitis and generalized tuberculosis in the absence of a macroscopic local conjunctival lesion. Rich believes that there is no greater susceptibility in humans towards infection with *Mycobacterium tuberculosis* than there is to *Mycobacterium bovis*.

Yates and Grange (1988) suggest that despite virtual eradication of bovine tuberculosis infection in cattle in the region, about 1% of bacteriologically proven tuberculosis cases in South-East England are still caused by bovine tubercle bacilli. In the period 1954-1957 in areas in Germany with high cattle reactor rates, up to 25% of tuberculosis cases in humans were caused by *Mycobacterium bovis*. By comparison only 2.7% of human tuberculosis cases in an area with low cattle tuberculosis incidence were related to infection with *Mycobacterium bovis* (Schliesser 1982).

Blood and Radostits (1989) write that complete eradication of bovine tuberculosis has not been achieved in any country of the world, but in many a state of virtual eradication with minor recrudescences can be claimed. The complex epidemiology of this organism, which has one of the broadest host ranges of all pathogens, complicates control efforts (Grange and Collins 1987).

The transmission dynamics of *Mycobacterium bovis* infection changed considerably from prehistoric times to the present. This was mainly associated with the rise of civilization. There are some reports which consider the possibility that after the domestication of cattle a mutation occurred in the bovine bacillus to create the human type (Lancaster 1990, Wadsworth 1984). Due to its chronicity, tuberculosis would have been able to perpetuate itself in small communities. But, it has not been detected in many modern day isolated communities, which leads Lancaster to the conclusion that it can be thought of as a common or even true human disease only after the development of agricultural societies. There is some evidence from prehistoric skeletons that it existed in simple early human societies, even though it was uncommon among such groups (Cohen 1989, Clark *et al* 1987).

Stead and Bates (1980) describe *Mycobacterium tuberculosis* as having the potential to cause epidemics when introduced into any susceptible populations. They write that early skin-testing data shows that most people in Europe eventually became infected, but only some of these developed disease. One possible explanation of this is that there are variations in natural resistance to tuberculosis, which over several generations resulted in the elimination of the relatively more susceptible population, although a simpler explanation would be that not all individuals were exposed to sufficient risk factors to precipitate the conversion of their subclinical infection into clinical disease.

First reports on the current tuberculosis pandemic are from 16th century England. Stead and Bates estimate that the disease requires about 300 years to complete its course in one geographic area. There is some data which suggests that the present pandemic began in 16th century England, reaching a peak in 1750 during the early beginnings of the industrial revolution. During this period of urbanization the life-style of people changed significantly and as a result the risk of person-to-person spread was considerably higher. From England it spread all over the world, reaching high incidence in western Europe in the early 1800s, in the late 1800s in North America and still has not reached its peak in some developing countries. Stead and Bates suggest that the downward slope of tuberculosis mortality was only little affected by tuberculin testing, BCG vaccination and early chemotherapeutic efforts until in 1952 isoniazid was introduced as a chemotherapeutic agent.

Mercer (1990) reviewed changes in tuberculosis incidence and mortality since the 18th century in England. He writes that the disease had become endemic in the towns of England by the 18th century and 'consumption', as the disease in humans was called, contributed about 15-20% of deaths in the London Bills of Mortality. Sharing of airspaces with infected individuals along with under-nutrition were likely to have affected the risk of clinical disease establishing from initial contact, while other living conditions affected the risk of mortality on re-activation of initially dormant disease later in life. During both centuries people in towns were likely to have contracted primary infection at an early age. Its destructiveness has to be seen in the context of the other epidemic diseases to which everyone was exposed in overcrowded living conditions. Mercer writes that suppression of tuberculin reaction has been found during scarlet fever, glandular fever, measles infection and probably smallpox. He points out that the actual physical disruption produced by the respiratory complications of such infections was probably even more important. During the second half of the 19th century there was a decline in mortality, but the population continued to have 100% contact with the tubercle bacillus. Mercer suggests that this decline could have been an indirect benefit of vaccination against smallpox, since this disease probably re-activated latent infection or weakened the resistance of the survivors. He does not believe that any immuno-genetic changes can account for the decline in tuberculosis death rate, particularly as the trends for non-respiratory forms of tuberculosis did not fall much in the second half of the 19th century. Mercer thinks that changes in transmission were rather unlikely to account for the decline in mortality from tuberculosis, as during this time the population in towns was increasing most rapidly but there were only slight improvements in general living conditions. The first countries to industrialise were also the first to experience the downturn in tuberculosis mortality. But even in the 1940s tuberculosis case-fatality was about 50%. Mercer notes that in addition to preventive measures used against tuberculosis such as vaccination and chemotherapy, changes in family size (initially among the middle classes), and in housing conditions among the less well-off, could have been involved with a reduction of transmission rates and severity of air-borne infectious disease.

Stead and Bates (1980) reviewed factors of importance in the epidemiology of *Mycobacterium tuberculosis* infection in humans. They consider industrialization and urbanization resulting in crowded living conditions as more important than any other single factor. Low socio-economic status appears to be associated with increased tuberculosis mortality. Poor nutrition is probably less important, as are racial differences. Stead and Bates emphasize that individual infection is more likely, given a sustained and intimate contact with infectious persons resulting in the probability of multiple exposures. Since infection with *Mycobacterium bovis* has become a rarity in cattle and humans, infection through inhalation is considered the most important transmission path. Size of the inhaled particle is more important than the quantity of organisms. Stead and Bates quote a landmark study by Riley and colleagues (1962) which demonstrated that infectious particles having the aerodynamic properties of droplet nuclei were capable of passing through the duct system from a hospital ward occupied by sputum smear-positive patients and infecting guinea pigs kept on the roof. Droplet nuclei are so small that they can stay constantly airborne and can rapidly disperse throughout an enclosed atmosphere. These particles are not filtered by simple gauze masks. It was shown that in guinea pigs and mice almost all tubercle bacilli that were inhaled as single organisms, reached the lung alveoli and produced a tubercle. Survival of aerosolised bacilli is brief, with only about 1 percent surviving for several hours. It was therefore concluded that effective prevention of transmission has to be targeted at these aerosolised particles. In a recent official statement the American Thoracic Society (Anon. 1990) points out that because of the extraordinary significance of airborne droplet nuclei for successful transmission of *Mycobacterium tuberculosis* any of the other methods which were once thought to be important for preventing infection such as disposing of clothes and bedding, sterilizing fomites, using caps and gowns and gauze or paper masks, boiling dishes, and washing walls are unnecessary because they have no bearing on airborne transmission.

Schliesser (1985) reviewed the epidemiology of bovine tuberculosis in humans. He emphasizes the importance of aerogenous as well as alimentary transmission from cattle to humans. Traditionally infection was attributed mainly to the consumption of milk from tuberculous cattle. Schliesser quotes work by Schmiedel (1970) which suggests that due to its high fat content which increases absorption into the lymph system, milk represents an ideal medium for alimentary transmission. Schliesser notes the accepted fact that pasteurization kills pathogenic bacteria in milk if the correct procedures are followed. In the absence of pasteurization, bacteria contained in milk products can remain infectious for extended periods of time. This can be up to 100 days in butter and 322 days in certain types of cheese. Schliesser states that meat from tuberculous animals may constitute a significant risk of infection if available for consumption. *Mycobacterium bovis* has even been isolated from mince meat. Schliesser quotes Jensen (1937) who considered the risk of aerogenous infection as being higher in a shed with tuberculous cattle than in a hospital with tuberculosis cases. Infection by direct contact would be a risk factor for certain occupations such as farmers, abattoir workers and veterinarians.

In Australia human tuberculosis caused by *Mycobacterium bovis* is now rare. About 10 cases are recorded every year. Most of them show pulmonary lesions and are usually older people, but in earlier times a number of younger abattoir workers probably did become infected by aerosol transmission from cattle (Patel and Streeton 1990). In New Zealand the annual incidence of tuberculosis infection (including *Mycobacterium tuberculosis* and *Mycobacterium bovis*) averaged 4.2 cases per 100,000 population during 1985-90. The risk of infection was higher in Maori and Pacific Islanders compared with people of European ethnic background (Stehr-Green 1992). During 1988 1.8% of a total of 228 *Mycobacterium* isolates from human tuberculosis cases were typed as *Mycobacterium bovis.* All 4 cases were between 41 and 60 years of age. In 3 cases it was possible to isolate *Mycobacterium bovis* from sputum, in all cases from pleural fluid and in 1 of the cases from a urine sample. One case each was reported from Hamilton and Wellington Area Health Board and 2 cases were identified in the Palmerston North area (Anon. 1989). During 1989 a total of 15 cases with specimens yielding *Mycobacterium bovis* isolates were reported by the New Zealand Communicable Disease Centre (Anon. 1989, Anon. 1990). Amongst these were three infants, 8 people older than 41 years of age, and 4 of intermediate age. 13 of the 15 individuals were males.

Concurrently with the recent epidemic of the human immunodeficiency virus (HIV) a resurgence of tuberculosis has been reported from many areas of the world where HIV infection is widespread. One example for which data are available is New York City (Brudney *et al* 1991). The authors discuss the possible reasons for this unexpected development. They found that the disease is a growing problem in impoverished populations, particularly in homeless people living in mass shelters. In this group non-compliance with the treatment regimen was found to be particularly common, further augmenting the disease problem. It was noted that despite the current belief that most tuberculosis cases in HIV patients are related to reactivation of old tuberculosis infection, there is also a substantial amount of primary tuberculosis. This is becoming more likely as there are more inadequately treated individuals with active tuberculosis crowded together with highly susceptible HIV-infected homeless people. In New Zealand an outbreak of tuberculosis in a ward of 30 psychogeriatric patients was reported, which was probably caused by a single undiagnosed fatal index case (Taylor *et al* 1991). The incident probably resulted in three deaths and the infection of 43.3% of the patients accommodated in the closed geriatric ward. Non-compliance with treatment instructions is also likely to result in the appearance of drug-resistant strains of *Mycobacterium tuberculosis.* This has recently been reported from New York prisons, where 13 HIV-infected inmates died due to tuberculosis treatment failure (Purvis 1991).

The occurrence of AIDS and tuberculosis has been termed 'the cursed duet' (Chretien 1990). Tuberculosis is considered the only AIDS-related opportunist disease that can infect healthy members of the community. Tuberculosis infection may hasten the onset of AIDS in otherwise asymptomatic HIV-infected individuals, possibly by several years (Festenstein and

Grange 1991). Contrary to the general belief that tuberculosis follows immunosuppression caused by HIV infection, it has been suggested that immune suppressant conditions such as tuberculosis, malnutrition and others may have preceded HIV infection and facilitated its transmission (Packard and Epstein 1991). The recent developments in human tuberculosis point to the continued importance of this disease, which was once considered to be nearly conquered (Rich 1951).

EPIDEMIOLOGY IN DOMESTIC ANIMALS

Cattle

The first confirmed description of *Mycobacterium bovis* infection in cattle is based on a report by Columella in the year 40 A.D.. In the 17th and 18th century in Germany "Perlsucht" (a term referring to the grape-like lesions) was considered a symptom of syphilis, which resulted in strict procedures for the disposal of the affected animals. When this misconception was corrected, the control measures were removed. It was considered possible to eat meat from tuberculous animals and there were then no obstacles in the way of the spread of the disease. It was not until Robert Koch identified *Mycobacterium bovis* that the relationship between "Perlsucht" and lung tuberculosis in cattle was established (Schliesser (1982).

Based on the tuberculin test, the first disease control programme which was successfully implemented was Bang's eradication scheme in Denmark in 1892. In Germany such a programme based on tuberculin testing every animal was not considered economically feasible. Ostertag's eradication scheme which relied on the detection of animals with open lesions was introduced in Germany in 1912 on a voluntary basis. In 1939 the scheme was discontinued, because it was found that reactor rates were higher in herds which took part in the programme. In 1952 a compulsory tuberculosis control scheme was introduced in West Germany which was based on identification of infected cattle using the tuberculin test. The number of tuberculosis-free herds increased from 9.9% in 1952 to 99.7% in 1961. Schliesser (1982) discussed the major factors associated with maintaining reactor rates in cattle at a low level. Major problem areas included the specificity of the tuberculin test and the risk of reintroduction into tuberculosis free herds. The latter, Schliesser attributes mainly to international trade of animals and their products and to transmission from *Mycobacterium bovis* infected humans. He also points out the differences between the epidemiology of tuberculosis in humans and domestic animals.

During the last 100 years in industrialized countries human living conditions have improved and infection rates have decreased despite the absence of a disease eradication programme. On the other hand, during the same period of time changes in domestic animal husbandry occurred (such as increased herd sizes, higher stocking densities and greater numbers of animal movements) which facilitated transmission and spread of infectious diseases. In Germany by 1975 such good progress had been made that it was possible to reduce herd testing to once every three years. Since then, the number of infected herds has steadily decreased, but the number of reactors within infected herds has increased (Schliesser

1985). Weber *et al* (1988) report that the proportion of reactors in infected herds increased with the time elapsed since the last tuberculin test.

In intensive production systems bovine tuberculosis is more common in dairy cattle than in beef cattle. This is related to the fact that dairy cattle are usually kept in sheds in close contact with one another whereas beef cattle are usually kept on pasture. The different age structure of the two major cattle production systems may also be of importance. Prevalence increases with age and dairy cattle are usually kept longer than beef cattle (Schliesser 1985). Francis (1958) quotes Villemin (1868) who observed higher prevalences of tuberculosis in those cattle which were kept indoors. In extensive cattle production systems in North and South America, in Asia and Africa tuberculosis usually is less prevalent. But in some situations (Africa, Australia) high incidences have been reported in range cattle. This may be related to aggregation of animals around watering points. Shortridge (1981) analysed the patterns of tuberculosis infection in a problem area in New Zealand. He considered congregation of animals around water ponds and dams as conducive to the spread of infection within herds.

Recent studies in Northern Ireland suggest that within- and between-herd transmission through aerosolised secretions may be of continued importance in the epidemiology of bovine tuberculosis. It was found that in cattle infected with *Mycobacterium bovis,* lung lesions and nasal excretion occurred frequently and could be diagnosed from 2 months after the last clear tuberculin test (McIlroy *et al* 1986, Neill *et al* 1988). Dunnet *et al* (1986) also mention in their report that analysis of breakdown data from Great Britain shows that tuberculosis is rarely transmitted to neighbouring herds. They conclude that the presence of non-reacting excreters is more likely to affect within- herd transmission. Dunnet *et al* mention the importance of the introduction of infection through purchases of tuberculous cattle. Especially in the early 1970s imported Irish cattle were related to a significant number of breakdowns in Great Britain. In Ireland in areas with a high incidence, transmission between cattle is considered the principal means of spread. In some areas other transmission paths are also thought to be of relevance (O'Connor and O'Malley 1989). Downey (1990) outlined the main reasons for failure of the Irish tuberculosis eradication programme in containing spread of infection. He mentions that one of the factors involved was residual infection within herds which had not been detected by tuberculin testing. A major reason for failure was spread from infected cattle to other cattle by movement and contact, especially under stressful conditions.

There have been few attempts to model the epidemiology of bovine tuberculosis infection within cattle herds. As part of the Australian Brucellosis and Tuberculosis Eradication Campaign (BTEC) a deterministic simulation of the interaction of bovine tuberculosis in range cattle was developed to evaluate the effect of different disease control strategies in northern Australia (Stoneham and Johnston 1987). In these areas in many large herds it had not been possible to eliminate infection despite immense testing and culling pressure. Herds appeared to clear up, but sometimes the disease re-emerged at even higher

incidence than the original level. The results of several simulation model runs suggest that the disease can be maintained at low levels in small matriarchal breeder groups (9 to 15 head) and is spread by wandering infected males. Disease build-up is generally slow except in situations where cattle congregate in very large numbers. Efficient mustering to ensure that all groups are represented in the yards for testing would probably be more important than test efficiency. It was recommended that when an infected female was detected all members of its social group should be culled to reduce the risk of undiscovered residual infection. The model was used to estimate the management pressure which had to be exerted for particular geographic locations to achieve tuberculosis eradication, depending on cattle density and water availability.

Livingstone (1985) developed a computer simulation model of tuberculosis in a cattle herd. He tested the hypothesis that tuberculosis can persist due to factors such as incomplete mustering, presence of anergic infectious animals in the herd and infrequent testing. Modelling results were compared with actual cattle TB testing information from the West Coast of the South Island, New Zealand. Progression of the disease after the addition of one infected animal was significantly slower in extensively farmed herds compared with those intensively farmed. It was concluded that infection cannot be maintained, given the current testing and culling pressure in this area, without an external source of infection.

Farmed Deer

In 1978, tuberculosis infection was reported from farmed red deer in New Zealand (Beatson 1985). It is now considered the most important bacterial disease in farmed deer in New Zealand and in the United Kingdom (de Lisle and Havill 1985).

It has been suggested that deer kept under farm conditions may be more susceptible to *Mycobacterium bovis* infection than are cattle. Given the circumstances with regard to behavioural and environmental factors, extensive lesions can develop rapidly which results in increased probability of spread within a herd (Clifton-Hadley *et al* 1991). As is the case with cattle, the pathogenesis of the disease is mainly dependent on the size of the infecting dose and the susceptibility of the host. The latter depending on variables such as genetic constitution, previous exposure to *Mycobacterium spp.*, nutrition, social status in the herd, handling stress and sex hormone levels (de Lisle *et al* 1985).

Recognizing the threat of tuberculosis to the deer farming industry, New Zealand, Denmark and Great Britain have all embarked on tuberculosis control or eradication programmes. They are mainly based on tuberculin skin testing and subsequent slaughter of reactors. Clifton-Hadley and Wilesmith (1991) write that due to the presence of endemic infection in wildlife in New Zealand and in the United Kingdom the eradication of tuberculosis in farmed deer may be difficult to achieve. Tuberculous captive deer have been implicated in New Zealand as a source of infection for possums which are an important wildlife reservoir for bovine tuberculosis (Livingstone 1988).

Other Domestic Animals

Prevalence of tuberculosis infection in small ruminants is believed to be linked to the disease frequency in other hosts such as cattle. In industrialized countries detection of tuberculous lesions in small ruminants is less common when disease levels within the cattle population decreases. In extensive animal husbandry systems transmission probabilities are low resulting in low overall prevalences (Schliesser 1985). In some instances prevalences of up to 5% have been observed in sheep flocks in New Zealand (Davidson *et al* 1981).

Disease levels in pigs also usually reflect the incidence in local cattle populations. Myers and Steele (1969) report that in 1921 in the U.S. 12% of hogs slaughtered under federal inspection were found to have tuberculous lesions. They note that in the mid west of the U.S.A. it was possible to trace 96% of swine carcass condemnations for tuberculosis to feeding of unsterilized skim milk or other dairy products or to keeping them together with cattle. Prevalence in pigs is thought to increase with age. The principal route of infection in the pig is the digestive tract, by consumption of milk or milk products, kitchen and abattoir scraps, and excreta from tuberculous cattle (Acha and Szyfres 1989). Transmission between pigs is considered epidemiologically insignificant, as lesions usually remain localized and pigs are slaughtered at an early age. High disease levels in cattle can result in prevalences of up to 20% in local pigs (Blood and Radostits 1989).

Lepper and Comer (1983) quote work by Snider and Cohen who found tuberculous lesions in four dogs and 24 cats out of 61 contacts on farms with *Mycobacterium bovis* infection. The authors concluded that on premises with tuberculosis infection surveillance of these species is recommended. Yet, it is unlikely that domestic dogs and cats represent a epidemiologically significant factor in the dynamics of tuberculosis infection.

EPIDEMIOLOGY IN WILDLIFE

Tuberculosis has been known as a serious clinical disease in wild mammals in captivity for more than a century (Thoen and Himes 1981). These authors write that it is widely distributed in wild mammal populations in the United States, where outbreaks caused by infection with *Mycobacterium bovis* have been reported mainly from zoos, game parks and primate colonies. Schliesser (1985) notes that in European countries sporadic incidents of bovine tuberculosis in wild mammals were mainly reported before eradication of cattle tuberculosis was achieved. Evidence from various countries in the world shows that given specific epidemiological circumstances significant levels of tuberculosis infection can be found in wild species such as buffalo, goats, pigs, deer, badgers and brush-tailed possums (Lepper *et al* 1983). The risk which these reservoirs of infection constitute for infection in domestic animals and man is difficult to estimate.

Badger

Badgers are an important reservoir of reinfection for cattle in the United Kingdom and Ireland (Zuckerman 1980, O'Connor and O'Malley 1989). The disease is considered to be endemic

throughout badger populations in both countries (Wilesmith 1991, Morris and Pfeiffer 1990). In Great Britain, local surveys of badger populations around areas with recently infected cattle herds revealed tuberculosis prevalences of up to 50% in localized areas (Wilesmith 1991). In the Republic of Ireland, during the period 1980-1989 17.4% of 3915 badgers examined showed evidence of tuberculosis infection. Infection was present in every county of the country (Dolan 1990). In a local post-mortem survey in an area with a continuing TB problem in the cattle population 48% of 30 badgers showed tuberculous lesions (McAleer 1990). Wilesmith (1991) reviewed the epidemiology of bovine tuberculosis infection in badgers based on the results from a longitudinal study in Gloucestershire, in the southwest of England. Most badgers get infected via the respiratory route. Submandibular abscesses are often found as the first clinical sign of infection. Transmission through bite wounds from territorial fights could account for a higher prevalence in males. Mother to cub transmission could be a major epidemiological factor. It is suggested that adult females are responsible for maintenance of infection within a population. A large proportion of excreting (ie infectious) tuberculous badgers survived for more than 12 months. Mortality induced by tuberculosis does not seem to have an effect on population density. Single badgers with advanced tuberculous lesions shedding large numbers of bacteria in their urine are thought to be the primary source of infection in cattle. Hence, the probability of transmission of infection from badgers to cattle is low and probably occurs mainly as a sporadic incident. The risk increases with increasing badger density (Wilesmith 1991).

In contrast to the findings from the above field study, Mahmood *et al* (1987) challenged badgers with bovine tubercle bacilli in an experimental study and found that intradermal inoculation was almost always successful and intratracheal challenge always failed. The badgers did show immune responses which enabled them to hold the disease in check for up to 22 months. They begin to shed organisms only in the late stages of the disease. Disease is found in local "pockets" of infection, which do not necessarily equate with individual social groups. They may involve a number of contiguous groups (Dunnet *et al* 1986).

Benham and Broom (1989) studied the interactions between badgers and cattle on pasture. They found that badgers were approached by cattle of all ages. Badgers preferred not to use pastures occupied by cattle and avoided approaching closer than 10-15m from cattle. They concluded that tuberculosis transmission would be more likely through badger products such as urine or faeces than direct contact. Other researchers found that badgers did not always avoid cattle (Kruuk 1989). Kruuk also found that badgers need short-grazed pastures to feed efficiently, which could bring them into contact with cattle. Badgers defend their territories ferociously. They form social groups which use the same territory and setts. Female offspring tend to stay with their mother's group. Young males stay for extended periods of time within their mother's social group. They eventually may emigrate into a vacancy in a neighbouring group. This tendency to stay close to their mother's home range could explain why infection has not spread evenly through areas with endemic infection (Cheeseman *et al*

1988). Stuart and Wilesmith (1988) consider that the social structure of the population, their subterranean existence, social grooming and group sleeping are all important factors for maintenance of infection in a badger population.

An attempt has been made to develop a simulation model of the population dynamics of the badger and the epidemiology of bovine tuberculosis (Anderson and Trewhella 1985). A simple mathematical model was developed which was mainly aimed at identifying areas where current knowledge was inadequate. It was recognized at that time that the understanding about the epidemiology of the disease was only limited. The modelling results suggest that average prevalence is positively correlated with badger density. The disease should be able to persist endemically at low badger population densities due to factors such as frequent pseudo-vertical transmission and long survival periods of infectious animals. Anderson and Trewhella (1985) suggest that a high risk of cattle TB breakdowns is probably associated with high tuberculosis prevalence in infected badgers, high cattle herd density on pastures close to good badger habitat and farm management practices such as allowing badgers access to cattle sheds, salt licks and water troughs. The authors predict that very substantial reductions in badger abundance are necessary to induce marked changes in prevalence within the badger population. However, moderate reductions in badger density would significantly reduce, but not eliminate the risk of cattle herd infections.

Brush-Tailed Possum

Between the beginning of the nineteenth century and the start of this century the Australian common brushtailed possum (*Trichosurus vulpecula* Kerr) has been introduced and liberated at numerous locations in New Zealand (Pracy 1962). The susceptibility of possums to *Mycobacterium bovis* infection was first experimentally demonstrated in 1948 by Bolliger and Bolliger (1948). In 1967 the first possum with tuberculous lesions to be identified in New Zealand was found by a trapper on a farm with a persistent tuberculosis problem. In this and later cases it was reported that in all tissues examined, large numbers of *Mycobacterium bovis* organisms were present and the animals were discharging organisms through open sinuses or the respiratory system (Ekdahl 1970).

As a result of these findings, in 1970/71 the New Zealand Ministry of Agriculture and Fisheries conducted an epidemiological survey of possum tuberculosis in the Buller and Inangahua Counties. The study had a duration of one year. Possum kill operations were done at monthly intervals. 4.9% of 5908 possums captured showed tuberculous lesions on post mortem examination. Monthly prevalence ranged from 2.5% in August to 9.35% in December. In mature possums there was a trend towards higher prevalences in summer than in the winter months, although the difference was not statistically significant. Cattle tuberculosis incidence rates declined from the beginning to the end of the survey. 65% (N=100) of tuberculous possums had lung lesions and 52.6% (N=81) had lesions in the axillary and pre-scapular lymph nodes. It was 2.3 times as likely that infection would be found in a possum population if infected cattle were grazing in the same area, although the

difference was not statistically significant ($p > 0.1$). A "spring rise" in cattle tuberculosis incidence was attributed to the fact that on the New Zealand West Coast it is common practice to graze herds in bush areas during winter months (Cook 1975).

In 1973/74 a one year study of *Mycobacterium bovis* infection in possums involving two-day trapping surveys at two-monthly intervals was conducted in the Hohonu Range, New Zealand, in a cooperative project between the New Zealand Forest Research Institute, Christchurch and the Ministry of Agriculture and Fisheries. An overall tuberculosis prevalence of 7.7% in a total of 1486 possums was found. Both tuberculosis prevalence and relative density of possums were highest on pasture and decreased from the bush/pasture margin to the remote forest. Tuberculous possums were clustered in small foci of 2-5 animals. It was suggested that indirect transmission through sequential or simultaneous den sharing might be a important infection route. Seasonal variation in prevalence was observed, with highest levels in autumn and winter along the pasture margin and in spring within the forest. It was suggested that possums were at higher risk of infection during summer and autumn months due to increased foraging and social activities and that the rise in detectable prevalence in winter reflects the length of the incubation period. It was found that infection was more likely in immature male possums than in immature female possums. The body condition of the possums appeared to be worse in infected than in uninfected possums. 29.5% of tuberculous animals showed lesions in the axillary lymph nodes and 55.4% in the lung (Cook undated, Coleman 1988).

In an experimental study on the course and pathology of the disease in possums it was possible to demonstrate initiation of infection by subcutaneous and intranasal inoculation and transmission through direct and close contact, both by aerosol and by mother - joey contact (pseudo-vertical). Disease developed rapidly to a fatal stage and lesions were typically widely disseminated. However, it was realized that the disease process might have been accelerated by reduced body defenses in captive possums and that the infection dose was very high (O'Hara *et al* 1976).

In 1981 the pathology and histopathology of *Mycobacterium bovis* infection in possums was reviewed by Julian (Julian 1981). Distribution of lesions was summarised, including data on 327 tuberculous possums necropsied in three different areas. In 62% (N=203) of the cases, superficial lymph nodes and in 61.8% (N=202) the lungs were involved in the disease process. The pathological appearance of affected somatic lymph nodes is typically a soft fluctuating abscess with a diameter of up to 4 cm containing semi-liquid lime green pus, which on occasion may form open sinuses to the exterior. In visceral organs and lymph nodes white to yellow nodules of up to 2 cm in diameter are most commonly seen. These lesions may be multilobular with a more caseous centre than is seen in typical somatic lesions. Miliary white lesions occur in lungs, liver, spleen or kidneys. In lungs, generalised grey-white consolidation of lobes or part of a lobe has been observed. Histopathologically, lesions consist typically of granulomatous tissue, with no distinctive fibrous capsule and with varying amounts of

amorphous eosinophilic debris centrally. Lymphocytes, plasma cells, macrophages, giant cells and many neutrophils in great concentration around the caseous material were observed in the reaction process. Large numbers of acid-fast organisms were present. Typical tubercles (including epithelioid cells, Langhans' giant cells and capsule formation) were rarely seen.

Results of a preliminary analysis of the data from the possum tuberculosis survey in the Hauhungaroa Ranges have been published previously (Hennessy 1986), but are analyzed more fully in this thesis. A mean prevalence of 1.4% was reported. There was no association between relative density estimates (derived from trapping and faecal pellet count data) and tuberculosis prevalence in possums. No statistically significant association between infection and sex, breeding ability and condition was found. Sexually mature animals were more likely to be infected than immature animals ($p < 0.05$). A correlation coefficient of 0.9 was observed between cattle tuberculosis incidence (averaged estimates for 11 zones) and possum tuberculosis prevalence. It was concluded that level of infection in cattle represents a good indicator for level of infection in adjacent possum populations. Hence, in areas with endemic tuberculosis infection in possums direct inference from presence of infection in cattle to presence of tuberculous possums would be possible.

Based on current knowledge of possum ecology and the epidemiology of tuberculosis in possum populations a deterministic simulation model was developed to aid the understanding of the disease problem, evaluate different options for control strategies and identify future directions of research. The model suggested that disease in possums has to be aggregated locally at high prevalence levels to persist at the relatively low overall prevalence which has been observed in possum population surveys all over the country. It showed that assuming immigration is insignificant, eradication of tuberculosis infection within a possum population is theoretically possible if 16% of the possum population is removed every year. The model predicts that control of tuberculosis in endemic areas is most likely to be successful using repeated single possum population control operations or an initial single control operation, followed by sustained cropping or vaccination. In the light of the high cost involved in intensive possum control operations it was suggested that separation of cattle from margins of high-density possum habitats, or ground control operations targeted at clusters of disease aggregation may be more cost-effective (Barlow 1989).

Feral Buffalo and Bison

Bovine tuberculosis is known to be endemic in feral swamp buffalo populations of the Australian Northern Territory. Hein and Tomasovic (1981) found a prevalence of 0.017 in 11322 buffalo examined during routine post-mortem examination at 2 abattoirs during 1979. This was a significant reduction compared with a prevalence of 0.16, which was reported by Letts (1964) in 1964. This reduction in prevalence was explained by Hein and Tomasovic (1981) as due to selective harvesting of mature animals for meat, resulting in removal of many tuberculous animals from the population. But as prevalence was highest along the relatively narrow coastal strip of land, which was mainly sampled during the earlier survey, differences

in habitat may provide another explanation for the decrease in prevalence. The authors suggest that lower population density of feral buffalo may affect transmission probability. The large proportion of cases with sole or predominant involvement of the thoracic organs suggests that as in cattle the respiratory route is the most important transmission path in feral buffalo. McCool and Newton-Tabrett (1979) indicate that 97% of buffalo tuberculosis in northern Australia is contracted via the respiratory route. They considered this finding rather surprising in view of the work by Tulloch (1978) on buffalo behaviour which showed that there was ample opportunity for both alimentary and respiratory infection. In fact, Tulloch was reported to have estimated buffaloes were spending on average 4 hours per day in intimate contact in communal wallows. McCool and Newton-Tabrett write that this extended period of close contact is not seen in northern Australian cattle when resting around watering points, only during the few minutes when they drink from crowded water troughs. They concluded with respiratory transmission being the main method of spread, sources of mycobacteria in the environment are probably unimportant. Hence, control of tuberculosis in feral buffalo populations should be possible through standard tuberculosis control measures.

Freeland and Boulton (in preparation) developed a model of the epidemiology of bovine tuberculosis in swamp buffalo in the Northern Territory of Australia. They included the effects of social groups into their model. Modelling results suggested that the mode of group size regulation has a major effect on prevalence levels. The importance of matriarchal breeder groups was stressed for maintenance of infection. Spread of disease would depend on the mode of group size regulation (emigration of matriarchal family clans). Small group sizes which resulted from migration of clans were likely to result in low prevalences. Infected males could potentially spread the disease during emigration or when visiting females from other social groups during breeding season.

Woodford (1982) studied the occurrence of tuberculosis in wild Cape buffalo in Ruwenzori National Park, Uganda. He found tuberculous lesions in 10% of 52 buffaloes from a random sample and 38% of 64 animals which were selected based on being in poor condition. *Mycobacterium bovis* was identified in 12 of 14 cases. Most cases appeared to be infected by respiratory transmission and no lesions were seen which could be ascribed to alimentary infection. The author explains this finding with the close herding habits of wild buffaloes and their propensity for wallowing in tight groups in small mud holes which facilitates droplet transmission. Woodford concludes that bovine tuberculosis could cause an annual mortality of about 1% in this particular area.

In 1961 Choquette *et al* (1961) reported that they found tuberculous lesions in 50% of 436 bison from Wood Buffalo National Park, Canada, which had reacted to the tuberculin test. They quote other work by Fuller who wrote that tuberculous lesions had been found in 39% of 1508 bison slaughtered between 1952 and 1956. The same authors refer to a report by Hadwen who during the period between 1923 and 1939 found tuberculous lesions in 53.4% of 12,005 bison slaughtered at Wainwright. Choquette *et al* examined another 500 bison from

Elk Island National Park, which were not tuberculin tested and did not show any lesions on necropsy. They conclude that in Wood Buffalo National Park current tuberculosis control based on tuberculin testing and slaughter of reactors would be at best a disease reduction program. More recently concern has been expressed again about the levels of tuberculosis infection in bison in Canada. Between 1983 and 1985 during a survey in and around Wood Buffalo National Park, Canada, 21% of 72 bison found dead showed tuberculous lesions on post-mortem. The results suggest that infection occurred primarily via the respiratory route. It was concluded that the disease was endemic within the population and that therefore there was a growing risk of spread to uninfected bison and cattle (Tessaro *et al* 1990).

Wild Deer

Infection with *Mycobacterium bovis* has been reported from a number of free-ranging deer species, as pointed out in a recent review by Clifton-Hadley and Wilesmith (1991). These authors and others report that there have been some incidents where infected wild deer were suspected of introducing infection into captive deer populations (Mackintosh and Beatson 1985). Mackintosh and Beatson write that in New Zealand a high proportion of wild deer captured or shot was found to be infected with *Mycobacterium bovis*. It has been suggested that in New Zealand where deer had been live-captured in many parts of the country and then traded for breeding purposes, infected animals probably provided a means for introducing tuberculosis infection into areas which previously had been free of tuberculosis.

Other Wild Animals

Wild pigs have been found to be infected with *Mycobacterium bovis* at significant levels in a number of countries. Letts (1964) confirmed *Mycobacterium bovis* infection in 54% of 149 tuberculosis-like lesions from a total of 260 wild pigs in Australia's Northern Territory which were autopsied. He associated this relatively high prevalence with the pigs living in close association with swamp buffalo. At the end of each dry season hundreds of old buffalo die, thereby providing food and a potential source of infection with *Mycobacterium bovis* taking into account the high incidence of tuberculosis in wild buffalo in the Northern Territory. Corner *et al* examined 751 wild pigs in Australia's Northern Territory and found a infection prevalence of about 19%. No pulmonary lesions were found. Corner *et al* concluded that the wild pig is probably an end host for *Mycobacterium bovis* and not a significant source of infection for cattle. Recently a survey was conducted in New Zealand, where 251 wild pigs were post-mortemed and 31% were found to have tuberculous lesions (Wakelin and Churchman 1991). The authors suggested that the disease possibly had spread between pigs by aerogenous transmission because 33% of infected pigs had either lung or bronchial lymph node lesions. This finding suggests a possible difference in the epidemiology of *Mycobacterium bovis* in Central Otago, New Zealand, because in general the importance of infection in pigs through the digestive tract is emphasized in the literature.

Other Species

Feral goats were found with tuberculosis prevalences of up to 31% within individual groups in areas with endemic tuberculosis in New Zealand (Sanson 1988). The epidemiological significance of bovine tuberculosis in goats is generally considered as minimal. In most cases it is related to the presence of a reservoir of infection in another species, such as the brushtail possum in New Zealand.

Woodford (1982) found that bovine tuberculosis infection was endemic in the wart hog population of Ruwenzori National Park, Uganda. He concluded that the disease must have been introduced with domestic cattle.

Infection in wild carnivorous species has to be expected in areas with endemic tuberculosis in important infection reservoir species such as for example the brush-tail possum, cattle and deer in New Zealand. Allen (1991) reviewed the occurrence of bovine tuberculosis in feral species other than cattle, possums and deer in New Zealand. He suggests that most of the few cases with bovine tuberculosis in feral cats and ferrets which were found in New Zealand were related to infection in at least one of the major reservoir species mentioned above. Cats and ferrets are unlikely to contribute significantly to the maintenance and spread of bovine tuberculosis infection.

CHAPTER 4

A CROSS-SECTIONAL STUDY OF *MYCOBACTERIUM BOVIS* INFECTION IN POSSUMS IN THE HAUHUNGAROA RANGES, NEW ZEALAND

INTRODUCTION

In 1982/83 the New Zealand Ministry of Agriculture and Fisheries conducted a survey on the prevalence of *Mycobacterium bovis* infection in populations of Australian brush-tailed possums (*Trichosurus vulpecula* Kerr) around the perimeter of the Hauhungaroa Ranges, Central North Island. The objective was to gain a better understanding of the epidemiology of tuberculosis both in possums and in cattle that graze areas adjacent to infected possums. This knowledge would then be used to improve the efficiency of the national tuberculosis control policy. The study was included into this thesis because it had not been completely analysed previously.

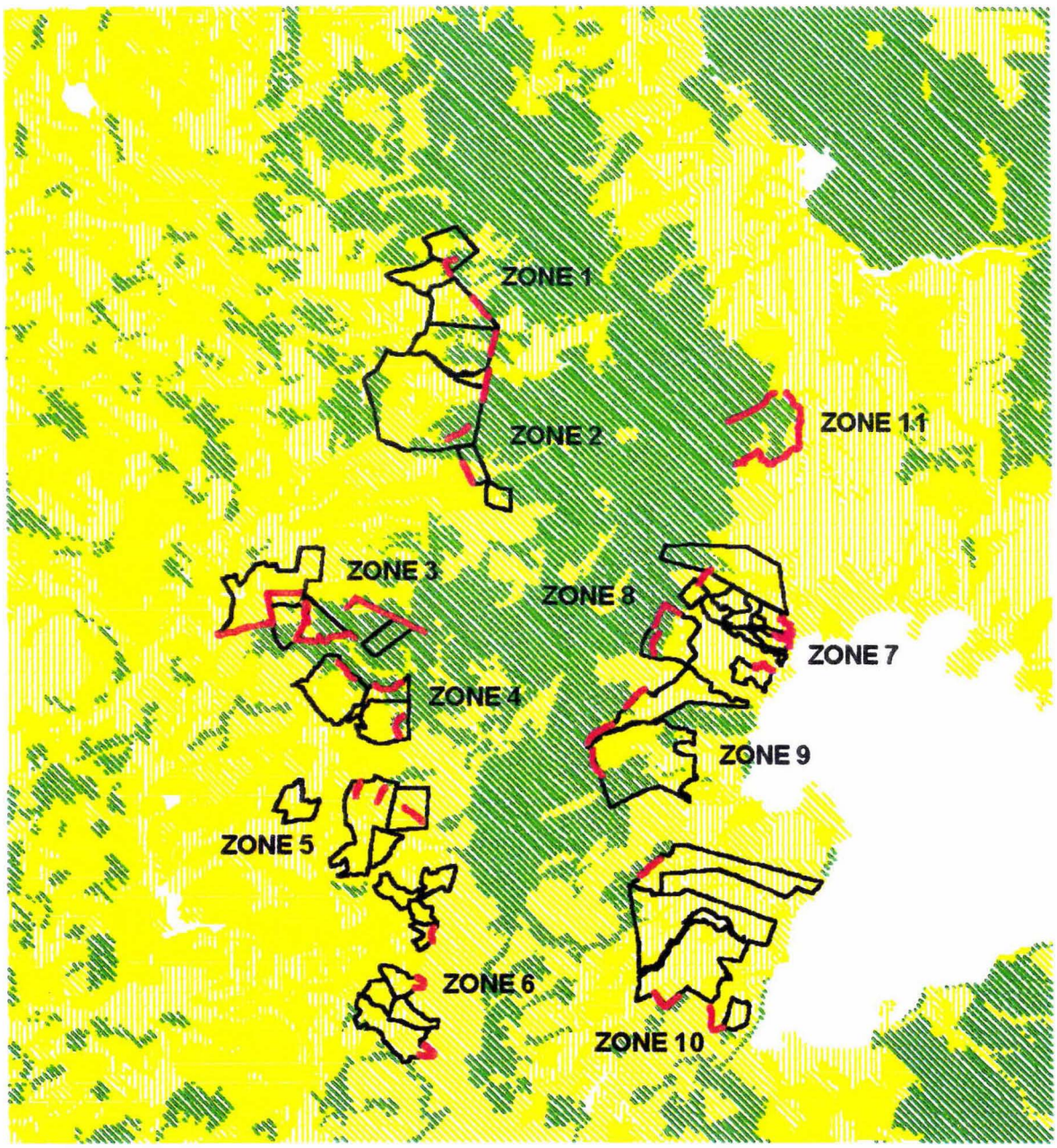
MATERIALS AND METHODS

Study Design and Data Collection

The survey area was divided into 11 geographic zones surrounding the Hauhungaroa Ranges, Central North Island. The zones include North Benneydale (zone 1), Eastern Waimiha (zones 2 and 3), Mangakahu (zone 4), Waituhi (zone 5), Punga (zone 6), North East (zone 7), North West (zone 8), Central West (zone 9) and South West Western Bays (zone 10) and a reference zone west of Arataki (zone 11; see figure 1). The last zone was within the Hauhungaroa forest, and during the study design it was intended to use this zone as a reference or standard population representing the prevalence in a possum population where cross-species transfer of infection was unlikely, since it had no direct contact with cattle or domestic deer.

Within each zone 3 base trap lines were placed along the bush pasture margin except in zone 11 which was located within the bush (see figure 1). Each base trap line comprised 100 traps at 20 m spacing. Additional trap lines of variable length and using varying catch methods were set adjacent to each base line to narrow the confidence intervals of the tuberculosis prevalence estimates. Base trap lines and their surrounding subsidiary trap lines were aggregated to subzones.

Figure 1: Vegetation map of the Hauhungaroa Ranges, Central North Island, with location of farms and base trap lines



On the base trap lines a total of 1100 traps was set for 4 subsequent nights in the period between November 2, 1982 and April 20, 1983. Information on the total number of trap nights and traps set on subsidiary lines is not available.

Possums were trapped using leg hold traps, cyanide baits and wire snares. For each base line, trapping results were recorded on a trap catch information sheet. For each possum captured, physiological parameters such as age class, sex, body weight and length and breeding status were recorded on a possum card. The animals were euthanased and a post mortem examination for the presence of tuberculous lesions was conducted. From animals tentatively diagnosed as infected with *Mycobacterium bovis* based on the presence of macroscopic lesions, formalin-fixed and fresh tissue samples were sent to the Ruakura Animal Health Laboratory for further examination. Animals were diagnosed as infected with *Mycobacterium bovis*, if acid fast organisms were present and histological examination showed pathological changes characteristic for tuberculous lesions. Lesions were considered to be "open", if open sinuses were detected or the lungs were involved in the disease process.

Data Analysis

The analysis of the data was conducted in three parts. The objective of step one was to gain a basic understanding of the ecology of possums living in the perimeter of the Hauhungaroa Ranges. This was considered to be of importance as the survey covered a large area with varying habitat characteristics, and this variability could possibly affect the ecology of local possum populations and thus modify the epidemiology of tuberculosis infection. Step two consisted of a descriptive analysis of tuberculosis infection in the study area. The third step dealt with the epidemiological analysis of the data. Standard statistical methods for the analysis of categorical and continuous data have been employed (Snedecor and Cochran 1989). For continuous variables, means together with their standard deviation were estimated (x = mean, +/- standard deviation). Pearson's correlation coefficient (r) and the Spearman's rank correlation coefficient were given as a measure of the strength of the linear relationship between continuous variables. The strength of the association between 2 dichotomous variables was expressed as an odds ratio (OR) (Martin *et al* 1987). Analysis of variance and Student's t-test were used to assess the statistical significance of the difference between means. Pearson's chi-squared statistic and Fisher's exact test of homogeneity were used for a statistical comparison of proportions. The software package PC-SAS version 6.04 (SAS Institute, Cary, North Carolina, U.S.A.) was used to perform statistical calculations.

Special reference has to be made to the analysis of the association between body condition and tuberculosis infection. Three different estimates of condition were used. First, general body condition was assessed subjectively on a scale from 1 to 3 by palpating along back and rump. Second, based on the amount of kidney fat, fat reserves were scored as excellent, good, poor or none. Third, body weight - controlled for length (nose to tail tip) - was used to compare possum populations stratified for factors of interest. With the first 2 subjective assessments variation between observers had to be expected. The relationship

between body weight W and length L is of the general form: $W = a*L^b$. The coefficients a and b were calculated by linear regression of ln$W$ on ln$L$. The exponent b describes the mean growth characteristics of a population (Bailey 1968, Bamford 1970, Taylor 1979). Analysis of covariance was used to test the effect of other factors on body weight, controlled for length (Crum 1986). A generalized least-squares approach was used to account for the unbalanced design of the data (Pedhazur 1982). Dummy variables and interaction terms were included to test for equality of slope and intercept respectively (Berenson *et al* 1983). It is noted that the nonorthogonal and nonexperimental design of the data requires cautious interpretation of the results of this analysis (Cliff 1987, Cramer and Appelbaum 1980). The association between the other two condition indices and tuberculosis infection controlling for confounding factors was tested for statistical significance using multiple logistic regression (Afifi and Clark 1984).

Possum density estimates were based on trap catch results from the 3 base trap lines within each zone and faecal pellet count information.

Four different methods were used to derive population density indices from trapping data. One estimate was based on faecal pellet count information, which was made available by the Forest Research Institute, Christchurch.

Relative density estimates were estimated both from pellet count information using the presence-absence technique described by Baddeley (1985) (PELLET) and from trap catch information using the method described by Batcheler *et al* (1967) (BATCHELER). The number of trappable possums was calculated from trap catch data using Leslie's regression method (LESLIE) described by Seber (1982) and the generalised removal model (OTIS) described by Otis *et al* (1978). The computer program "CAPTURE" was used to apply the generalised removal model (White 1978). The program "CATCH" developed by the Forest Research Institute, Christchurch, was used to derive another absolute density estimate (CATCH) based on a maximum likelihood approach to estimating probability of captures, described by Seber (Seber 1982). For the purposes of this analysis the standard assumptions (population closure, constant catch effort, equal probability of capture and the catch being proportional to population size) were assumed to be fulfilled (Eberhardt 1978, Davis and Winstead 1980). It is recognized that the assumption of equal catchability may have been violated due to factors such as changes in weather and trap shyness. The catch effort varied slightly over the period of 4 trapping days due to trap interference by non-target species or traps being sprung. However these violations are unlikely to have been serious. Using each of the five methods described, density estimates were derived for each base trap line.

A measure of true prevalence was obtained by taking into account the sensitivity and specificity of the diagnostic method (Schwabe *et al* 1977).

Results of cattle tuberculosis testing during the testing season 1982/83 were available through records from the New Zealand National Tuberculosis Data Base. The spreadsheet

software QUATTRO (Borland International, Scotts Valley, CA, U.S.A.) was used to calculate measures of tuberculosis incidence for cattle herds grazing areas which were included in the study (Kleinbaum *et al* 1982).

Data was analyzed on a IBM compatible micro computer using the database management software PANACEA (Pan Livestock Services, Reading, England) and the statistical package PC-SAS (SAS Institute Inc., Cary, N.C., U.S.A.).

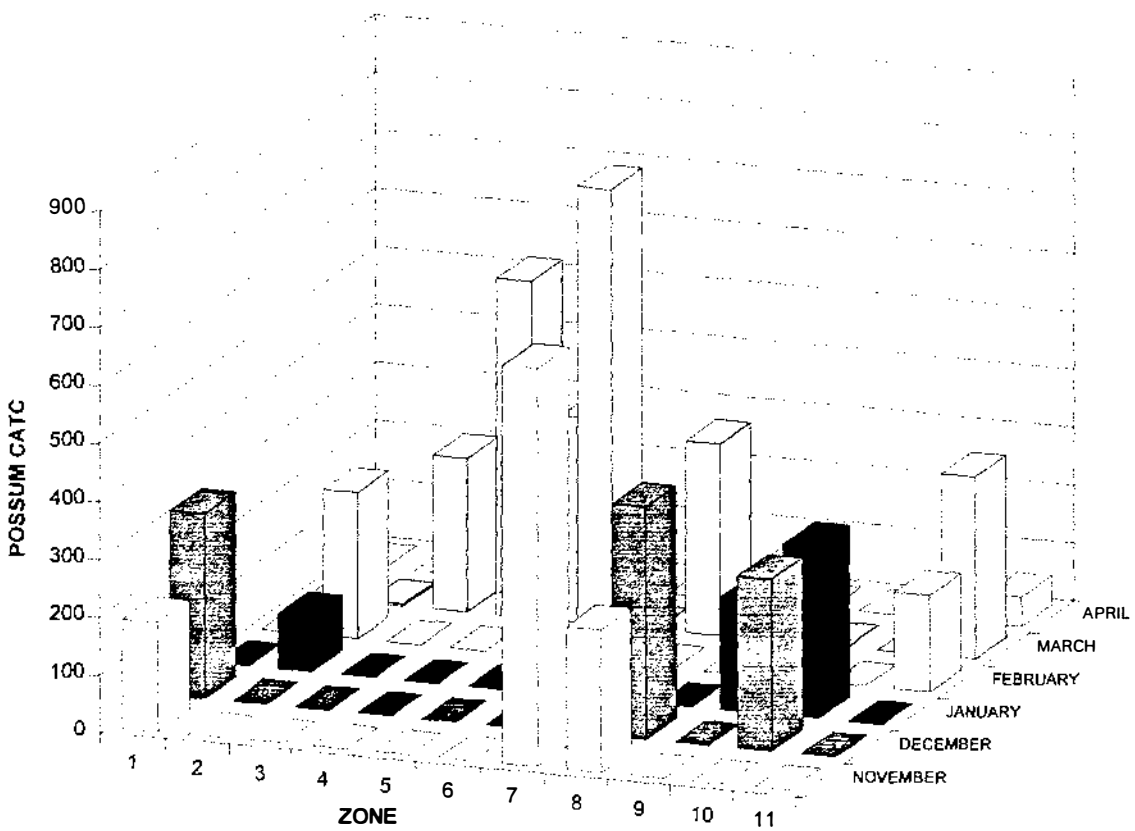
RESULTS

Trapping Statistics

In total, 6083 possums were caught at 4491 trap sites. Fifty percent (N=3062) were caught using leg hold traps, 49% (N=2994) using cyanide baits, 0.3% (N=20) with wire snares and 0.1% (N=7) were found dead. Forty-three percent (N=2636) of the possums were caught on the base trap lines. A total of 2638 possums was captured along the base trap lines.

Eighteen percent (18.5%, N=1124) of the possums were captured in November, 16.8% (N=1023) in December, 10% (N=989) in January, 23.7% (N=1441) in February, 30.5% (N=1855) in March and 0.8% (N=51) in April. In geographic zone 1 data collection started in November and finished in December, in zone 2 it started in December and finished in March, in zones 3 to 5 all the data was collected in March, in zone 6 in February, in zone 7 in November and March, in zone 8 in November, December and March, in zone 9 from January to March, in zone 10 in December and January, and in zone 11 from February to April. (see figure 2)

Figure 2: Possum catch stratified by geographic zone and month



Ecological Characteristics of the Possum Population under Study

Characteristics of the Total Population

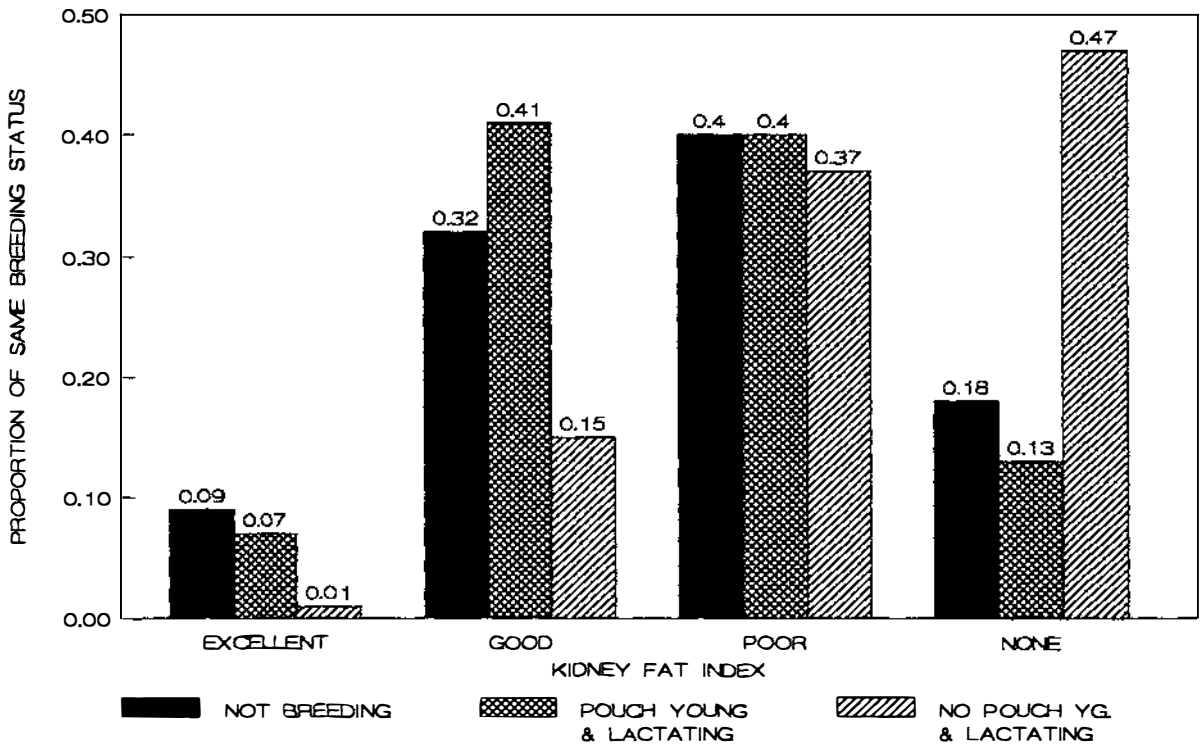
Forty-six percent (N=2795) of the animals were females. Thirty-two percent (N=1935) of the possums were immatures. The proportion of immatures among total females (34%, N=941) was significantly higher than the proportion of immatures among males (30%, N=994; χ^2=8.22, 1df, $p < 0.01$; see table 1).

Table 1: Possum catch stratified by sex and age

	FEMALES		MALES		
AGE CLASS	N	% row	N	% row	TOTAL
IMMATURE	941	48.6	994	51.4	1935
MATURE	1854	44.7	2294	55.3	4148
TOTAL	2795	46.0	3288	54.0	6083

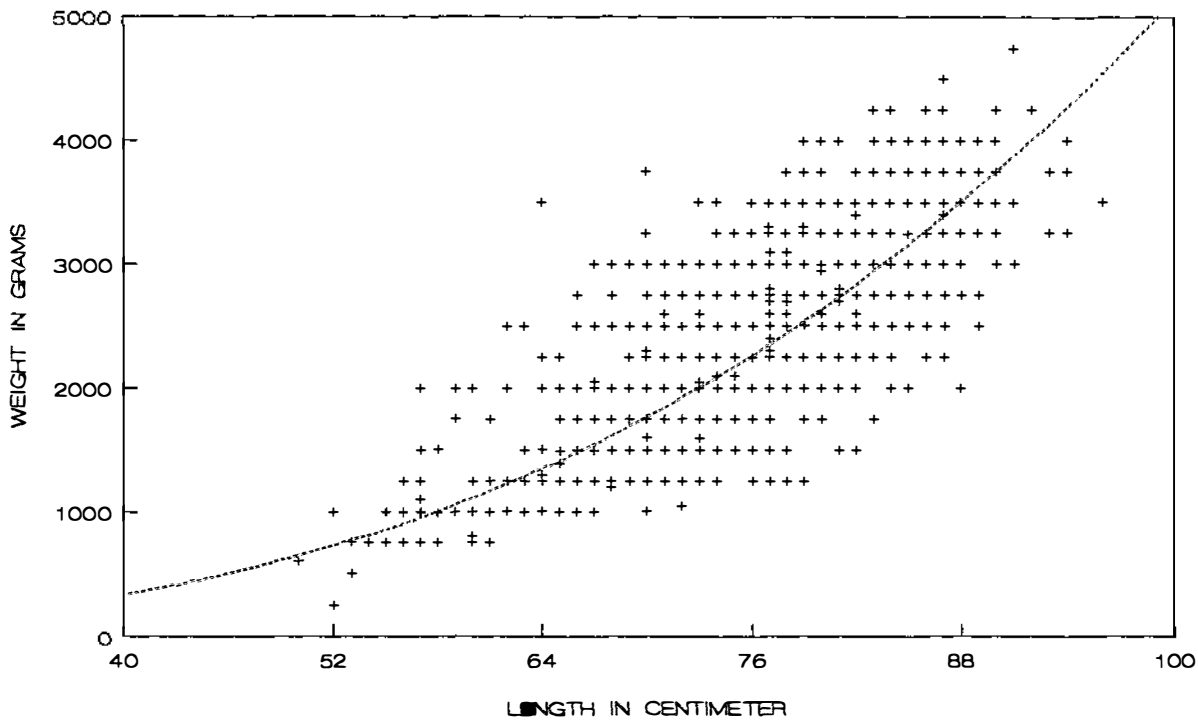
Twenty-three percent (N=430) of adult females had pouch young. Thirty-five percent (35.2%; N=653) of adult females had a pouch young or showed signs of lactation. General body condition was good in 47% (N=2852) of the animals, average in 47% (N=2872) and poor in 5.9% (N=357). On the basis of the amount of kidney fat, 7.9% (N=261) of the cases were in excellent, 31.5% (N=1037) in good, 38.1% (N=1256) in poor and 22.5% (N=742) in very poor condition. There was no difference in general body condition between age and sex classes. Adult possums were 4.8 times more likely to have a good or excellent kidney fat index than immature animals (χ^2 = 487.7, 1df, p = 0.000). Female possums in good body condition were more likely to have a pouch young than adult female animals in average or poor condition (χ^2=23.7, 2df, p = 0.000). The kidney fat index tended to be worse in adult females without pouch young but with signs of lactation than in adult female animals with pouch young or in non breeding individuals (χ^2=137.1, 6df, p = 0.000; see figure 3).

Figure 3: Kidney fat index and breeding status in adult female possums



The average weight (x_w) and length (x_l) of adult animals was significantly lower in females than in males (females: x_w=2640g +/- 487, x_l=789cm +/- 49.7; males: x_w=2820g +/- 574, x_l=793cm +/- 55.1; t_{WEIGHT}=10.21, p=0.0000; t_{LENGTH}=3.063, 4142df, p = 0.0027). Weights and lengths of immature animals did not vary significantly between sex classes (x_w=1580g +/-460; x_l=689cm +/- 67.3). The following equation describes the length-weight relationship for all possums in the sample excluding breeding adult female animals: $W = 0.00538 * L^{2.99}$ (R^2=0.64, $F_{1,5637}$=9981.2, p = 0.000; see figure 4). The linear regression of the logarithmically transformed variable weight on the logarithmically transformed variable length explained 68.3% of variation in ln *weight* ($F_{1,5637}$=12142.5, p = 0.000).

Figure 4: Weight-length relationship in possums

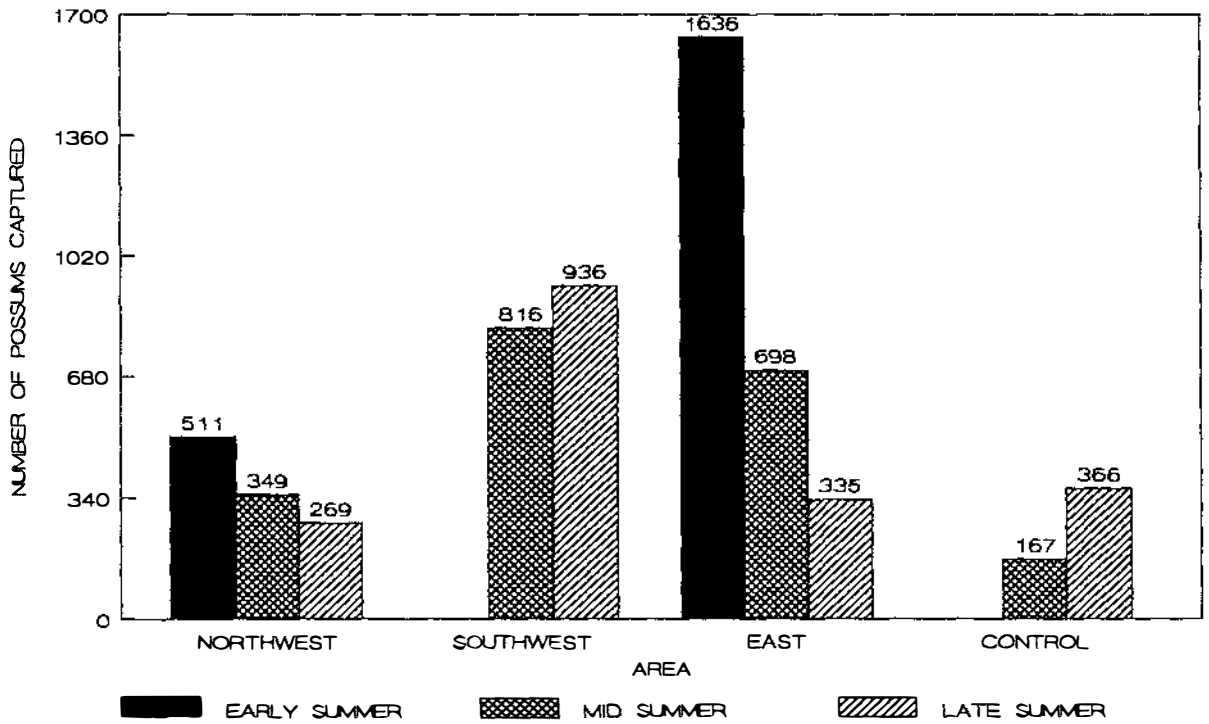


Comparison of Geographically Grouped Populations

Because animals were collected from various geographic zones in different months (figure 2), the month of data collection and the geographic location were considered to be potentially confounded. To account for probable differences in habitat which might in turn modify possum ecology, the zones of the study were grouped into four main areas according to their geographic position relative to the Hauhungaroa Ranges. Zone 1 to 3 comprised the north-west area (NW), no. 4 to 6 the south-west (SW) and no. 7 to 10 the east area (E). The reference zone no. 11 was counted as a separate area (R). The months of data collection were grouped as early summer (ES) for November - December, mid summer (MS) for January and February and late summer (LS) for March - April. Data from the south-west and the reference

zone were only collected in mid and late summer. Figure 5 shows the distribution of possum catch stratified by area and part of summer.

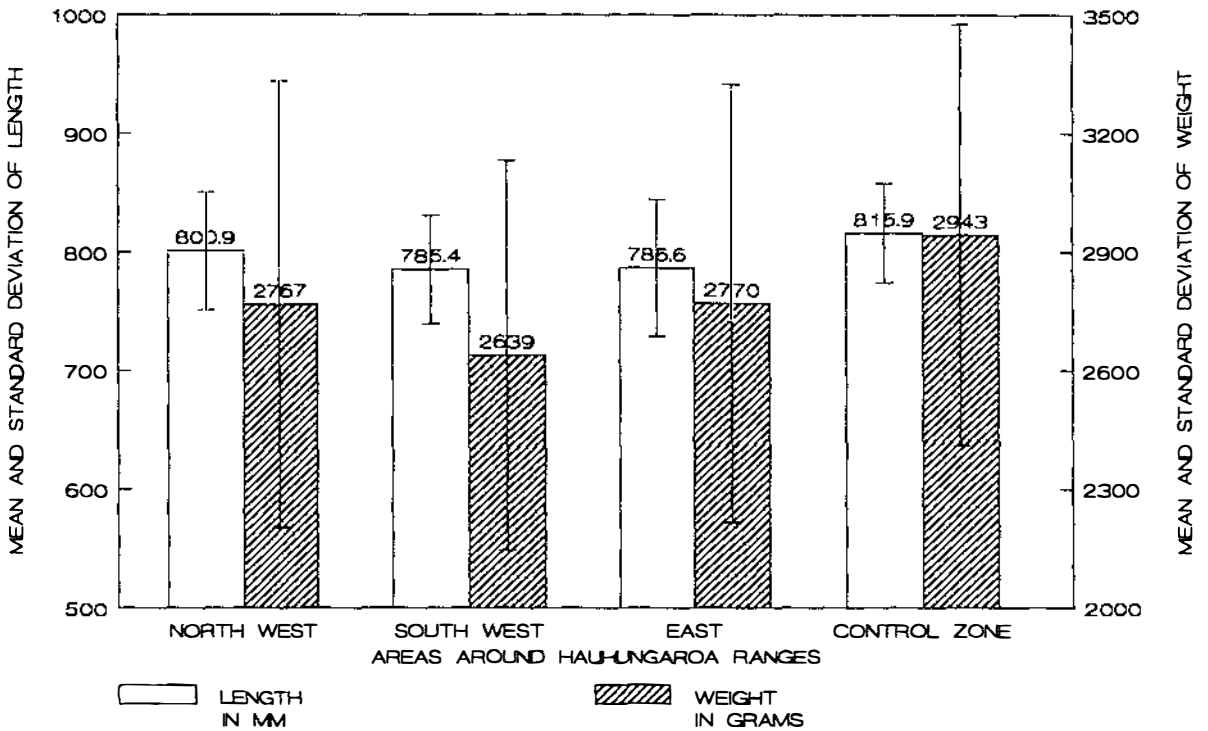
Figure 5: Possum catch stratified by geographic area and part of summer



Analysis on the basis of the areas showed that the proportion of possums assessed as being in poor body condition was significantly higher in the south-west and the east (SW: 7.9%, N=92; E: 7.3%, N=194) than in the north-west and the reference zone (NW: 3.2%, N=54; R: 3.2%, N=17; χ^2=196.8, 6df, p = 0.0000).

Average length in adult possums was shortest in the south-west and the east (SW: 785.4mm +/- 45.9, N=1144; E: 786.6mm +/- 57.8, N=1886) compared with the north-west and the reference area (NW: 800.9mm +/- 49.8, N=767; R: 815.9 +/- 41.9, N=347; $F_{3,4140}$=44.6, p = 0.0000). The average weight of adult animals (excluding adult females with pouch young) was lowest in the south-west (2639g +/- 492, N=1146) and highest in the reference zone (2943g +/- 533, N=347). Average weights of animals in these 2 areas were significantly different from average weights of possums captured in the east and north-west with 2770g (+/- 556, N=1886) and 2767g (+/- 564, N=765) respectively ($F_{3,2434}$=37.97, p=0.0001). Figure 6 shows average length and weight of adult possums stratified by geographic area.

Figure 6: Average weights and lengths of adult possums stratified by geographic area



Controlling for month of data collection (by including only data from February and March 1983) adult animals (excluding females with pouch young) caught in the north- and south-west of the Hauhungaroa Ranges (NW: 2596g +/- 519, N=364; SW: 2622g +/- 466, N=1109) were lighter than those from the east and the reference zone (E: 2763 +/- 601, N=636; R: 2932g +/- 527, N=329; $F_{3,2434}$=37.97, p = 0.0001). Slopes of the equations describing the weight-length relationship were statistically significantly different between eastern areas (b=2.84 +/- 0.037), the areas west of the Hauhungaroa Ranges (SW: b=3.156 +/- 0.057; NW: b=3.148 +/- 0.065) and the reference area (b=3.56 +/- 0.102; R^2=0.69, $F_{7,5631}$=1812.3, p = 0.0000).

In the south-west 51.7% (N=906) of the animals were females, in the north-west 46.2% (N=522), in the east 41.7% (N=1114) and in the reference zone 47.5% (N=253; see table 2).

Table 2: Possum catch stratified by sex and geographic area

	FEMALES		MALES		
AREA	N	row %	N	row %	TOTAL
SOUTH-WEST	906	51.7	846	48.3	1752
NORTH-WEST	522	46.2	607	53.8	1129
EAST	1114	41.7	1555	58.3	2669
REFERENCE	253	47.5	280	52.5	533
TOTAL	2795	46.0	3288	54.0	6083

In the south-west 34.6% (N=606) were immature possums, in the north-west 32% (N=361), in the east 29.3% (N=782) and in the reference zone 34.9% (N=186). The proportion of immatures was not significantly different between sex classes in each area except in the east (33.4% in females, 26.4% in males; χ^2=15.47, 1df, p = 0.0000; see tables 3a and 3b).

Table 3a: Possum catch stratified by age class and geographic area

	FEMALES				
	IMMATURES		MATURES		
AREA	N	row %	N	row %	TOTAL
SOUTH-WEST	315	35	591	65	906
NORTH-WEST	163	31	359	69	522
EAST	372	33	742	67	1114
REFERENCE	91	36	162	64	253
TOTAL	941	34	1854	66	2795

Table 3b: Catch of male possums stratified on age class and geographic area

	MALES				
	IMMATURES		MATURES		
AREA	N	row %	N	row %	TOTAL
SOUTH-WEST	291	34	555	66	846
NORTH-WEST	198	33	409	67	607
EAST	410	26	1145	74	1555
REFERENCE	95	34	185	66	280
TOTAL	994	30	2294	70	3288

Separate analyses for the 2 age groups revealed that in adults the east had the lowest proportion of females (E: 39.3%, N=742) compared with the other areas (NW: 46.7%, N=359; SW: 51.6%, N=591; R: 46.7%, N=161; χ^2=45.8, 3df, p = 0.0000; see table 4). No statistically significant difference was found in juvenile possums (χ^2=4.83, 3df, p = 0.185). The percentage of males within adult possums decreased from 59.2% (N=901) in early to 52.8% (N=724) in mid and 53.4% (N=669) in late summer (χ^2=14.58, 2df, p = 0.0007).

Table 4: Catch of adult possums stratified on sex and geographic area

	ADULTS				
	FEMALES		MALES		
AREA	N	row %	N	row %	TOTAL
SOUTH-WEST	591	51.6	555	48.4	1146
NORTH-WEST	359	46.7	409	53.3	768
EAST	742	39.3	1145	60.7	1887
REFERENCE	162	46.7	185	53.3	347
TOTAL	1854	44.7	2294	55.3	4148

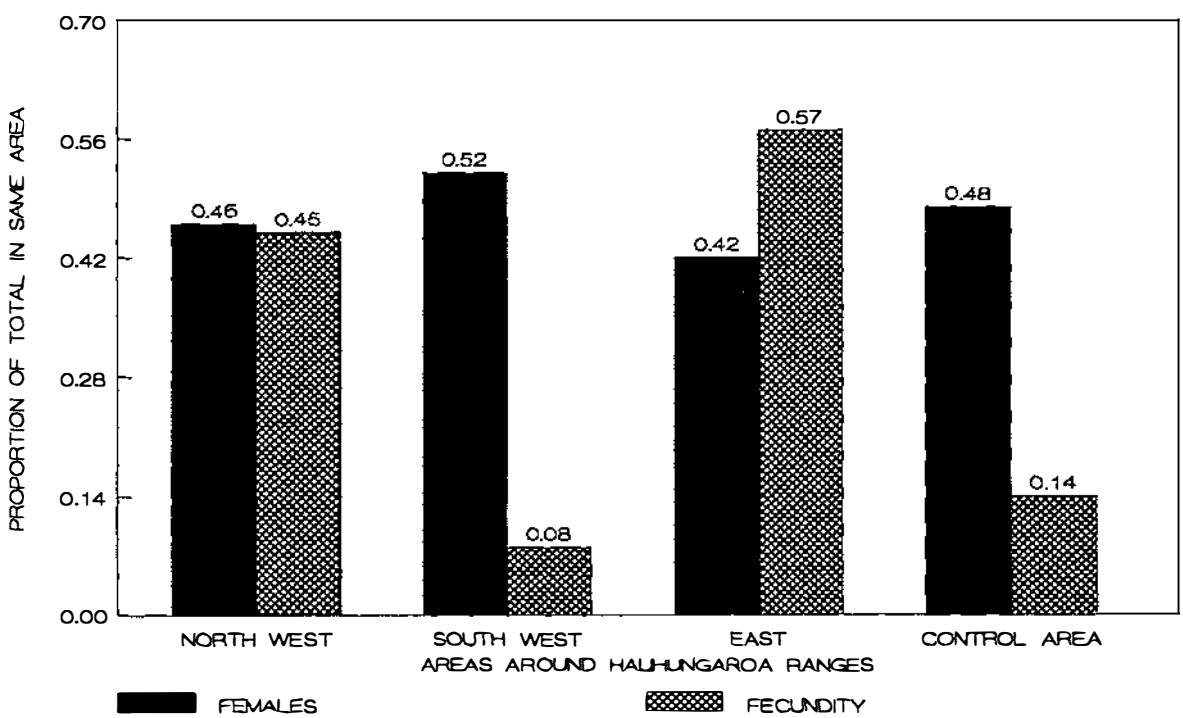
The proportion of female adult possums with pouch young was 37.6% (N=279) in the east, 27% (N=97) in the north-west, 11.1% (N=18) in the reference zone and 6.1% (N=36) in the south-west (χ^2=199.7, 3df, p = 0.0000; see table 5). The proportion of females with pouch young decreased from 39.4% (N=245) in early summer through 18.2% (N=118) in mid to 11.5% (N=67) in late summer (χ^2=145.7, 2df, p = 0.0000). Separate analyses for each area revealed that this effect was not present in the reference area and the south-west (p > 0.1). In the north-west the proportion of possums with pouch young decreased significantly from 31.6% (N=53) in early and 37.5% (N=39) in mid summer to 5.8% (N=5) in late summer (χ^2 = 27.5, 2df, p = 0.0000). In the east the proportion of female possums with pouch young decreased significantly from 42.3% (N=192) in early summer to 28.3% (N=54) in mid and 34% (N=33) in late summer (χ^2=11.9, 2df, p = 0.0026). Including only observations from mid summer the reference area and the south-west had the lowest proportion of possums with pouch young (R: 6%, N=3; SW: 7.3%, N=22) compared with north-west and east (NW: 37.5%, N=39; E: 28.3%, N=54; χ^2=100.5, 3df, p = 0.0000).

Table 5: Breeding status of female adult possums stratified by geographic area

	WITH POUCH YOUNG		NO PY, LACTATING		NO PY, NOT LACTATING		
AREA	N	row %	N	row %	N	row %	TOTAL
SOUTH-WEST	36	6.1	13	2.2	542	91.7	591
NORTH-WEST	97	27.0	66	18.4	196	54.6	359
EAST	279	37.6	149	18.9	323	43.5	742
REFERENCE	18	11.1	4	2.5	140	86.4	162
TOTAL	430	23.2	223	12.0	1201	64.8	1854

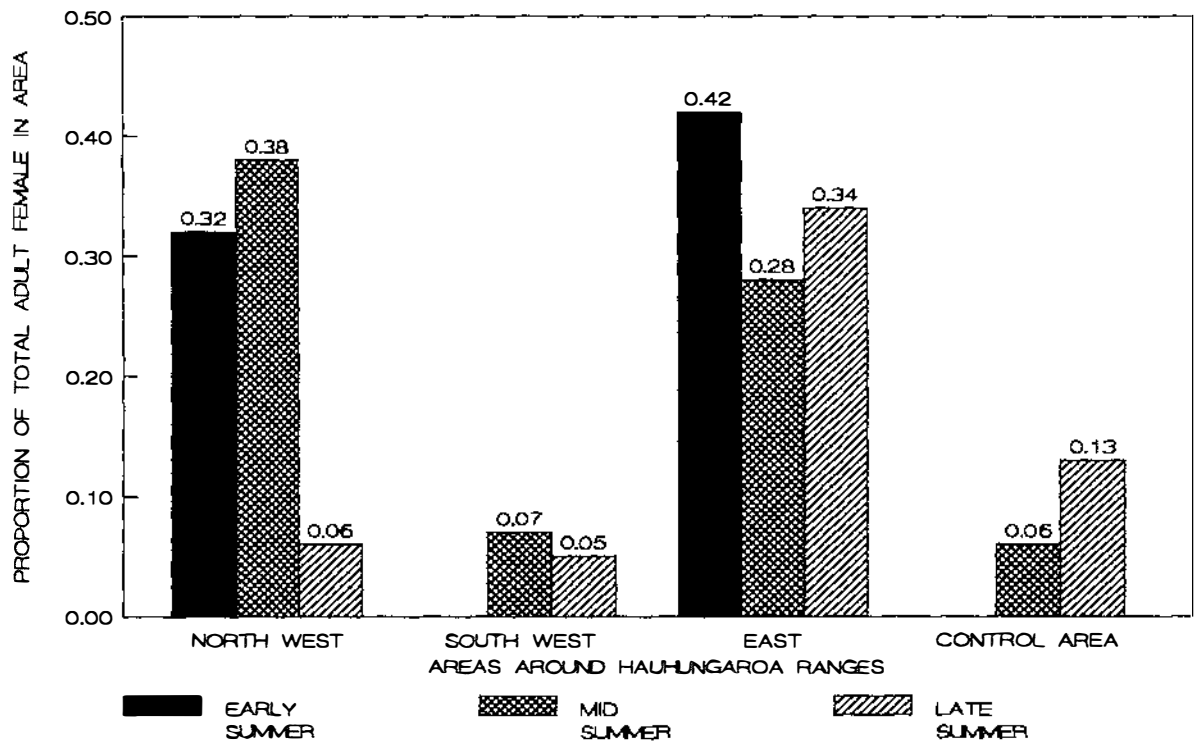
The proportion of breeding female adult possums (with pouch young or with signs of previous lactation) was 56.5% (N=419) in the east, 45.4% (N=163) in the north-west, 13.6% (N=22) in the reference zone and 8.3% (N=49) in the south-west (χ^2=384.2, 3df, p = 0.0000; see table 5 and figure 7).

Figure 7: Proportion of breeding females in total adult females and proportion of female in adult possums stratified by geographic area



Sixty-seven percent (N=417) of adult female possums were breeding (meaning presence of pouch young and/or signs of lactation) in early summer, 22.5% (N=146) in mid summer and 15% (N=90) in late summer (χ^2=422.2, 2df, p = 0.0000). Stratification on area showed that the percentage of breeding females in the reference zone was 6% (N=3) in mid and 17% in late summer (N=19; χ^2=3.5, 2df, p = 0.1703), in the east 66% (N=302) in early, 37.2% (N=71) in mid and 47.2% (N=46; χ^2=50.8, 2df, p = 0.0000) in late summer, in the south-west 10.2% (N=31) in mid and 6.3% (N=18; χ^2=3.08, 2df, p = 0.2146) in late summer and in the north-west 68.5% (N=115) in early, 39.4% (N=41) in mid and 8.1% (N=7; χ^2=86.5, 2df, p = 0.0000) in late summer (see figure 8).

Figure 8: Proportion of breeding females in total adult females stratified by geographic area and part of summer



The proportion of breeding females captured with a pouch young was 58.3% (N=243) in early, 80.1% (N=117) in mid and 74.4% (N=67) in late summer (χ^2=26.6, 2df, p = 0.0000). In the 2 areas where data was available for all three summer categories the results are as follows: In the north-west, 44.4% (N=51) of breeding females were carrying a pouch young in early summer, 92.7% (N=38) in mid and 71.4% (N=5) in late summer (χ^2 = 29.4, 2df, p = 0.0000). In the east 63.6% (N=192) of breeding females were captured with a pouch young in early summer, 76.1% (N=54) in mid and 71.7% (N=33) in late summer (χ^2=4.64, 2df, p = 0.0982). There was a statistically significant correlation between proportion of breeding females in total adult females and the proportion adult females in total adult possums on a line basis (r = -0.17; p < 0.05).

Analysis of the weight-length relationship was extended by the inclusion of age class, sex and part of summer as additional classification variables in a analysis of covariance. Only age class contributed significantly to a reduction in variance. The final model for all animals except females with pouch young explained 76.4% of the variation in logarithmically transformed weight ($F_{9,5629}$=2032.61, p = 0.0000). It included logarithmically transformed body length (ln *L*), area and its interaction term with ln *L*, age class and its interaction term with ln *L*. The slope of the average growth curve was steeper for immature possums (b = 2.61 +/-0.05, t=52.98, p=0.0000) compared with mature animals (b = 1.97 +/- 0.05, t=40.67, p = 0.0000). A separate model was fitted to the data for mature animals only. The final model included ln *L*, area, sex, part of summer and their interaction terms with ln *L* (R^2 = 0.386, $F_{13,3695}$=178.33, p = 0.0000). Slopes were different for adult males (b = 2.08 +/- 0.07, t=31.41, p=0.0001) and adult females (b = 1.71 +/- 0.08, t=21.41, p = 0.0001).

Analysis of proportion of breeding females in total adult females per subzone did not show a significant association with any of the different density estimates. The rank correlation varied between -0.07 for OTIS and CATCH, -0.10 for LESLIE, -0.11 for PELLET and -0.24 for BATCHELER (N=33; p > 0.1).

Comparison of Population Density Indices

The estimates for the different population density indices are shown in figures 9a and 9b. Note that the subzones shown in the graphs are not contiguous.

Figure 9a: Possum population density indices based on PELLET and BATCHELER

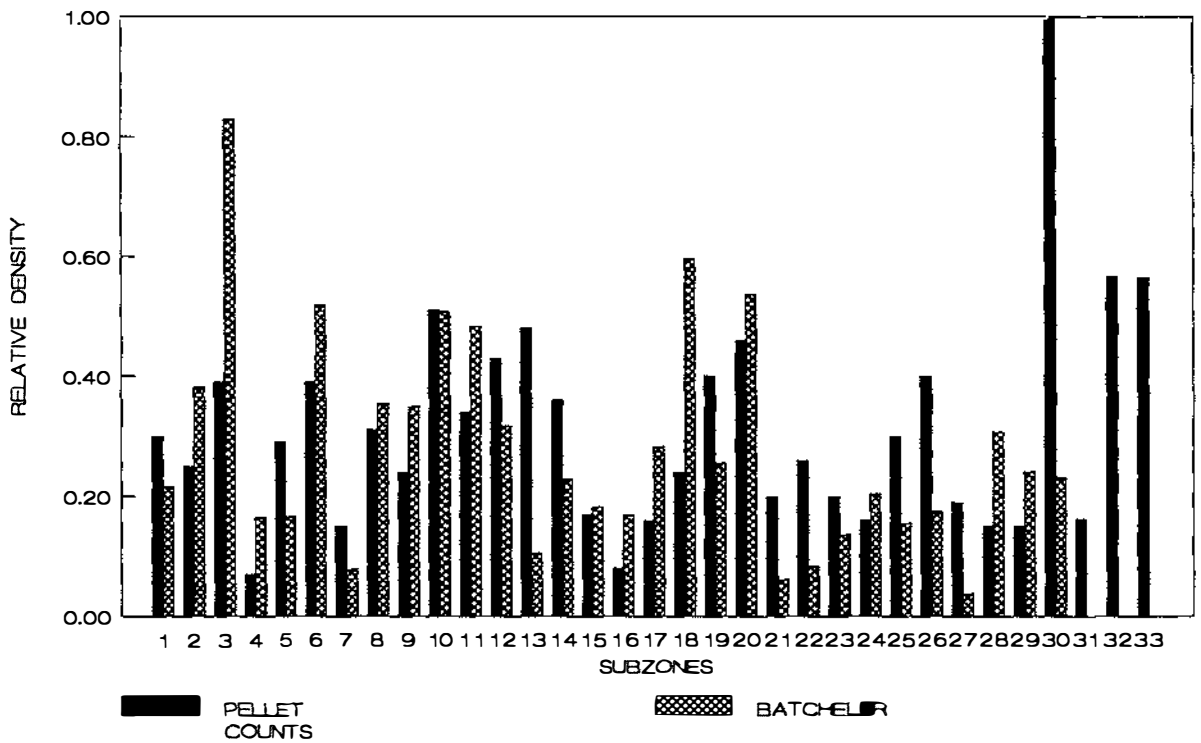
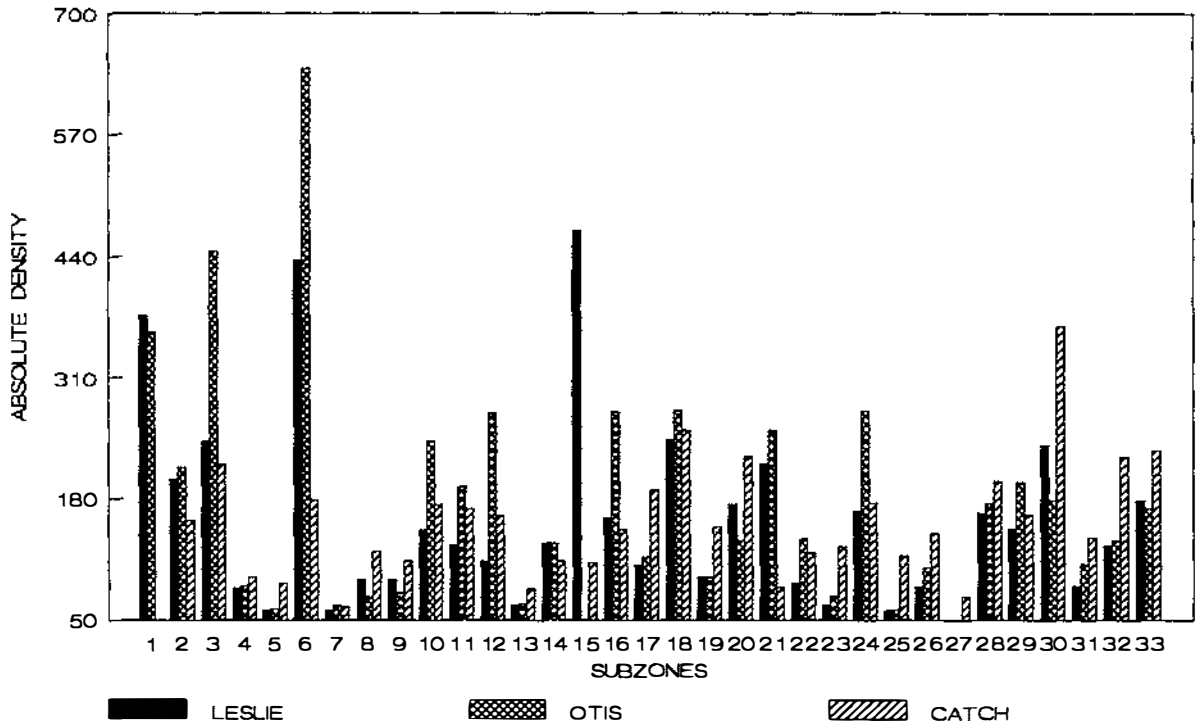


Figure 9b: Possum population density indices based on OTIS, LESLIE and CATCH



There was a statistically significant rank correlation coefficient of 0.88 between the estimates of OTIS and LESLIE (N=32, $p < 0.01$) and of 0.77 between BATCHELER and CATCH (N=33, $p < 0.01$). The rank correlation between LESLIE and BATCHELER was 0.5264 ($p < 0.01$). The coefficients of the other correlations between density indices were less than 0.5. The mean population density per trap line for PELLET was 0.31 (+/- 0.21), for BATCHELER 0.29 (+/- 0.19), for LESLIE 160 (+/- 103), for OTIS 184 (+/- 127) and for CATCH 151.7 (+/- 66.4). The total number of trappable possums around the base lines derived from LESLIE was 5121, from OTIS 5710 and from CATCH 5006. The average of number of trappable possums was lower in the reference zone and the east (LESLIE: 135.9 +/- 56.6, N=14; OTIS: 150.6 +/- 62.6, N=14) than in the north- and south-west (LESLIE: 178.7 +/- 127.5, N=18; OTIS: 211.9 +/- 159.2, N=17; for LESLIE: $F_{1,30}=1.36$, $p = 0.2525$; for OTIS: $F_{1,29}=1.83$, $p = 0.1858$). The density estimates based on trapping data (BATCHELER) gave the lowest figure for the east (E: 0.20 +/- 0.13, N=12), the south- and north-west in the middle (SW: 0.32 +/- 0.17, N=9; NW: 0.34 +/- 0.23, N=9) and the highest for the reference zone (R: 0.43 +/- 0.23; $F_{3,29}=1.85$, $p = 0.1608$). The density estimates derived using CATCH were highest for the reference zone (198.7, +/- 51.8, N=3) and lowest for the north-west (116.9, +/- 61.96, N=9). The south-west and the east ranged in the middle with 156.2 (+/- 50.4, N=9) and 162.7 (+/- 77, N=12) respectively.

Possum Tuberculosis

Post mortem examinations were done on all 6083 possums. Samples for confirmatory laboratory examination were taken from 142 (2.4%) of possums considered suspect for

tuberculosis. Of these animals 128 (2.1% out of total) had been tentatively diagnosed as being infected with *Mycobacterium bovis* based on post mortem findings. A total of 76 possums was diagnosed as infected with *Mycobacterium bovis* histopathologically and by detection of acid fast organisms. One of these animals had not been suspected to be infected with *Mycobacterium bovis*. The TB prevalence estimate based on the total sample of 6083 possums is 0.0125 with a 95% confidence interval from 0.009 to 0.015. 55.3% (N=42) of diseased animals had "open" lesions.

During the Hohotaka possum survey tissue samples from 26 possums with TB suspect lesions had been collected and submitted for diagnostic examination to MAF Animal Health Laboratory Wallaceville (Anon. 1989). Taking the culture result as the definitive measure of disease status, smear and histological examination together achieved a sensitivity of 65% and a specificity of 100%. Using these figures true TB prevalence in the Hauhungaroa survey would be 0.0192 (1.9%), if the samples had been cultured for the presence of *Mycobacterium bovis*. The 95% confidence interval of this estimate reaches from 0.016 to 0.023.

Tuberculous possums were found in 51.5% (N=17) of 33 subzones. No evidence of tuberculosis was detected in the sample taken from zones 3, 4 and 11 (reference zone).

Epidemiology of Tuberculosis in Possums

Differences in Condition, Breeding Status, Sex and Age Class

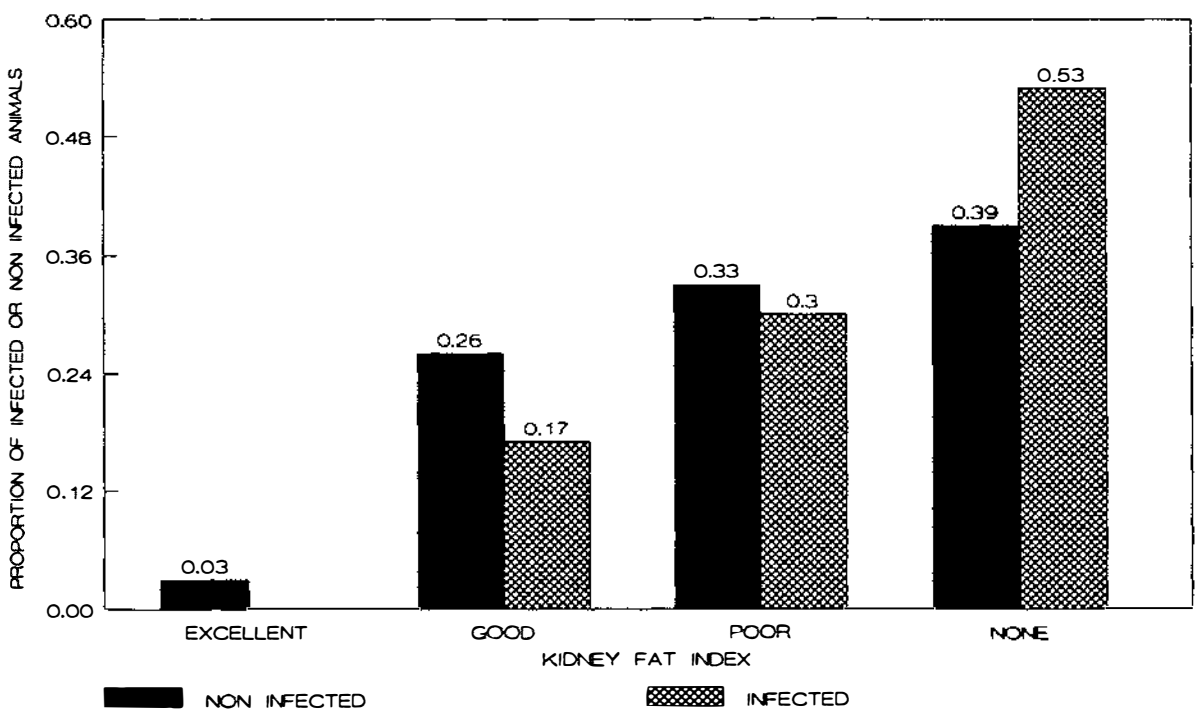
To reduce bias due to confounding habitat factors in the following paragraphs, only animals from trap lines where TB infected individuals had been captured were included in the statistical analysis.

Sixty-three percent (N=48) of possums with tuberculous lesions were of male sex. Adult possums were 1.9 times as likely to be infected as immature possums (χ^2=4.16, 1df, p = 0.0413). Prevalence was 0.054 in males and 0.039 in females. Adult female possums were 3.64 times more likely to be infected than immature female animals (χ^2=4.1, 1df, p = 0.0429). Immature male possums were more likely to be infected than immature females (OR = 3.12; χ^2=3.17, 1df, p = 0.0751). Five percent (4.9%, N=25) of adult females and 5.8% (N=38) of adult males were infected (χ^2=0.46, 1df, p = 0.4974). For both age classes average body length controlled for sex was statistically significantly different between tuberculous and non-tuberculous animals ($p < 0.01$; see table 6). TB prevalence was higher in breeding females than in non-breeding adult females, but the difference was not statistically significant (OR = 1.99; χ^2=2.13, 1df, p = 0.1441). It increased from 0.035 (N=286) in non-breeding individuals, 0.054 (N=92) in animals without pouch young but signs of lactation to 0.074 (N=135) in possums with pouch young (χ^2=3.1, 2df, p = 0.212). Using multiple logistic regression it was found that controlling for age class, possums with a kidney fat index of none or poor were 2.29 times as likely to be found infected as were animals with an index of excellent or good (95% confidence interval: 1.29 - 4.17; Likelihood Ratio Statistic for Model fit: χ^2 = 13.23, p = 0.0013; see figure 10).

Table 6: Average body length and tuberculosis infection status

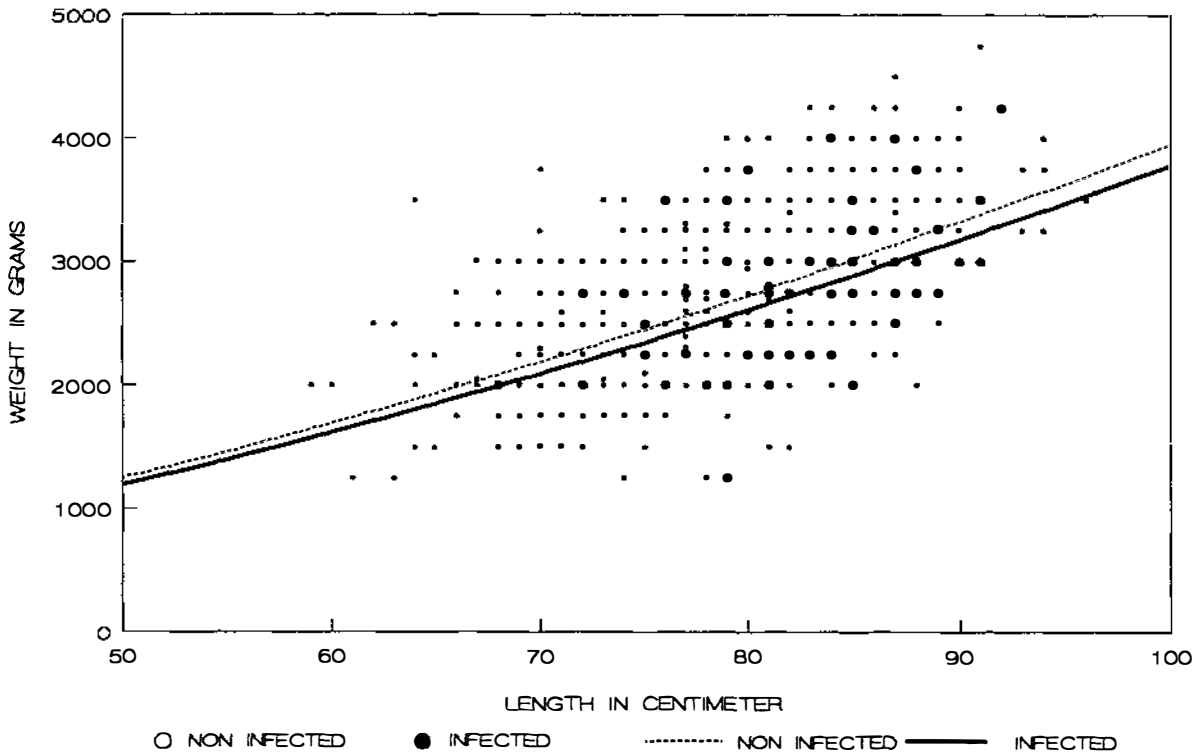
	IMMATURES			MATURES		
INFECTION STATUS	MEAN	SEM	N	MEAN	SEM	N
TB	726.5	18.1	10	824.0	6.57	62
NON - TB	690.3	3.8	230	790.4	1.58	1107
ALL	691.8	3.76	240	792.2	1.55	1169
TEST STATISTIC	F=5.62, 1df	p=0.0181		F=24.19, 1df	p=0.0001	

Figure 10: Tuberculosis infection status and kidney fat index



The slope of the weight-length relationship (controlling for age class, sex and part of summer) was statistically significantly different between infected possums (b = 1.74 +/- 0.28) and non- infected animals (b = 2.23 +/-0.07; R^2=0.76, $F_{11,1477}$=437.9, p = 0.0000; see figure 11). Logarithmic transformation of both body weight and length did not improve model fit.

Figure 11: Tuberculosis infection and weight-length relationship in adult possums



Comparison between Geographic Areas and Part of Summer

Prevalence of TB infection in possum populations using all available data in the north-west and the east was 3.0% (N=34) and 1.5% (N=40) respectively. It was statistically significantly higher than the prevalence of 0.1% (N=2) in the south-west (χ^2=41.94, 2df, p = 0.0000). Restricting the sample to the trap lines where infection was present, tuberculosis prevalence was 9.8% in the north-west, 3.9% in the east and 0.8% in the south-west (χ^2=28.18, 2df, p = 0.0000). Around trap lines with presence of tuberculosis infection, prevalence in immature possums increased from 0.015 in early, and 0.038 in mid to 0.0417 in late summer (χ^2=2.42, 2df, p = 0.2988). In adults prevalence was 0.057 in early, 0.056 in mid and 0.041 in late summer (χ^2=0.68, 2df, p = 0.7104). During early and mid summer there was a higher prevalence in males (0.064, N=34) than there was in females (0.053, N=22, χ^2=0.48, 1df, p = 0.4888). In the south west of the Hauhungaroa Ranges the average proportion of females among total adult possums was higher around trap lines where infection was present (0.64 +/- 0.25) than around those free of infection (0.50 +/- 0.31; t=1.91, 243df, p = 0.0590). In the same area within trap lines with infection there was no significant difference in average proportion of females among total adult possums between infected and non infected trap groups (t=0.54, 19df, p=0.5965). For trap lines with infection present, average proportion of breeding animals in total number of adult females on the basis of aggregated data for groups

of 10 neighbouring traps was 0.62 (N=43) if infection was present and 0.47 (N=149) if infection was not present (t=2.05, 190df, p = 0.0427).

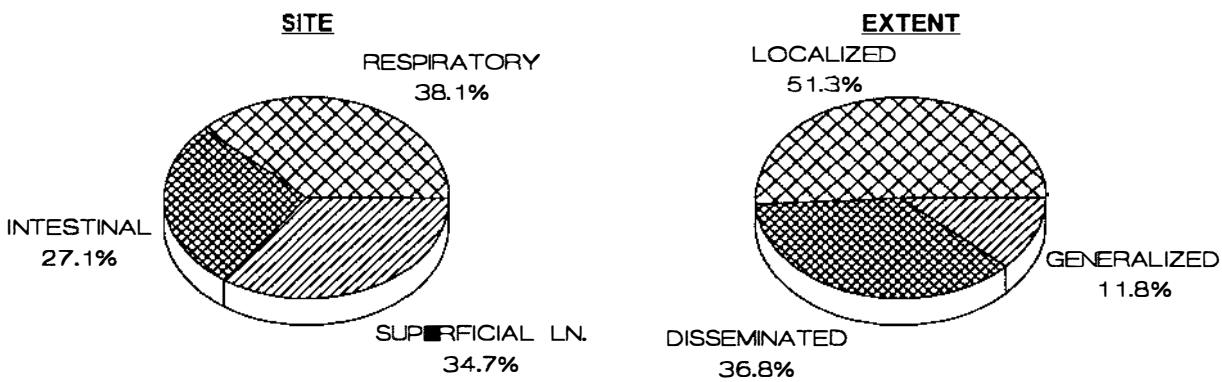
Average tuberculosis prevalence within groups of 10 traps where infection was present was statistically significantly higher if animals with open lesions were found (open lesions: 0.36 +/- 0.29, N=33; closed lesions: 0.22 +/- 0.15, N=22; t=1.99, 51df, p = 0.0515). There was a positive correlation between the proportion of open lesioned possums among total animals captured and tuberculosis prevalence (r = 0.78, $F_{1,53}$=80.8, p = 0.0000) within groups of 10 neighbouring traps with infection present.

The strength of correlation between possum tuberculosis prevalence and none of the different population density indices was statistically significant.

Distribution of Lesions in Tuberculous Possums

Lesions found during post mortem examination were grouped according to location within the body of the animal. Spread within the body was estimated according to the different locations macroscopically identified as being involved in the disease process. Of diseased animals 59% (N=45) had lesions in the respiratory tract (lungs and/or mediastinal lymph nodes), 42% (N=32) in the intestinal tract (intestines and/or abdominal organs and/or mesenteric lymph nodes) and 54% (N=41) in the superficial lymph nodes (axillary and/or inguinal lymph nodes; see figure 12). Fifty-five percent (N=42) of tuberculous possums had "open" lesions. Lymph node tissue was involved in the disease process in 77% (N=59) of cases.

Figure 12: Spread of disease within body in tuberculous possums



In 21% (N=16) of infected possums macroscopic pathological changes were found only in the superficial lymph nodes, in 17% (N=13) only in organs of the respiratory tract, in 17% (N=13) only in the intestinal tract, in 18% (N=14) in respiratory tract and peripheral lymph nodes, in 12% (N=9) in respiratory and intestinal tract and in 11% (N=8) the disease was generalized (see figures 13a and 13b). Thirty-seven percent (37.5%, N=6) of animals with only lesions in superficial lymph nodes had open sinuses. If the disease process was localized, 48.7% (N=19) had lesions only in the local lymph nodes. Of 42 animals with only

single lesion sites 38.1% (N=16) had lesions in the superficial lymph nodes, 13% (N=13) in the respiratory tract and 13% (N=13) in the abdominal tract (see table 7).

Table 7: Distribution of single lesion sites

	IMMATURES		MATURES				
			MALES		FEMALES		
SITE OF LESION	N	%	N	%	N	%	TOTAL
RESPIRATORY	2	25.0	7	22.6	4	30.7	13
ABDOMINAL	3	37.5	5	23.8	5	38.5	13
SUPERFICIAL	3	37.5	9	42.9	4	30.7	16
TOTAL	8	100	21	100	13	100	42

Figure 13a: Spread of disease within body in tuberculous possums stratified by age class

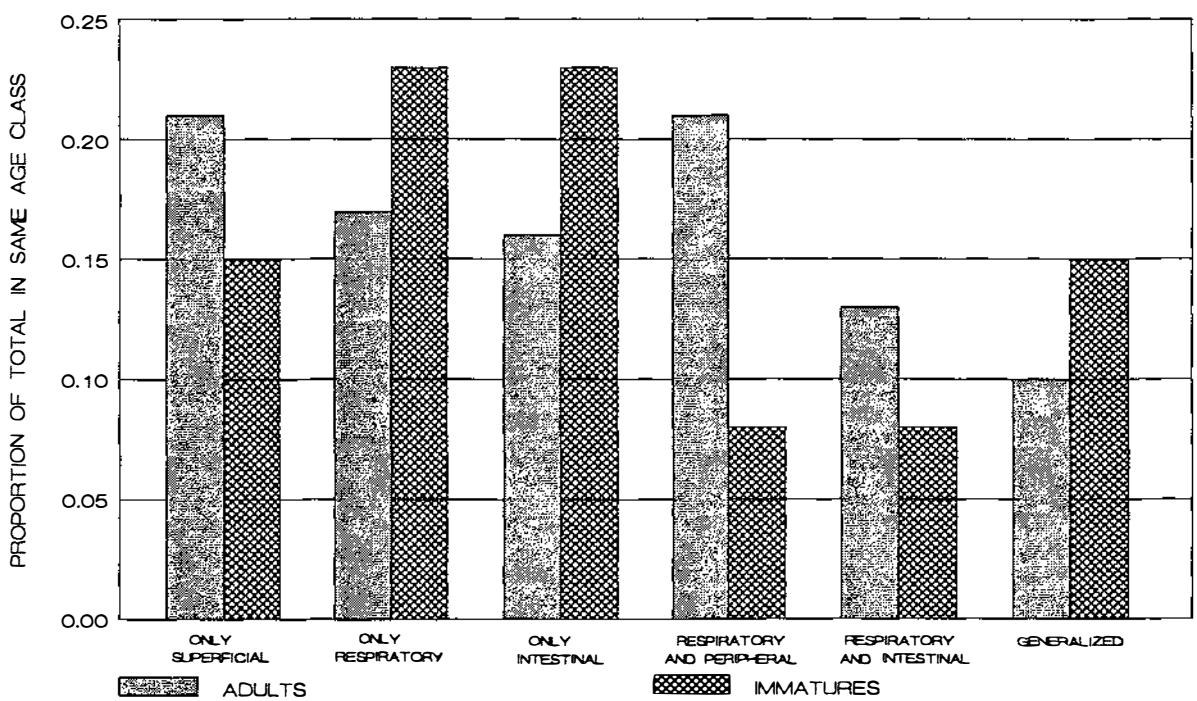
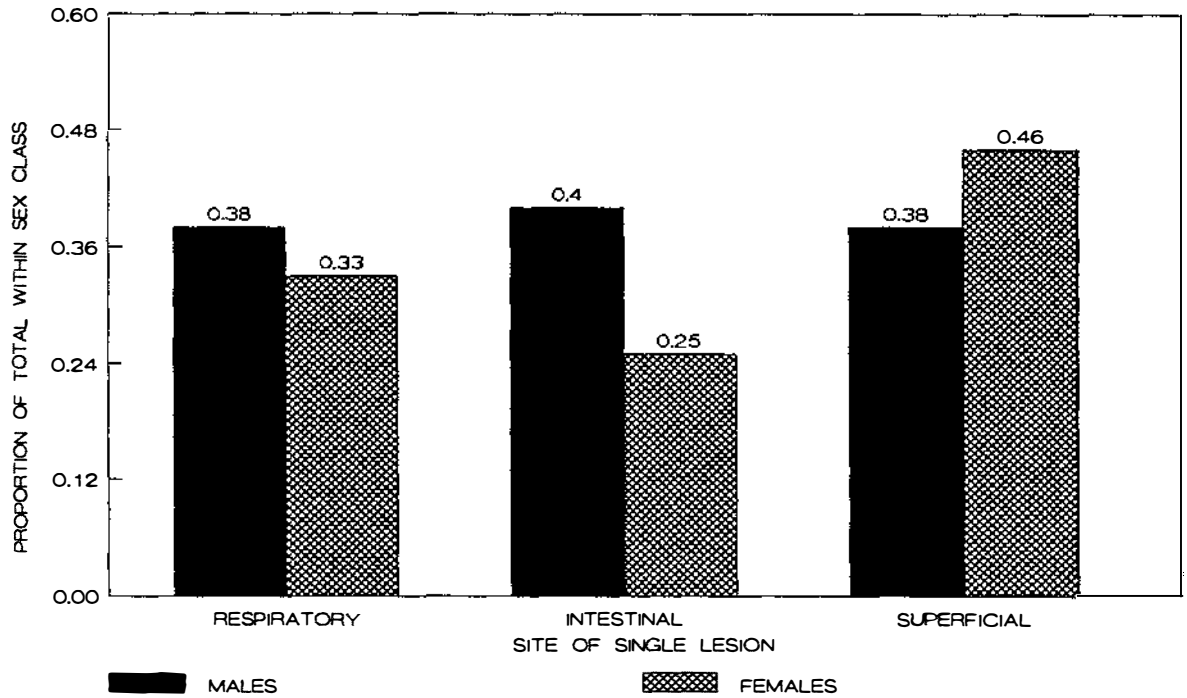


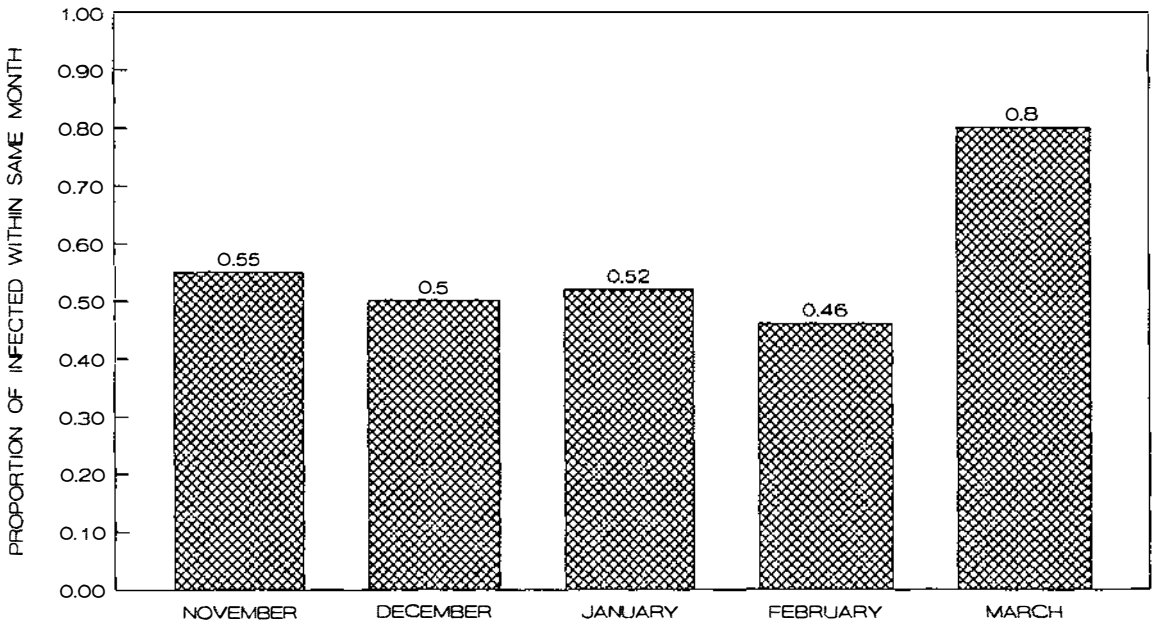
Figure 13b: Proportion of single site lesions stratified by sex class



In 87% of animals with generalized tuberculosis (N=8) kidney fat index was poor or nil. Of animals (N=13) with only the respiratory tract involved in the disease process, 69% had a kidney fat index of poor or nil. If only the intestinal tract was involved, it was 77% (N=13) and if only peripheral lymph nodes were involved, all animals (N=16) had a kidney fat index of poor or nil. The further disseminated the disease within the body the higher was the probability of finding open lesions (Chisquared test for linear trend: χ^2=4.66, p = 0.0308). In animals with open lesions condition was worse if the disease process was further disseminated.

In 50% (N=5) of infected male immature possums the disease process was already disseminated or generalized. In all female immature infected possums (N=3) only localized lesions were found, and there was no further spread within the body in any case. Cases with involvement of superficial lymph nodes were more prevalent in infected immature males (60%, N=6) than in immature females (33.3%, N=1, Fisher's exact test of homogeneity: p = 0.3671). Lesions in the respiratory tract were equally frequent in both sexes. Intestinal tract lesions are more common in adult female infected possums (48%, N=12) than in adult males (34.2%, N=13,). In early and mid summer the disease process was disseminated or generalised in 47% of the cases (N=31) and in late summer in 60% (N=6; p = 0.3343). In early and mid summer 51% (N=34) of tuberculous possums had "open" lesions and in late summer 80% (N=8; Fisher's exact test of homogeneity: p = 0.1703; see figure 14).

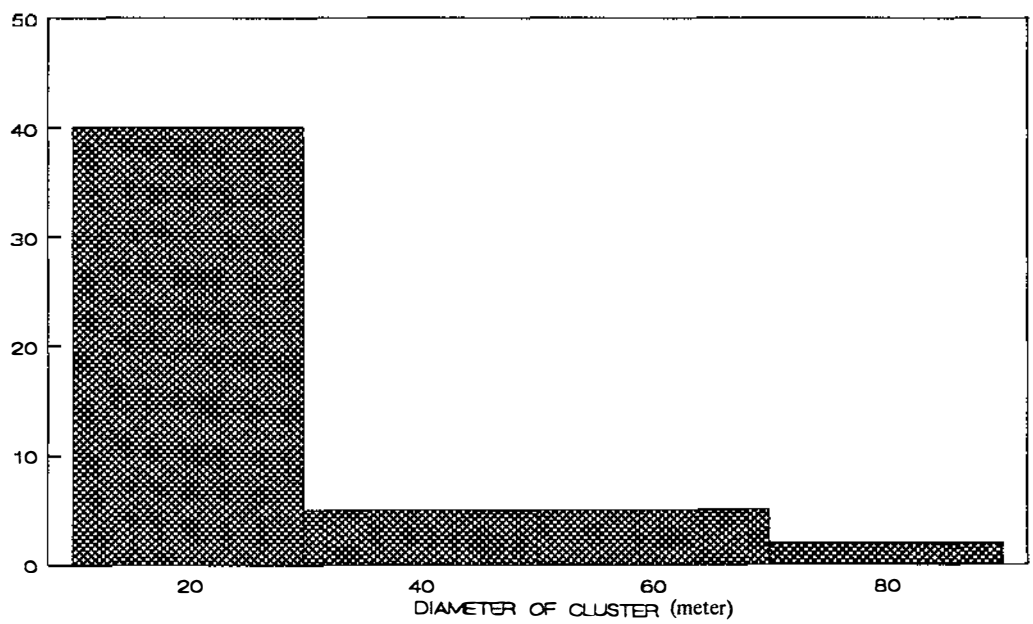
Figure 14: Proportion of possums with "open" lesions stratified by month



Spatial Patterns of Infection

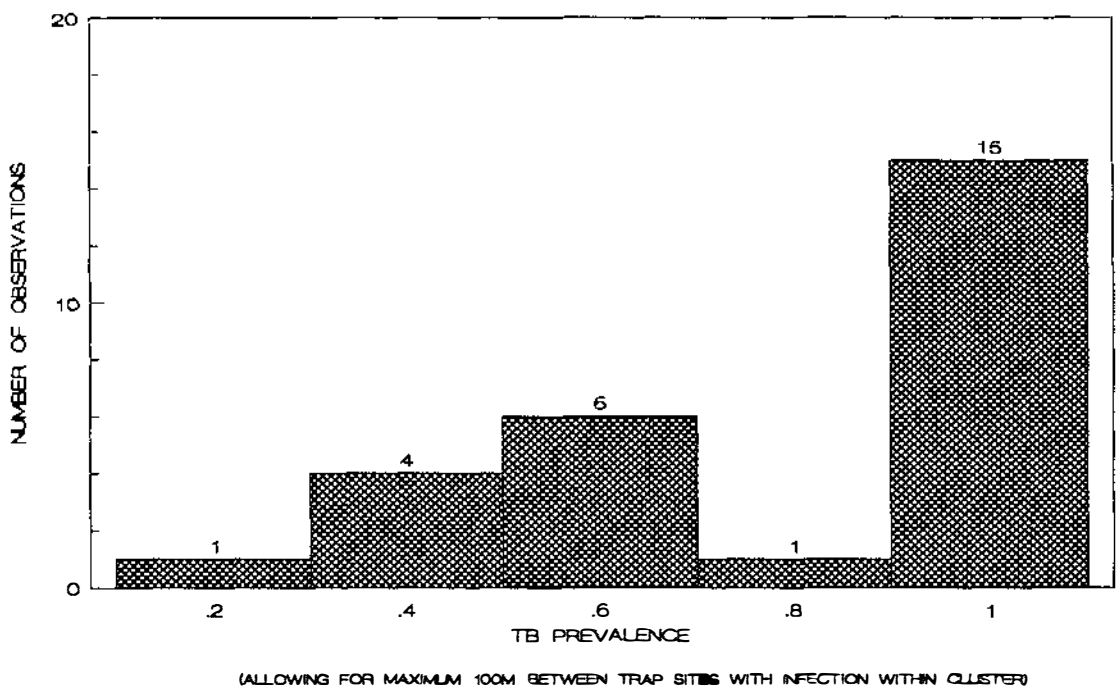
Infection was detected at 71 of 557 trap sites where possums were captured. Forty-one of these trap sites were located on the base trap lines. Of these, 30 trap sites with an infected trap site adjacent on the same line were included into distance calculations between local clusters of *Mycobacterium bovis* infection. The average distance between trap sites with TB infection present was 400.7m (+/- 781.4). In a frequency distribution of the distance between trap sites 63.3% (N=19) of the traps with infected possums were within a distance of about 200m. 43.3% (N=13) of the trap sites with infected possums were within a distance of 100m. Note that in the following calculation of clusters of TB infection it is not known whether the trap lines did cross a particular cluster right through the middle or if they just touched it on the outer edge. Therefore the figures may misrepresent the true situation. For estimation of size of clusters of TB infection a maximum distance of 100m from one trap with infection to the next trap with infection was allowed. Sets of contiguous trap sites with tuberculosis infection with a maximum of 100m between trap sites with infection were considered local clusters of infection. The distance between the traps at both ends of the clusters was used as an estimate of cluster size. Based on this calculation, 27 clusters of TB infection were detected along the trap lines and the average size of a cluster was 32.6m (+/- 24.8; see figure 15).

Figure 15: Histogram of size of clusters of tuberculosis infection



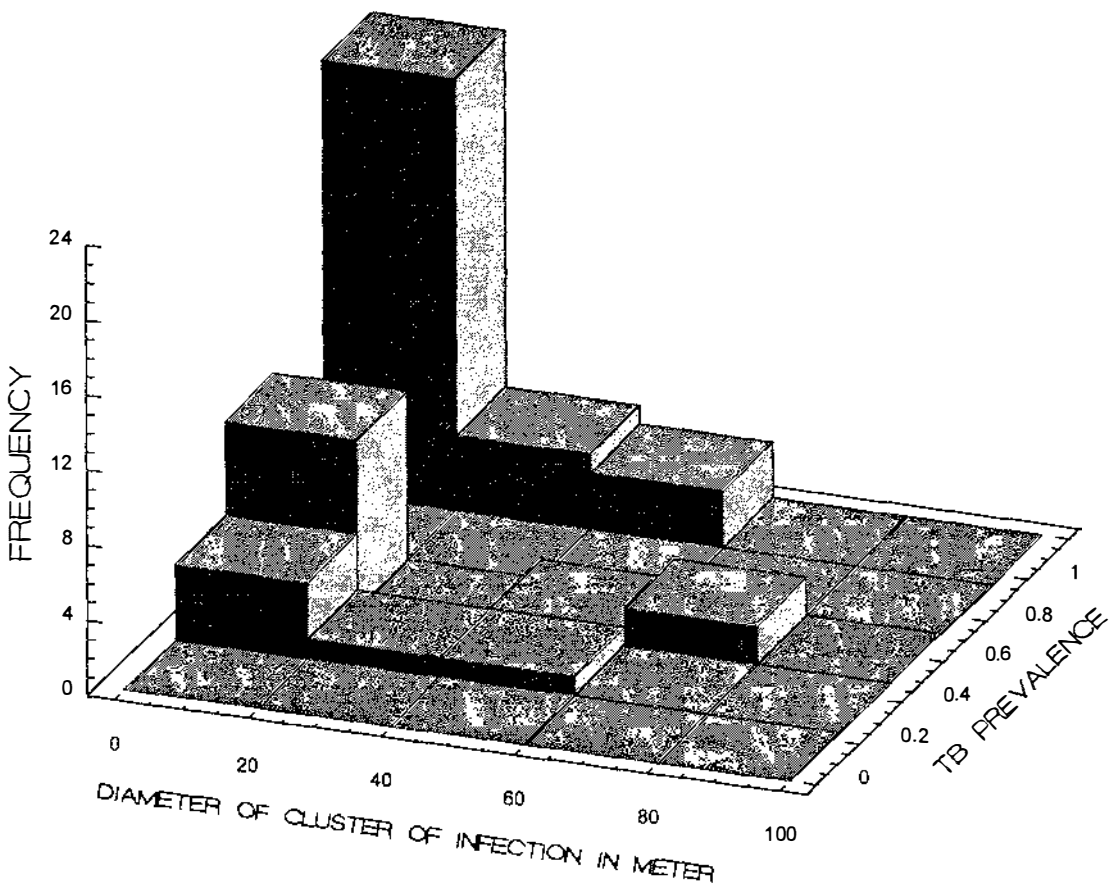
Seventy-four percent (N=20) of the clusters consisted only of one trap site. One cluster covered an area of at least 120m. Average prevalence within clusters of infection was 0.76 (+/- 0.29). 55.6% (N=15) had a prevalence of 100% and 40.7% (N=11) had a prevalence between 0.20 and 0.50 (see figure 16).

Figure 16: Histogram of tuberculosis prevalence within clusters of infection



Average prevalence of cases with open lesions was 0.30 (+/-0.35). In 44.4% (N=12) of the clusters no animal with open lesions was found. There was no statistically significant correlation between prevalence measures and cluster size (see figure 17).

Figure 17: Tuberculosis prevalence within clusters of infection and size of cluster



Average distance between trap sites with TB infection was shorter if animals with open lesions had been caught in a particular trap (150.7m +/- 39.6, N=28) than if only animals with closed lesions were identified (707m +/- 284, N=14; t=2.71, 14df, p = 0.0169).

Possum and Cattle Tuberculosis

Cattle tuberculosis infection was present in 93% (N=28) of 30 subzones. In 46.4% (N=13) of the subzones with cattle infection no evidence of tuberculous possums was found. Mean cattle tuberculosis incidence was significantly higher in subzones where possum tuberculosis was present (0.087 +/-0.05, N=15) compared with subzones where no tuberculous possums had been captured (0.03 +/-0.04, N=15; $F_{1,24}$=15.14, $p = 0.0007$). Figure 18a displays the spatial distribution of cattle tuberculosis incidence and possum tuberculosis prevalence overlaid on a contour map with farm and trap line locations.

There was a statistically significant correlation coefficient between average possum tuberculosis prevalence per subzone and average cattle tuberculosis incidence on adjacent properties in the years 1982/1983 ($r= 0.44$, $p < 0.05$). Exclusion of one observation with extreme residuals resulted in a reduction of the correlation coefficient ($r=0.36$, $p < 0.05$; see figure 18b).

Figure 18a: Contour map of the Hauhungaroa Ranges with cattle and possum tuberculosis information, farm and trap line locations

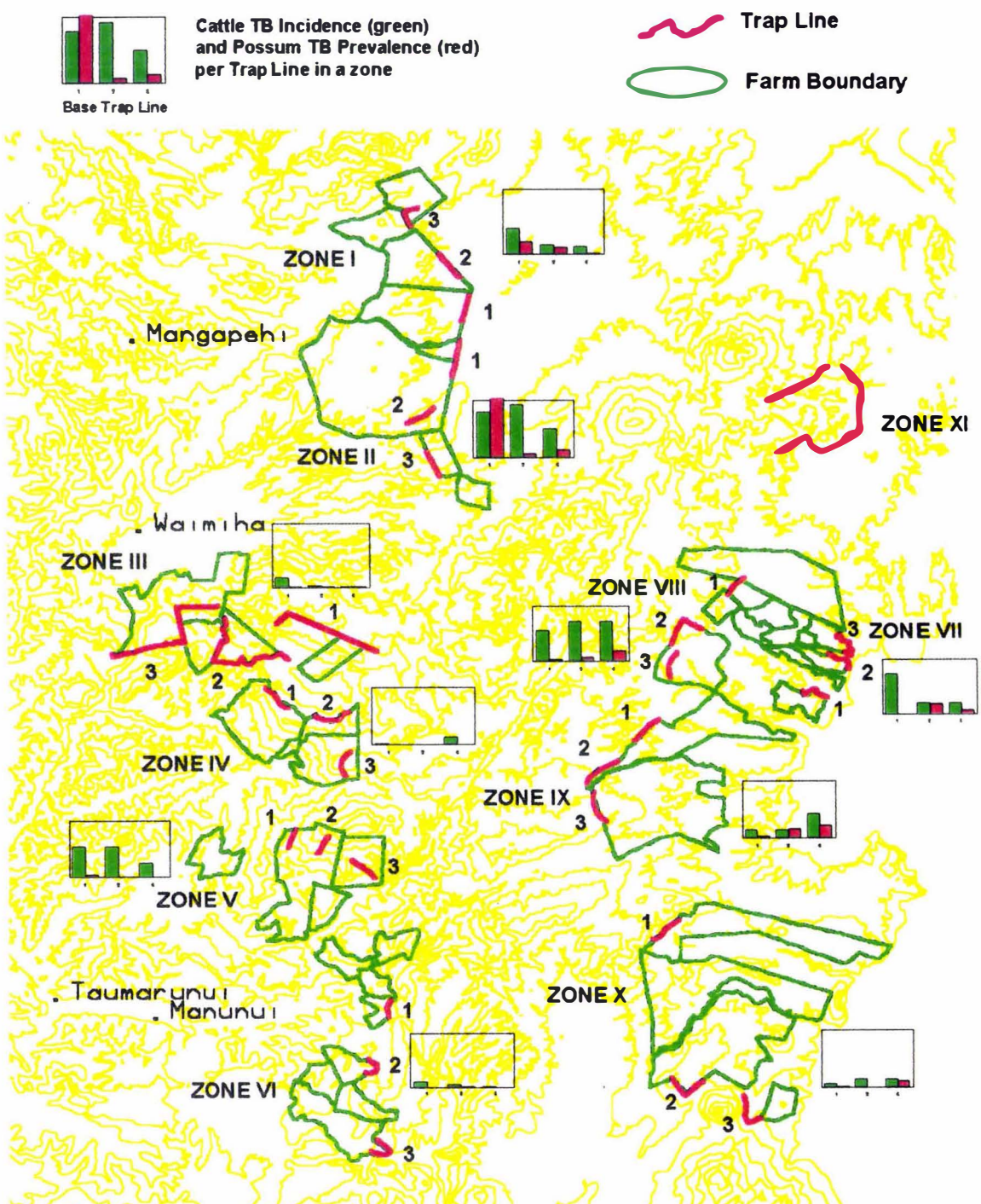
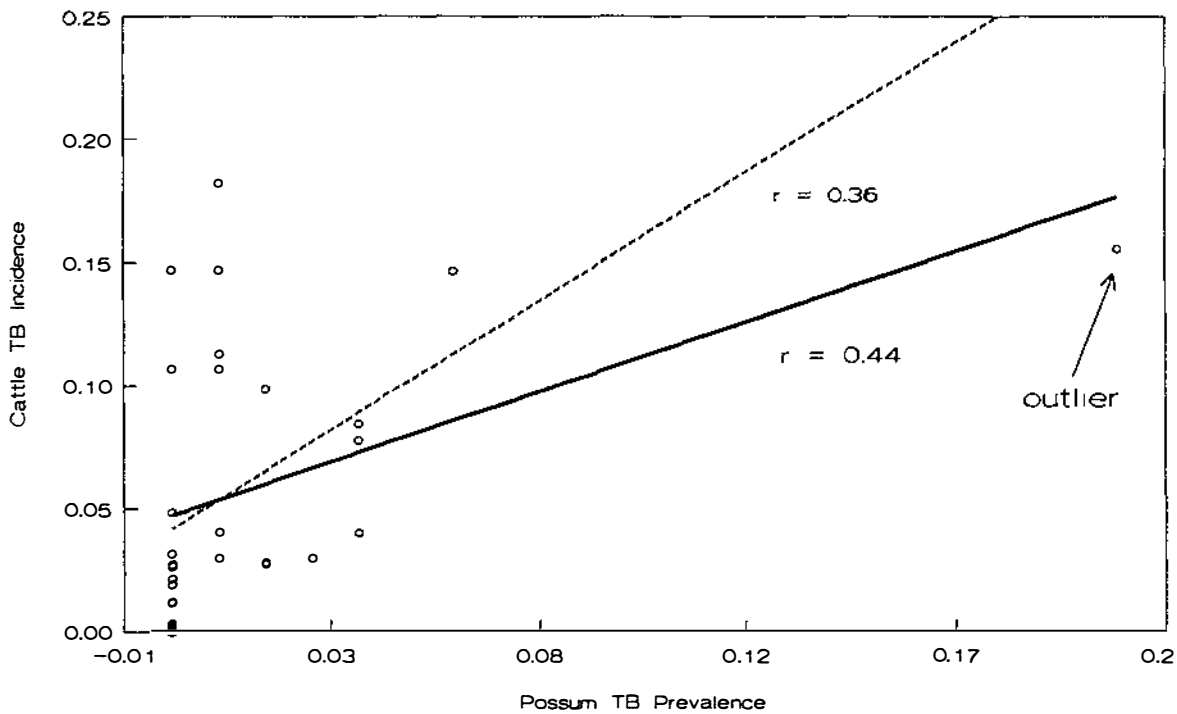
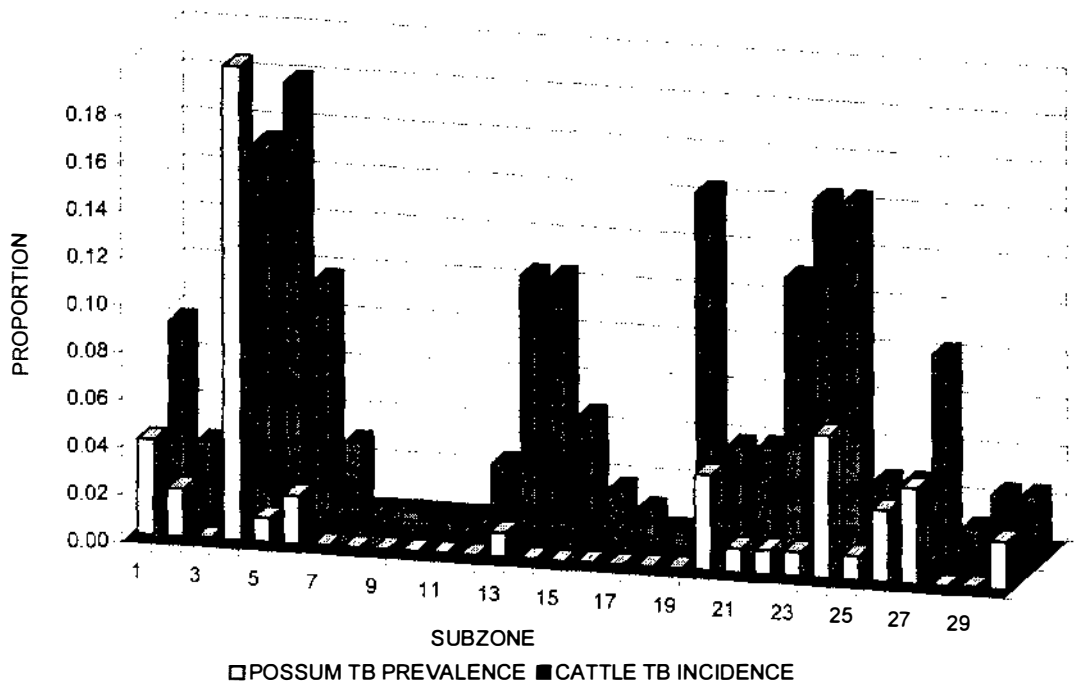


Figure 18b: Correlation between cattle tuberculosis incidence and possum tuberculosis prevalence



Cattle tuberculosis incidence rate was positively correlated with fecundity in the geographically associated possum population ($r=0.6$, $p < 0.05$; see figure 19).

Figure 19: Possum tuberculosis prevalence and cattle tuberculosis incidence by subzone



(*note* that the subzones are not contiguous)

DISCUSSION

Limitations on Interpretation

This study had been carried out some years ago but not comprehensively analyzed, although sufficient information was still accessible to allow most analyses to be completed. During the data analysis it became clear that the study design made it impossible to eliminate confounding between certain important variables in the analysis. This limits the weight which can be given to some of the results, since they may not accurately reflect field differences. There appears to be a strong seasonal influence on the ecology of possums and probably also on the epidemiology of *Mycobacterium bovis* in possum populations. Data was collected in 11 different zones at different times of the year, and this made a comparison between geographical areas difficult since area and time of year are seriously confounded.

When the study was underway it was realized that it was not possible to catch sufficient tuberculous possums on the main trap lines. It was decided that additional traps had to be placed around the main lines. Because of the way this was done, it was not possible to use this additional information for analysis of possum population density or spatial clustering of the disease, as no information on trap spacing and catch effort was available. Thus while the majority of the findings reported represent a valid assessment of a cross-sectional ecological and epidemiological study of a tuberculous possum population, these design limitations must be borne in mind in interpreting the data.

The diagnosis of *Mycobacterium bovis* infection was based on the presence of acid fast organisms and histopathological examination. However, it is recognized that a totally reliable diagnosis of tuberculosis requires isolation and identification of the organism from exudates, body discharges, or from lesions (Thoen 1984).

Ecology of Possums in the Study Area

Comparing figures for adult females it appears that on average possums in the Hauhungaroa Ranges (2640g; 789cm) were slightly longer and heavier than in the Orongorongo Valley (2320g; 757-794) (Bell 1981). Average body weight was similar compared to that reported from a study in the Central King Country (2610-2790g) and lower than in an area at Mt.Misery in Nelson Lakes National Park (2821-3118g) (Clout and Gaze 1984, Cowan and Rhodes 1988).

Bamford described the weight-length relationship in a sample of 200 possums from Styx valley, Westland, with the equation $W = 0.0125 * L^{2.81}$. He found no difference between sexes (Bamford 1970). Fraser fitted equations with different slopes and constants to weight-length data from low, moderate and high density populations in a survey in Copland valley, Westland. In the Hauhungaroa survey the equation for data from eastern areas had the flattest slope which suggests that these could be high-density areas with well-established populations and limited food resources. In contrast, the western parts and the reference area

may represent lower population densities. Fraser also observed a difference in slope between sex classes and explained this by a better utilisation of food resources by males (Fraser 1979).

In total, 46% of possums in the sample were females. A dominance of males has been reported from earlier studies in various areas. Stratification on the 4 different geographical areas in the Hauhungaroa Ranges shows that there is marked variation in the proportion of females. It ranges from the sample being dominated slightly by females (51.7%) in the south-west of the Hauhungaroa Ranges to a minority of females in the east (41.7%). Separate analysis for adult possums reveals that 39.3% of adults in the east were females. In the same area the proportion of immatures within males was significantly lower (26.4%) than in the other three areas which average 33.7%. In the east 61.3% of the catch results originated from early summer, in the north-west 45.3% and in the other two areas trapping commenced in mid summer. Examination of the catch results for the east reveals that in early summer 40% of possums captured were females and that the proportion increased towards late summer to 46%. Dominance of males in samples from extinction trapping can be explained by their greater mobility compared with females (Coleman and Green 1984). The observation of the low proportion of immatures in the east is explainable by the fact that the majority of the trapping was undertaken in early summer when the immature possums still lived close to their mothers. In fact, in early summer 67% of all adult females showed signs of lactation or had a pouch young. Possums generally have a distinct seasonal breeding pattern, with an autumn peak of births between March-June and a spring peak in about September-November, the latter in most areas in New Zealand being less pronounced or even absent (Kerle 1984). Around the Hauhungaroa ranges there appears to be a difference in breeding pattern between areas, in that the north-west and the east have a more pronounced spring birth peak than the south-west and the reference area. This could explain the low fecundity in the last two areas, which were sampled before the autumn birth peak. In an ecological study in King Country spring breeding occurred in 47% of adult females (Cowan and Rhodes 1988). The finding that adult females in good body condition were more likely to have a pouch young has been described by Bell from the Orongorongo valley. He reported that lower growth rates delayed the onset of breeding and females in poorer physical condition bred less successfully (Bell 1981).

The above results show that there was a confounding element of both sampling season and area in the data. Variation between the different zones in catch effort with regard to time of the year when trapping was done further complicated the analysis.

The trapping data did not seem to satisfy the assumptions of the methods used for population density estimation, so that none of the measures seemed to be reliable enough to be included in any further analysis.

Epidemiology of Bovine Tuberculosis Infection in Possums

A tuberculosis prevalence of 0.0125 was found in the total sample of 6083 possums. The diagnosis was based on post-mortem findings which were confirmed by histopathological and microscopical examination for the presence of acid-fast organisms. This technique has a low sensitivity as has been shown by a comparison of these methods with bacteriological culturing based on diagnostic results from another possum tuberculosis study (Forest Research Institute 1989). A corrected prevalence of 0.02 assuming a diagnostic sensitivity of 65% seems to be a more reliable estimate. An evaluation of possum autopsies showed that 71% of possums infected with *Mycobacterium bovis* had gross lesions (Hennessy 1986). This suggests that the prevalence estimates derived from this study are likely to be an underestimate as only animals with gross lesions on post mortem had been subjected to laboratory examination. A similar prevalence figure was found in 1988, when a survey in an area adjacent to zone no.6 was conducted and a prevalence of 0.023 was estimated (Forest Research Institute 1989). Tuberculous possums were found in 46% of subzones, which suggests that disease was not clustered in a "macro" geographical sense. However, only at 13% of trap sites with possum captures were tuberculous lesions detected in animals. More than 40% of trap sites with infection were within 100m and more than 60% within 200m from another trap site with an infected possum. This indicates that tuberculosis infection is clustered at a "micro" geographical scale. Such patchiness of infection in endemic situations has been reported for fox rabies and attributed to stochastic effects in the dynamics of the disease (Sayers *et al* 1985, Mollison and Kuulasmaa 1985). The results of estimation of cluster size have to be interpreted cautiously as sampling on a single trap line provides only limited information other than frequency of clusters. Prevalence within patches of infection averaged 0.76. This estimate was probably inflated by small samples taken from clusters which were touched on the outer edge by a single trap site. Reliable information for estimation of cluster size and prevalence of tuberculosis infection requires the data to be collected on a sampling grid.

Overall prevalence was higher in adult male possums than it was in adult females. The higher prevalence in mature possums compared with juveniles could be related to a longer exposure to risk of infection, and possibly also to different mechanisms of transmission. During the early summer months prevalence was higher in adult possums than in juvenile animals. There was no such difference in mid and late summer. Prevalence in immature possums increased from early to late summer. In immature possums, bodies of infected animals were on average longer than they were in uninfected animals. Within the age class of immature possums, there was a higher prevalence in males than in females. Until early summer young juveniles (shorter body length) would have been exposed to some of the transmission factors (e.g. mating, fights) only for a relatively short time compared with older juveniles or adult animals, as they mainly interacted socially with their mothers. But during the months following independence, especially the juvenile males would be at increased risk of developing disease while trying to establish their own activity area. It is known that

immature possums, particularly of male sex, have a tendency to disperse at about 9 months and later (Clout and Effort 1984). Most of the immature possums captured in this study were probably born in around May-June 1982 (note: average age of maturation varies between 1 and 2 years of age (Gilmore 1969, Crawley 1973, Kerle 1984), so the true age of possums classified as juveniles will fall in the range of 6 to 18 months, and dispersal began in the following mid-late summer (January-April 1983). If infection is present at a "macro" geographical scale, the probability for non-infected juveniles to come in contact with *Mycobacterium bovis* organisms would be higher once dispersal commenced. During the dispersal phase a non-infected immature possum might pass through a number of different local populations, use a relatively large number of different den sites and may have a number of antagonistic contacts with resident possums. In turn, an infected juvenile could start new foci of infection during dispersal. Mollison (1987) demonstrated in mathematical simulation experiments that long-distance infectious contacts, though individually of low probability, can contribute significantly to the advance of an epidemic. It has been found in epidemiological studies of rabies in foxes that juvenile dispersal is one of the factors which ensure spread of the disease (Tinline 1988). Coleman (1988) reported similar sex-age differences from a survey in the Hohonu Ranges. But Cook (1975) did not find any sex differences within the two age classes during a survey in Buller and Inangahua Counties. In adult possums prevalence was slightly higher in males during early and mid summer than it was in females. Adult male possums were also more trappable in early summer than adult females. This suggests that adult males were more active during early summer, which resulted in an increased risk of infection. On average, tuberculous adult possums had a longer body than non-tuberculous adult possums. Body length is not an appropriate indicator of age, but this result may hint at a higher prevalence in older animals.

In mature female possums prevalence was higher in animals with pouch young. There have been reports of immunosuppression in mature pregnant female mammals (Griffin 1988) (Griffin and Davis 1985, Outteridge 1985). The drain on the mother's reserves while raising the young to a body weight at weaning of about 56% of her own weight may represent an intrinsic stress factor which increases the likelihood of lowered resistance to the establishment of infection, the progression of disease, or both (Tyndale-Biscoe 1984). Groups of possums in which infection was present appeared to have a higher proportion of females with young at the time of capture than groups free from infection. The nature of any cause-effect relationships cannot be discerned from this data, but it does appear that the mother - pouch young association is of importance in the dynamics of tuberculosis infection at a local level. The young suckles in the pouch for about 5 months, subsequently rides for another 2 months on the back of the mother and afterwards stays for a while in close proximity to the mother. During this long period of physically close association between mother and young allogrooming occurs frequently and is only gradually reduced with increased independence of the young (Biggins 1984). By nine months the mother shows increasing antagonistic

behaviour towards the young, until it gets driven away from the den (Tyndale-Biscoe 1973). Thus, during the nursing period the probability of successful pseudo-vertical transmission from mother to young would be relatively high. If they survive long enough, infected juveniles, particularly the males, would be able to spread infection during dispersal.

There appears to be an association between condition (estimated through an assessment of kidney fat) and tuberculosis infection status. Tuberculous possums are more likely to be of poor condition than non-tuberculous animals. Analysis of weight-length relationship indicates that infected possums were lighter than uninfected animals. Tuberculosis infection has to be seen as a dynamic rather than static disease process, with a number of factors influencing progression. Assuming that in possums the outcome of *Mycobacterium bovis* infection is fatal in almost all cases, the length of the interval between infection and death depends largely on the state of the host-defence mechanisms. The latter, in turn, are influenced by extrinsic and intrinsic factors such as environmental stress and body condition. Therefore, condition in tuberculous possums will almost certainly be poor in the later stages of the disease process, but poor condition can also be a predisposing factor for a rapid generalization within the body (Schliesser 1985). It is unlikely that in possums a long-term equilibrium will develop between host and parasite, which typically would be the case for the chronic disease process in cattle and humans (Corner and Presidente 1980, Wake and Morgan 1986). In experimentally infected badgers, animals started to lose weight during the second year post infection (Pritchard *et al* 1987). Studies of experimental infection in possums have suffered from a significant bias due to stress induced immunosuppression in captive animals. Possums were weak and emaciated at about 4 to 8 weeks post infection (O'Hara *et al* 1976, Corner and Presidente 1981). In the data from the present study no statistically significant association between body condition and spread of infection within the body was observed. However, possums with generalized tuberculosis infection were more likely to be in poor condition. An effect of tuberculosis on body condition has to be expected if the function of important organs is compromised by (for example) extensive lung lesions. But in cases with a single superficial lymph node affected, body condition may not necessarily be different from non-infected animals. These mildly affected animals are unlikely to have reduced activity level compared with uninfected individuals and therefore are likely to be an important source of infection for other possums and possibly cattle, provided they are excreting organisms. Edwards *et al (*1971) investigated the relation between body build, tuberculous infection and clinical tuberculosis in a sample of 823199 U.S. navy recruits. They concluded that the major, if not the only, factor in the relation between physique and tuberculosis must be -after infection- susceptibility to developing clinical disease.

Analysis of data on organ involvement in the disease process can be used to draw inferences about the importance of various potential transmission paths of *Mycobacterium bovis* infection. On post mortem examination 54% of cases had lesions in the superficial lymph nodes and 59% in the respiratory tract. In a review of data collected in 3 different

studies it has been reported that 62.7% of 327 tuberculous possums had lesions in superficial lymph nodes and 62% in the lungs (Julian 1980). In 48.7% of cases with localized lesions, lymph nodes were the only sites involved in the disease process, whereas in 77% of all tuberculous possums lymph nodes were among the sites affected. This supports the traditional view that lymph nodes are a predilection site of *Mycobacterium bovis* infection. The meshwork of the lymph node trabeculae entraps the bacteria while filtering body fluids and provides them with an environment which is ideal for mycobacterial growth (Thoen and Himes 1986). If infection is detected early, the involvement of a regional lymph node together with the initial focus of infection is a typical finding in tuberculosis and has been described as the Ghon focus, the primary complex of Ranke or the localisation rule of Cornet (Francis 1958, Dungworth 1985). It is possible that lesions in the regional lymph node are more evident than at the initial focus of infection (Röhrer 1970). In possums superficial lymph nodes involved in the disease process are typically enlarged and palpable. They can be used as diagnostic indicators of moderate sensitivity in longitudinal studies. Open sinuses from lymph node abscesses are thought to be of particular importance for the spread of disease between possums. The post mortem information collected during this study unfortunately classifies sinuses and lung lesions as "open". Therefore it is not possible to look at the frequency of open sinuses in all tuberculous possums. But 37.5% of animals with only lesions in superficial lymph nodes can be assumed to have had open sinuses.

Lesion sites confined to one area of the body (as for example a typical primary complex) have been used as indicators for potential routes of infection (Francis 1971, Lepper and Pearson 1973, Thoen and Himes 1986). Given the low sensitivity of the post-mortem techniques used in the current study the following results should be interpreted cautiously. It is unlikely that the location of a primary complex would have been identified using the post-mortem procedures applied in the study. In 38% of tuberculous possums with single lesion sites, lesions were found only in the superficial lymph nodes, which may be attributable to infection through the skin. However other pathogenetic mechanisms for these lesions must also be considered since, a primary complex associated with the skin is only rarely seen in other species infected with *Mycobacterium bovis* - except in the cat where bacteria can be distributed over the skin during grooming (Weiss 1983). Thirty-one percent of the single lesion cases had only lesions in the respiratory tract and this could be associated with aerosol infection. It has been reported that aerosol droplets and droplet nuclei containing a few bacteria may float in the air for considerable periods of time, depending on environmental conditions. They can be inhaled into bronchioles and alveoli if they are small enough to to escape the mucociliary mechanisms of the bronchi. From human experience it can be concluded that the disease is not as highly infectious as some viral diseases. Even under the worst overcrowded and substandard conditions the infection rate between close contacts varied between 25% and 50%. Stead and Dutt (1988) summarize that it appears that exposure generally must be close and sustained, the environment heavily laden with droplet nuclei and

the prospective host should be unprotected by previously activated immune mechanisms if an infection sufficient to produce disease is to be established. Aerosol infection is considered to be the most important transmission path in cattle, goats, sheep and humans. Respiratory infection seems to be the most important infection path in badgers. It could be explained by the conditions in badger setts, where dust particles provide an ideal vehicle for transmission of disease (Gallagher *et al* 1976). 31% of single lesioned tuberculous possums showed only lesions inside the abdominal cavity which is most likely to be related to infection through ingestion. Mycobacteria seem to be able to diffuse into the lymphatics of the lamina propria of the small intestine and to be transported by phagocytes into the mesenteric lymph nodes (Thoen and Himes 1986). In horses, pigs, cats and fur bearers ingestion is reported to be the predominant transmission path (Schliesser 1985). An understanding of the social behaviour of the possums is required for interpretation of these findings.

Probability of transmission of *Mycobacterium bovis* infection between possums is increased by any kind of social interaction resulting in direct (and possibly also indirect) contact with each other. Possums are generally believed to be solitary animals (How 1978). They usually feed alone (Smith and Lee 1984). Simultaneous den sharing and allogrooming mainly occur between mother and young (Kean 1967). Sequential den sharing is not uncommon and its frequency depends on den site availability in the habitat and on population density (Cowan 1989). There have been reports of cases of simultaneous den sharing in adult possums, but it seems to be rather unusual (Green and Coleman 1987, Fairweather *et al* 1987, Cowan and Rhodes 1988). Depending on the weather conditions possums spend between 13.6% and 85.7% of their active period, which starts shortly before sunset and finishes shortly before sunrise, in or close to their den site (Ward 1978). During this time they intensively wash and groom their fur by spreading saliva using their forearms and syndactylus claws (Biggins 1984). While foraging the possums apply various methods of scent marking to demarcate their home ground. Kean (1967) found that on release possums marked their outward path at short intervals by pressing their chest gland against branches or other prominent features. He reports that the odour of the sternal gland permeates shelters and can be detected along tracks, particularly in places where tree trunks are traversed. Biggins *et al* (1978) observed in a captive possum population a dominant male chest marking a juvenile female possum. Quite often animals respond to scent-marks by a combination of gnawing and licking, as well as remarking the site. The use of the sternal gland for marking could be of particular relevance for the transmission of *Mycobacterium bovis* infection as it would explain the high proportion of animals with single lesion sites in the axillary or subscapular lymph nodes. Possums do not seem to have exclusive territories and they maintain a system of shared tracks (Crawley 1973). It is believed that their marking activities mainly serve to enhance their own familiarity with their home ground, which adult possums probably occupy for the rest of their lives (Biggins 1984). Under high population densities such as in New Zealand the home ranges of individual animals may extensively overlap, as it is impossible to

defend them (Kean 1967, Crawley 1973). Current information on dominance hierarchies suggests that adult females are dominant to males and that co-dominants avoid each other (Green 1984). There are conflicting views regarding the mating behaviour of possums. Some authors claim that the males are promiscuous based on encounters with females with overlapping home ranges (Smith and Lee 1984). Others report that there seems to be a mate-defence system where males form temporary 'consort' relationships with oestrous females (Jolly *et al* 1984). When mounting, the male temporarily grasps the back of the neck of the female with his teeth. He clings to the female with his forelimbs circling her thorax or abdomen. While climbing to the female's back, the male commences rapid up-and-down movements of the forepaws. Based on this mating behaviour, infection could be transmitted from male to female through skin lesions, as well as by the respiratory route. Antagonistic encounters are most frequent in the breeding season, and occur mainly between adult males. The animals usually adopt a bipedal threat posture with outstretched forelimbs and extended claws. They fight by grasping the opponent with the forepaws and using their teeth as principal weapons for lunging bites to the head, shoulders and neck of the opponent (Biggins 1984).

Open sinuses from lymph node abscesses would provide a source of infection in situations of simultaneous or sequential den site sharing, for transmission from mother to young, during fights, mating and in relation to marking behaviour. The high proportion of possums with lesions in superficial lymph nodes could be related to the same modes of interaction. Infection through the respiratory tract requires a sustained exposure to small droplets with bacterial load. Since simultaneous den sharing does not seem to be usual except for mother and joey, transmission by the respiratory route between adults could occur in antagonistic exchanges when possums exhale explosively, and possibly due to the creation of aerosols from contaminated material in dens. The gastrointestinal system would be a potential portal of entry in relation to allogrooming, selfgrooming (after contamination during marking, and after denning in an infected site) and marking behaviour (licking on marks). The intake of food contaminated with *Mycobacterium bovis* seems to be unlikely as a significant source for infection as tuberculosis bacteria have an estimated survival of 6-14 days in daylight and between 7 and maximally 56 days on pasture (Mitscherlich and Marth 1984). Variable results have been achieved in several studies exposing cattle and guinea pigs on *Mycobacterium bovis* contaminated pasture. Maddock (1984) grazed 6 guinea pigs for 3 days on pasture which had been sprayed with an emulsion of tubercular lungs on the preceding day and none of the animals got infected. He had to increase contamination and graze the guinea pigs longer to infect 8 out of 10 Guinea pigs in another trial. If pasture contamination were the dominant form of transmission between possums, it would be unlikely that infection would be as strongly clustered as has been found in this and other studies.

Immature male possums were more likely to have disseminated or generalized tuberculous lesions than immature females. The numbers of possums were too small to test this result reliably for statistical significance. But male immatures seem to be under more stress than immature females, which are less likely to disperse. Hence, their immunological defence mechanisms could be compromised. There was a trend suggesting that in immature males superficial lymph nodes were more likely to be involved in the disease process than was the case in immature females. If lesions in superficial lymph nodes are an indicator of cutaneous infection, males seem to be at higher risk for this mode of transmission. It could be explained by injuries resulting from antagonistic encounters which appear to be more frequent between males. Adult females commonly showed more lesions in the intestinal tract than adult male possums. There does not seem to be any difference in behaviour between the two sexes which could serve as an explanation for this finding. It may be linked in some way to interaction between mother and young. The proportion of cases with disseminated or generalized tuberculous lesions increased from early-mid to late summer. Feeding and weather conditions are favourable during summer and the body defence mechanisms may be able to contain infection within the primary complex. In late summer, during the stressful mating season immunological resistance is likely to be compromised and haematogenous or lymphatic spread of *Mycobacterium bovis* organisms would result in disseminated or generalized tuberculous lesions.

A correlation coefficient of 0.36 between possum tuberculosis prevalence and cattle tuberculosis incidence does not allow any conclusions to be drawn regarding *Mycobacterium bovis* transmission between the two species. The discrepancy between this finding and the correlation of 0.9 in a preliminary analysis of this survey data is an example of the ecological fallacy (Hennessy 1986, Last 1988). For each zone average possum prevalence across a number of trap lines and average cattle tuberculosis incidence across a number of farms had been used in the correlation estimation which resulted in an under-estimation of the true variation in the data.

This survey does not provide reliable information on tuberculosis prevalence in possum populations in potential contact with cattle herds on particular properties. It is not known to what extent the farm possum sample is representative of the possum population on these properties and whether herd management practices allowed contact between the sampled possum population and cattle from the property. In 46.4% of infected herds no tuberculosis infection was detected in the sample from the possum population. It can be concluded from the data that cattle tuberculosis incidence was higher if possum tuberculosis was present in the possum population. There was a relatively strong correlation of 0.6 between fecundity in possums and cattle tuberculosis incidence. As has been explained above, it appears that pseudo-vertical transmission may be an important factor in the dynamics of *Mycobacterium bovis* infection in possums. Hence, the fecundity estimate may be more reliable than the post-mortem result as an indicator for presence of tuberculosis infection in an endemic disease

situation. It is quite possible that this correlation coefficient was influenced by the confounding factor "sampling season".

The analysis of the data from the Hauhungaroa survey has identified some important factors in the epidemiology of *Mycobacterium bovis* infection in possums despite the shortcomings of the study design. The true tuberculosis prevalence was probably close to 2.0% which is a value similar to the estimates reported from other studies. Disease occurred in relatively infrequent patches of high prevalence resulting in this relatively low prevalence estimate over the whole survey area. Male juveniles had a relatively high prevalence of tuberculosis and they are known to disperse from their mother's home range, therefore they are very likely to contribute significantly to spatial spread of tuberculosis infection. Breeding females appear to have an increased probability to develop tuberculous lesions. In view of the prolonged relationship between mother and young, pseudo-vertical transmission may be an important factor in the dynamics of infection. Possums with tuberculous lesions do not seem to be impaired in their activity until the terminal stages of the disease process. This results in an unaltered behaviour pattern of infected animals, which together with the high proportion of lesions in superficial lymph nodes would increase the probability of infection for other possums. Disease transmission paths associated with cutaneous infection seem to be of particular importance especially in adult males as the high proportion of lesions in superficial lymph nodes within single lesioned cases suggests. There was seasonal variation in tuberculosis prevalence which may be related to environmental or social stress factors. The data from this survey provided little evidence of a causal relationship between possum and cattle tuberculosis infection. The understanding of the factors affecting the interaction between tubercle bacilli and possums as the host, is still very limited. Estimates of the probabilities and temporal aspects involved in the causal chain between exposure, infection and disease in possums are needed to develop efficient control strategies using simulation models. Several of these aspects of the epidemiology of bovine tuberculosis in possums are followed up in the longitudinal study of the temporal and spatial dynamics of the disease in a possum population reported in the next chapter.

CHAPTER 5

A LONGITUDINAL STUDY OF BOVINE TUBERCULOSIS IN POSSUMS AND CATTLE

INTRODUCTION

In April 1989, a longitudinal study of the epidemiology of bovine tuberculosis in possums and cattle was started as a cooperative project between the New Zealand Ministry of Agriculture and Massey University. The project is still under way and will probably terminate in 1994. The study has three different phases. Phase I was a preliminary exploratory phase which took from April 1989 until October 1989. Phase II consisted of monthly five day trapping sessions designed to measure the dynamics of the possum population and of tuberculosis infection in possums and cattle, and finished in May 1991. During phase III, three day trapping sessions are being conducted at monthly intervals. This phase commenced in June 1991 and will continue until spring 1994, making routine measurements. Studies of possum behaviour and disease transmission mechanisms are also being conducted to answer specific questions raised by earlier results. The analysis reported here is based on the data collected during the first 22 visits, between April 1989 and January 1991.

MATERIALS AND METHODS

Selection of Study Site

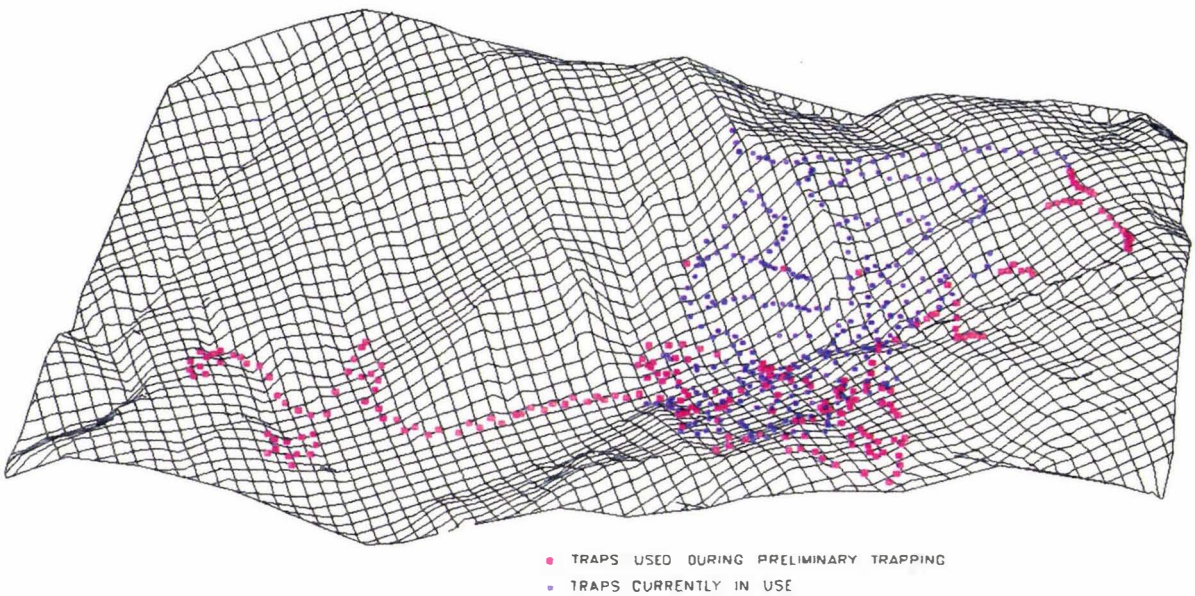
The study had to be undertaken in an area where *Mycobacterium bovis* infection was known to be present in the possum population. Incidence of *Mycobacterium bovis* infection in cattle populations was used as an indicator for the presence of infection in associated possum populations. The Ministry of Agriculture and Fisheries recommended the area of Castlepoint in the Wairarapa district, because it had a history of continuing tuberculosis problems in cattle and no possum population control operations were planned within the foreseeable future. The study farm was selected based on a recommendation by Mr. A.J. Harris, of the MAF Quality Management district office in Masterton. He had conducted the tuberculin testing in the area during previous years and had also done some possum hunting in the same area. On 14th October 1983 and 10th August 1985 Mr. Harris visited the farm and shot two possums which when examined proved to have tuberculous lesions. One of the possum was shot in "Corner" and the other one in "Back Flat" paddock on the study farm. On 20th July 1986 he shot a further tuberculous possum in "Backdrop" paddock close to the fence line of the neighbouring farm. The beef cattle herd on the selected farm had been tuberculin tested in May 1989, and 17 reactors were detected in 194 cattle (see table 8). The proportion of reactors was highest in the steer herd, with 10 out of 31 animals positive. The steers were kept throughout the last 6 months prior to testing in "Back Drop" and the surrounding paddocks. Taking these findings, plus ecological habitat factors and the farm manager's cattle husbandry practices into consideration, it was decided to screen "Back Flat", "Corner" and "Backdrop" paddocks for the presence of tuberculous possums. During the first four visits of phase I, this area was examined for accessible trap sites and screened for tuberculosis (TB) infected possums by trapping along the pasture/bush margins (see figure 20). In the light of these preliminary findings, decisions were made on what part of the area would be covered during the following

two project phases with the available manpower, taking into account evidence on the presence of any clusters of TB infected possums.

Table 8: Bovine tuberculosis history of cattle herd on study farm from 1979 until 1989

Episode Date	Episode Type	Test No.	No. Tested	CFR	Reactors	Post Mortem	Status	Slaughter Date	Slaughter No.	TB No.	NVL No.
18-May-89	Final PHT	17	59	2	2	p	Infect MC	06-Jun-89	2	2	
11-May-89	Part HT	17	135	15	15	p	Infect MC	06-Jun-89	15	11	4
03-Nov-88	Sale T		17	0	0		Infect MC				
26-Sep-88	Sale T		26	0	0		Infect MC				
16-Aug-88	Tb Cull				1		Infect MC				
30-Jun-88	Tb Cull				1		Infect MC				
23-Jun-88	Final PHT	16	72	2	2	n	Transition	12-Jul-88	2		2
16-Jun-88	Part HT	16	91	0	0		Clear MC				
27-May-88	Sale T		53	0	0		Clear MC				
10-Dec-87	Whole HT	15	58	0	0		Clear MC				
17-Sep-87	Sale T		50	3	3	p	Infect MC				
28-Aug-87	Misc Test		29	1	1	p	Infect MC				
26-Feb-87	Sale T		19	0	0		Infect MC				
16-Dec-86	Tb Cull				1		Infect MC				
28-Nov-86	Final PHT	14	59	5	5	p	Infect MC				
17-Oct-86	Part HT	14	61	0	0		Infect MC				
05-Sep-86	Sale T		16	0	0		Infect MC				
12-Dec-85	Whole HT	13	97	7	7	p	Infect MC				
04-Nov-85	Sale T		11	0	0		Infect MC				
21-Feb-85	Sale T		36	0	0		Infect MC				
29-Nov-84	Whole HT	12	93	5	5		Infect MC				
29-Mar-84	Whole HT	11	109	1	1		Infect MC				
10-Nov-83	Final PHT	10	31	0	0		Infect MC				
04-Nov-83	Part HT	10	51	1	1		Infect MC				
31-May-83	Tb Cull				1		Infect MC				
20-May-83	Final PHT	9	37	1	1		Infect MC				
12-May-83	Part HT	9	83	6	6		Infect MC				
13-Jan-83	Whole HT	8	89	3	3		Infect MC				
15-Jul-82	Final PHT	7	66	0	0		Infect MC				
02-Jul-82	Part HT	7	40	3	3		Infect MC				
23-Jun-82	Tb Cull				1		Infect MC				
11-Mar-82	Whole HT	6	114	1	1	p	Infect MC				
23-Jan-82	Misc Test		2	0	0		Clear MC				
13-Nov-81	Whole HT	5	80	0	0		Clear MC				
21-Sep-81	Sale T		23	0	0		Infect MC				
03-Apr-81	Whole HT	4	172	4	4	p	Infect MC				
07-Nov-80	Final PHT	3	53	0	0		Infect MC				
15-Sep-80	Part HT	3	89	1	1	p	Infect MC				
22-May-80	Sale T		28	1	1	p	Infect MC				
19-Apr-80	Final PHT	2	58	2	2	p	Infect MC				
17-Apr-80	Part HT	2	96	0	0		Infect MC				
20-Dec-79	Final PHT	1	94	1	1	p	Infect MC				
15-Nov-79	Part HT	1	70	5	5	p	Infect MC				
08-Oct-79	Sale T		20	0	0		Infect MC				
10-Sep-79	Sale T		14	0	0		Infect MC				

Figure 20: Location of all traps which were used during the three project phases draped over digital terrain model of the area

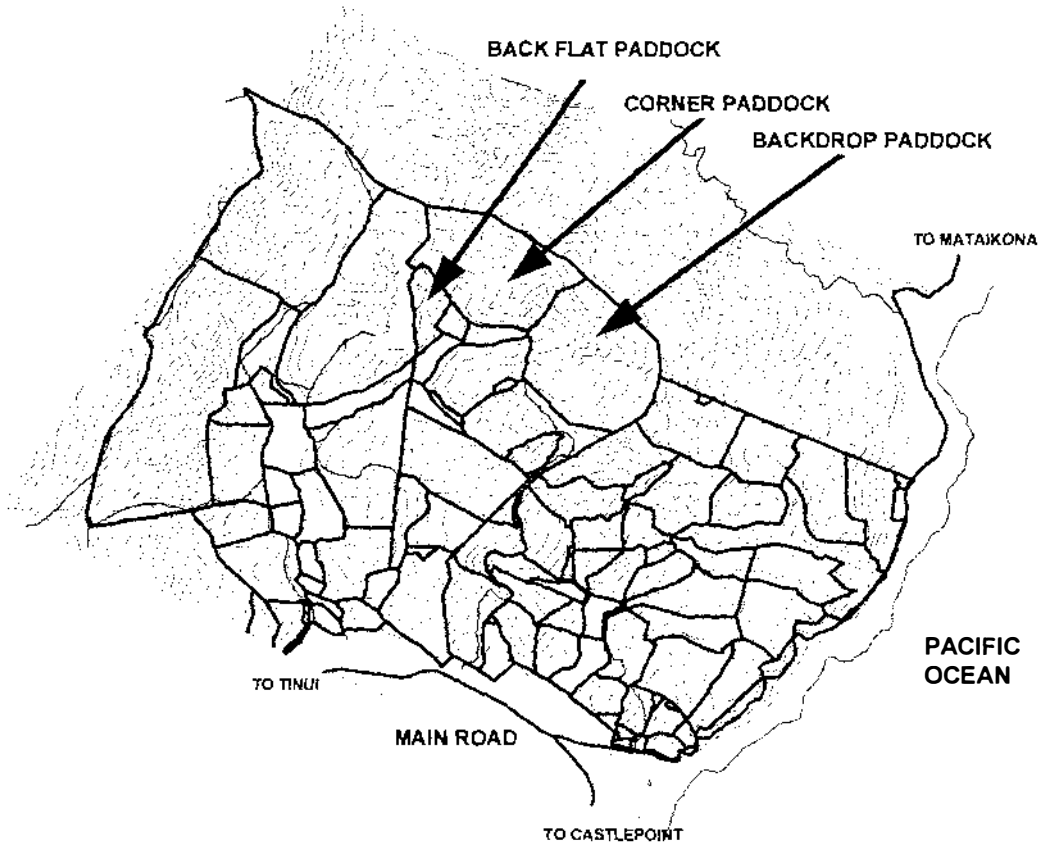


Field Procedures

Study Site and Study Design

The project area is located on a farm in the Wairarapa district, southern part of the north island, New Zealand. The study site occupies an area of 21 hectares within a 40 hectare paddock which is being grazed by beef cattle (see figure 21). The vegetation consists predominantly of native manuka trees and introduced gorse bushes, with pockets of native broadleaf forest in favoured sites. The manuka trees have been partially cleared from the site (about 30-40% open space), and there is improved pasture over most of the study site, under and among the manuka trees. The site comprises a central valley and surrounding slopes and steep hillsides. Multiple water courses drain to a creek which runs down the central valley. Eighteen months after the study began, the paddock was bisected by a new fence to separate a water catchment protection area from the more open grazing area of the paddock. Possum dens are predominantly on one side of the fence (from which cattle have since been excluded) whereas possum grazing areas are on both sides of the fence.

Figure 21: Paddock boundaries for Waio farm draped over a contour map



The project is a capture - mark - recapture study. Monthly five day trapping sessions were conducted from April 1989 until January 1991. A trapping grid with a total of 295 cage traps is centred on the sites where TB infected possums were found during the preliminary visits (see figure 22b). Three different models of treadle operated cage traps have been used. One model was operated by a foot trigger, and in the other two models the bait was attached to the triggering mechanism (see figure 22c for most frequently used trap model). The traps provided shelter through a metal sheet covering the top of the cage. All three models have been designed by the Department of Scientific and Industrial Research (DSIR) specifically for the purpose of possum trapping. A lured bait was used to attract possums to the trap. The bait has been pieces of apple coated with a mixture of flour and cinnamon powder.

Figure 22a: Photograph of the study area

Figure 22b: Trapgrid draped over digital terrain model of the study area

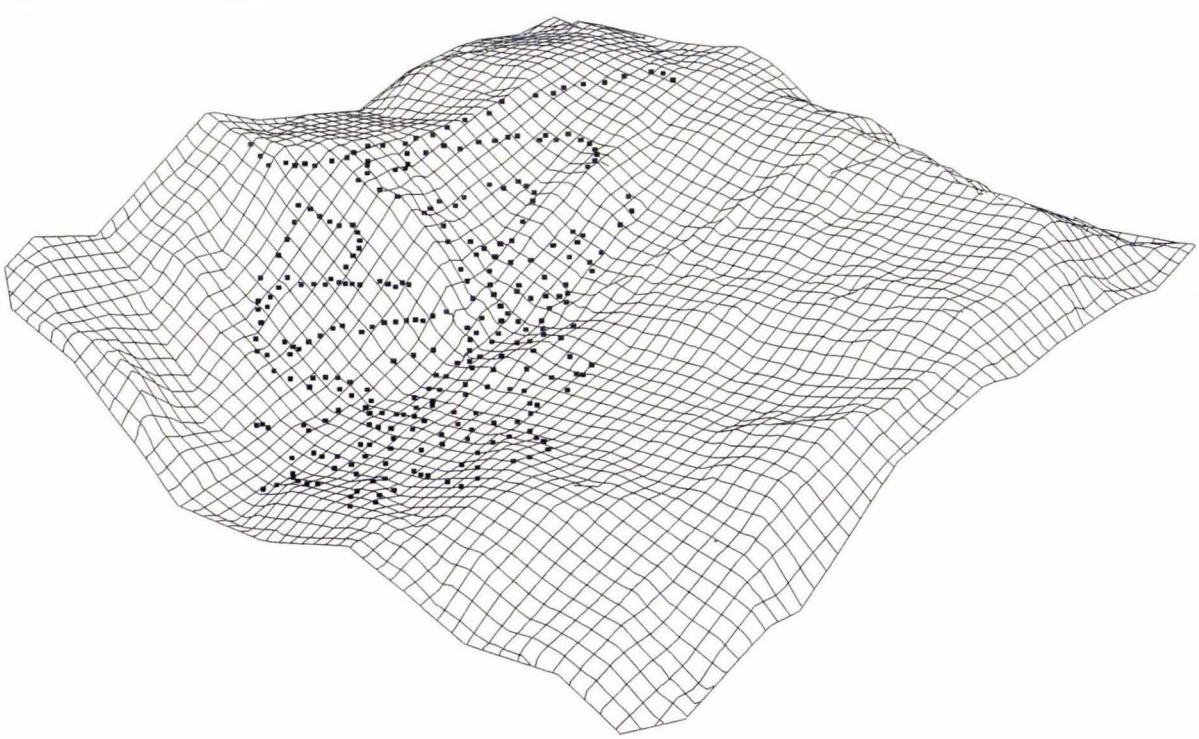


Figure 22c: Possum captured in most commonly used trap model

General Procedure for Animal Examination

Every morning the trap lines were checked for possum catch. Each animal found in a trap was immobilized by injection of an anaesthetic. Ketamine hydrochloride at an average dose of 35mg/kg was shown to be effective. The anaesthetic was injected intramuscularly into the quadriceps or triceps muscles.

Sequential blood samples were collected throughout the study from each individual possum, with a minimum interval between collections of two months. A blood sample of 4 to 5 ml was required to provide about 3 aliquots , each of 0.5 ml serum, for a possum serum bank.

Each animal was marked on the ears for recognition by attachment of monel-metal eartags (from Bird Banding Office, Department of Conservation (DOC), Wellington), punching of notches and tattooing (see figure 23, Stonehouse 1978). Pouch young with suitable ear size were tagged with small ear tags (fish tags) until they were independent and could be given permanent tags.

Figure 23: Possum with ear notches and ear tag

Once immobilized and sedated, each animal was examined both visually and by palpation of all superficial lymph nodes and other potential lesion sites detectable from the exterior. If this gave any reason to suspect infection with *M.bovis*, further samples were taken from the animal as described below, and submitted for culture. Pritchard *et al* (1986) report that clinical sampling methods will detect badgers with more advanced lesions, and the probability of detection is improved by sequential sampling.

Information on the animal and trap catch was recorded on special forms which were developed and refined during the preliminary visits (see appendices I and II). The information recorded on the possum card included the following data: possum number (ear tag), observer, date, trap site, type of trap, fur colour, sex, age category, total length, tail length, weight, condition, presence of pouch young (sex, weight, head length, total length), result of palpation, etc.. The forms were printed on "wet paper".

Possums were assigned to one of four age groups (pouch young, dependent immature, independent immature and mature). In addition an index of tooth wear was used for assessing age (Winter 1980, Cowan and White 1989). Body measurements were used for animals of up to 12 months to get a more accurate age estimate (Lyne and Verhagen 1957, Crawley 1973). Maturity was assessed in males based on testis width (Tyndale-Biscoe 1955, Gilmore 1969). The criterion of maturity in female possums was invagination of the pouch (Clout and Efford 1984). Sex was determined in pouch young starting from 12mm head length by means of the presence of pouch or scrotum rudiments (Tyndale-Biscoe 1955).

Details on Sample Collection

Blood Collection

The blood was collected with a 5ml syringe and a 0.8mm x 50mm needle by cardiac puncture, after which it was transferred into a glass tube (vacutainer). Haemolysis was minimised by leaving the blood sample to clot at the site of collection for about 3 - 4 hours. After centrifugation the sera were divided into 0.5 ml aliquots. These were stored in 2ml autoanalyzer cups in a deep freezer (at -15 to -20^{o}C). The blood and the serum was handled under clean conditions to minimise bacterial contamination.

Samples have been subjected to ELISA tests at CSIRO Division of Animal Health, Parkville, Australia and the Ministry of Agriculture, Fisheries and Food Central Veterinary Laboratory, Weybridge, United Kingdom.

Collection of Other Specimens

In cases where infection with *M.bovis* was suspected, further samples had to be taken. A post mortem examination was required for possum carcases found dead in and around the study area. In cases where tuberculosis was suspected, tissue samples were taken and sent for laboratory examination at the Central Animal Health Laboratory, MAF Quality Management, Wallaceville. A detailed report of the necropsy results using a special form was prepared (see appendix III). Sterile instruments were used for each individual examination. The lower jaw was removed from every dead possum found in the area, further processed and stored for later age determination (Clout 1982).

From open lesions small volumes of discharge were obtained by a swab, and transferred to transport medium for shipment. From closed lesions detected by palpation an attempt to aspirate fluid by sterile puncture was made, and the fluid was then immediately transferred to transport medium. The transport medium containers were kept in a refrigerator until despatch.

At post mortem examination a tissue sample was taken from lesioned possums considered suspect for infection with *M.bovis*, and placed in a plastic container with screw top. The sample for histopathological examination was cut into pieces of about 1 cm thickness, placed in a plastic container and preserved by adding 10% formalin. The sample for bacteriological examination was collected with as little contamination as possible and placed in a plastic container with screw top. This sample was stored in a refrigerator until despatch.

Other Investigations

Radio telemetry was used to locate den sites (Ward 1972, Thomas 1982). A radio transmitter was fitted to each apparently diseased animal (Ward 1972). Selected animals caught within the expected home range of the diseased animals were also radiotagged. In addition, if spare transmitters were available, as many animals as possible found in the vicinity of the infected group were tagged to identify denning patterns of the sub-population which included an apparent cluster of infected animals. Special consideration was given to locally born juvenile

possums. Because of technical limitations on the number of frequencies which could be used, a maximum of 50 possums was carrying radiotags at any one time. Two-stage transmitters in standard and longlife configuration (SIRTRACK Electronics, DSIR Land Resources, Havelock North, New Zealand) were attached to animals using neck collars. The animals were tracked using three-element yagi antennas and a portable radiotelemetry receiver (Model CE 12, Custom Electronics, Urbana, Illinois, U.S.A.). Data was recorded using a specifically designed form (see appendix IV).

Culture isolates of *Mycobacterium bovis* from possums, cattle and other wild species were typed using DNA restriction endonuclease analysis by the Central Animal Health Laboratory, Wallaceville (Collins and de Lisle 1984). Restriction patterns of isolates from cattle, possums and other species were compared to allow inferences regarding transmission (Collins 1986).

Incidence of tuberculosis infection was monitored in a group of about 20 - 30 beef cattle, kept principally on the study site. Each animal in the herd was tuberculin tested at 3 monthly intervals. Cattle reacting to the tuberculin test were removed from the herd at yearly intervals starting from the first test (24/10/89). Cattle removed from the study herd were subjected to an extensive post-mortem examination at the slaughter house.

Data Analysis

Pollock's (1982) capture-recapture design which is robust to unequal probability of capture was used for the estimation of population parameters in this study. Estimates of population size are calculated for each visit using Otis *et al*'s (1978) population models. The assumption of a closed population which is required for these models should hold for this short time period. Model 'm_h' of the set of models available in the computer program CAPTURE utilizes the Jackknife estimator and was considered most appropriate for estimation of population size in this study. It allows for heterogeneity of capture probabilities, but is sensitive to variation in capture probabilities over time. Unequal capture probabilities in possums have been described by Cowan (1987).Survival estimates between visits have been calculated using the Jolly-Seber method. The survival rate estimator of this method is less sensitive to violation of the assumption of equal catchability than the population size estimator (Pollock *et al* 1990, Pollock 1982). The computer program JS (M. Efford, DSIR Land Resources, Dunedin, New Zealand) was used for the calculations.

Home range estimates were calculated (based on information on both trap catch results and den locations) using the computer software TELEM88 version 9.90 (Coleman and Jones 1988). The convex polygon, the 95% ellipse and the harmonic mean (30%, 60% and 90% contours) methods were used (White and Garrott 1990). A center point of each home range was calculated using the arithmetic mean method (Lair 1987, Hayne 1949). A mean activity radius was estimated as the average distance of each animal location from the arithmetic center (Hayne 1950). Estimates were compared between adult sex classes for animals with at

least 40 locations available using the t-test and the Mann-Whitney test (Snedecor and Cochran 1989). The index of dispersion test and the standardized Morisita index of dispersion were used to analyze overlap of activity areas. These methods are based on event counts obtained over a sparsely sampled area. The standard deviation of the mean of the individual estimates depends on the spatial pattern of the events being counted. Randomly placed spatial processes follow a poisson distribution, hence the variance would equal the mean of the counts. If they are uniformly spaced the variance would be much less than the mean. If the events are aggregated the variance will be greater than the mean. The goodness of fit of the poisson distribution to the observed data is measured using the z - approximation to the chi^2 value (Krebs 1989, Diggle 1983).

Aerial photographs of the study area were taken from an aeroplane on November 13, 1989 by the Department of Survey and Land Information, Wellington. From these photographs a digital terrain model was digitised based on a 50m grid over the whole farm including major breaklines in the area of the study site. A 1:2500 scale orthophoto of the study site was used to map trap sites and den site locations, which were then digitised. A 1:7500 scale orthophoto covering the whole farm was used to map dispersal movements. A digital database of map information was created using the geographic information system (GIS) PC-Arc/Info version 3.4D (Environmental Systems Research Institute, Redlands, California, U.S.A.). The geographic data was organised into layers of information with the same feature type (point, line, polygon) and thematic grouping of features. The GIS was used to produce maps and combine information from different coverages for statistical analysis. Summary maps of the spatial distribution of total capture and the proportion of tuberculous possums at a each location were produced using the geostatistical method "kriging" for calculation of unbiased spatial interpolation estimates (Oliver and Webster 1990, Burrough 1986). The GIS was used to provide coordinate locations of individual trap sites which were linked to the respective summary information (such as total possum capture or proportion of tuberculous possums) using the database management software PARADOX version 3.5 (Borland International, Scotts Valley, California, U.S.A.). The interpolations were done using the geostatistical software GEOEAS version 1.2.1 (U.S. Environmental Protection Agency, Las Vegas, Nevada, U.S.A.).

Distances between all point locations such as den sites and centers of activity were calculated using the geographic information system PC-Arc/Info. Measurements were compared using the t-test and Mann-Whitney test. For each den site values of 3 variables (location slope, height above sea level and aspect) were generated using the following sequence of procedures in PC-Arc/Info. A triangulated irregular network model (TIN) was derived from the digital terrain model of the study area. Slope and aspect were estimated and height above sea level was interpolated from the TIN. A polygon coverage based on the TIN triangles with the respective information in the polygon attribute table was then generated. A

coverage with the point locations of the den sites was overlaid on top of this coverage to retrieve the respective polygon attribute information.

Time-space clustering of the occurrence of tuberculosis cases in possums was analysed using the Mantel regression method (Mantel 1967). This statistical test measures proximity of pairs of cases in space and time. Wartenberg and Greenberg (1990) compared statistical power of the most frequently used methods for detection of space-time clustering. They found that cross-product statistics such as Mantel's space-time regression were most useful and robust. The authors emphasize the importance of regions with elevated risk (hot-spot models) and trends of risk from a point source (clinal models) when investigating environmentally induced disease. Using an inverse distance transformation for emphasising small distances improved the ability to detect hot-spot patterns. Both untransformed and inverse distances were used during the present analysis. Separate analyses were conducted to investigate possible clustering within age and sex classes. Distances were calculated based on locations and dates, where and when animals were captured for the first time with clinical disease. For possums which were found dead and tuberculous lesions were first detected at post mortem examination, the location where they were found was used for the distance calculations. Scatterplots were used for displaying the bivariate distribution of measures of closeness. A linear regression line was fitted to the plotted points with 95% confidence limits on mean predicted values.

Average weights of possums were compared using repeated measures analysis of variance (Pedhazur 1982). A split plot approach was used to allow the inclusion of data from individual possums with missing values for particular visits (Snedecor and Cochran 1989). Sex stood for the block and season for the sub-plot treatment. Individual possums were considered the replicates. The hypothesis for the block treatment effect (or its interaction with sub-plot) was tested using replicates (or their interaction with sub-plots) nested within blocks instead of the overall residual mean squares. Procedure GLM of the statistical software package SAS was used for the analysis. The interpretation was based on Type III sums of squares which correspond to Yates' weighted squares of means analysis and allow a comparison of main effects in the presence of interaction. The results of the analysis have to be treated cautiously as a design with empty cells is likely to result in unequal correlations between repeated measurements (Littell *et al* 1991).

When analyzing event histories of a sample of individuals, the observations possess two features - censoring and time-varying explanatory variables - which create major difficulties for standard statistical procedures. Various methods which are grouped under the term survival analysis were used for analyses where the response variable is time-related. In general terms the dependent variable is measured as time to either 'failure' or 'loss' (censoring) (Cox 1972). The Kaplan-Meier product limit estimator was used to estimate cumulative failure probabilities (Peto *et al* 1977). Survivor function estimates were plotted against time, yielding a profile of the probability of surviving each month, given survival until this month.

The Wilcoxon and the Log-Rank test were used to test homogeneity between strata using the SAS procedure LIFETEST (Anon. 1988, Lee 1980). Proportional hazard regression analysis was used to estimate hazard rates adjusted for covariates and their interactions. As the data was collected on a discrete time scale the discrete logistic model available in the SAS Procedure PHREG was applied (SAS 1991, Singer *et al* 1991, Allison 1982). The start time for survival was based on date of entry into the study (visit when first captured). As in most studies of this kind, right but not left censoring was taken into account in the analysis. The maximum length of data collection was determined by the end of the study period which was considered in this analysis. Observations were considered as right censored in a way which depended on the objectives of the individual analyses. When analyzing time to death, possums which disappeared during the course of the study were included as "failures" if they were caught for the last time up to and including visit 18. Visit number 18 was chosen as the threshold value because it seems likely from earlier experience that if these animals had still been resident within the study area they most likely would have been recaptured between visits 18 and 22. When interpreting the results of this analysis it has to be kept in mind that possums which disappeared could have died, emigrated or have been trap shy. When time to development of lesions was analyzed animals which had disappeared were considered right censored observations. The proportional hazards regression model allows the inclusion of time-constant and time-varying covariates. Time-varying covariates were used to control for change in environmental conditions and physiological factors such as body weight and pregnancy status. The values for time-varying covariates for each risk set were retrieved from the data set using SAS programming statements (SAS 1988). As the animals could not be examined on every visit, observations in a particular risk set with missing values were replaced by values from the last examination. A stepwise regression approach was used to determine the 'best' model based on the likelihood ratio chi-square test. As suggested by Hosmer and Lemeshow (1989) for stepwise model-building strategies, a significance level of 0.15 for entry of variables into the model was chosen. Variables were removed if their significance level dropped below 0.20. Hazard rate ratios (which can be interpreted as relative risks) and their 95% confidence intervals were calculated for each covariate in the final model (Kahn and Sempos 1989). Effects which were included into the final regression model were tested for first order interaction with other variables in the model. The assumption of proportionality of the hazard rates for the different strata of each covariate was assessed by testing the likelihood ratio statistic of the product of the log transformed time variable and a covariate (Statistics and Epidemiologic Research Corporation 1990). If the interaction effect between predictor and time was statistically significant, it was included into the model to ensure appropriate estimation of the regression coefficients. Algebraic interpretation of the coefficients as scaled risk profiles are useful only if the proportional hazards assumption is met (Singer *et al* 1991).

Meteorological data was obtained from the New Zealand Meteorological Service, Wellington, New Zealand for the weather station "D06921 Castlepoint Lighthouse". The station is located at a distance of about 12.5 km from the study site. Meteorological data available includes rainfall, temperature, relative humidity and wind direction according to climatological form Met 301 (Anon. 1979). The information was missing for the months January, March, May and June 1990. Measurements for the most important meteorological variables were analyzed retrospectively to determine how representative they were with respect to long term trends, cyclical and seasonal patterns. Monthly meteorological data collected since 1972 was used for this comparison. When analyzing the influence of seasonal factors, the period December- February was grouped together as summer, March - May as autumn, June - August as winter and September - November as spring.

Statistical association with cumulative incidence for meteorological and population parameters was examined for lags of up to two months. Data on potential risk factors was stratified into covariate classes for each visit with the number of incident cases as the response variable and the number of animals at risk as the denominator for each month. The numbers of cases during each month relative to the population examined was small enough to be considered a poisson variate (McCullagh and Nelder 1989). Variables which were considered potential risk factors were included in a multivariate analysis using stepwise poisson regression (Kleinbaum *et al* 1988). SAS procedure LOGISTIC was used to conduct the analysis as outlined in Vine *et al* (1990). Parameters of the regression estimates were calculated using iteratively reweighted least squares. Model fit was assessed using Akaike's Information Criterion (SAS 1990). Entry and removal of variables in the model was based on significance levels of 0.10. Variables had varying numbers of missing values. In order to maximise the number of observations, analyses were rerun including subsets of variables which turned out to be statistically significant in the previous step.

Multiple correspondence analysis was used to graphically explore the association between categorical variables. It is a combination of a mathematical technique to explore the structure of a contingency table and a graphical technique, where the derived structure is illustrated in a diagram with points representing the categories of the variables (Anderson 1990). By simultaneous consideration of multiple categorical variables this technique allows the user to reveal the structure and patterns inherent in the data, which it would be difficult to detect in a series of pairwise comparisons. Each variable contributes a set of points based on its categories which are located in euclidean space. Inertia describes the spatial variation within each set of points and is calculated as the weighted sum of squared distances from the points to their respective centroid. Distances between points in the same set indicate similarities and differences among them with respect to their attributes. Points near the center of the display have undifferentiated profile distributions (Hoffman and Franke 1986). Association between category points from different variables is not based on distances, but on being found in approximately the same direction from the origin (SAS 1988). Each dimension

represented by one axis in a joint plot (or correspondence analysis diagram) accounts for a proportion of total inertia, which in turn determines the number of dimensions necessary to adequately represent the data matrix. Hoffman and Schaffer emphasize that correspondence analysis is not appropriate for hypothesis testing. They write that the method suffers from the "curse of dimensionality". There is no method to conclusively determine the number and combination of dimensions to plot and inspect. It is seen as a flexible, but subjective technique requiring considerable input from the researcher.

Statistical analyses were conducted using the software packages EPI-INFO version 5.02 (USD, Incorporated, Stone Mountain, Georgia, U.S.A.), SOLO version 3.0 (BMDP Statistical Software Inc., Los Angeles, California, U.S.A.), PC-SAS version 6.04 (SAS Institute Inc., Cary, North Carolina, U.S.A.), EGRET (Statistics and Epidemiology Research Corporation, Seattle, Washington, U.S.A.) and the computer spreadsheets Quattro Pro version 3.0 (Borland International, Scotts Valley, CA USA) and Microsoft Excel (Microsoft Corporation, Redmond, Washington, U.S.A.). Graphics and charts were generated using the above mentioned software packages and edited using DrawPerfect version 1.1 (WordPerfect Corporation, Orem, Utah, U.S.A.), Harvard Graphics version 3.0 (Software Publishing Corporation, Mountain View, California, U.S.A.) and Microsoft PowerPoint for Windows version 2.0 (Microsoft Corporation, Redmond, Washington, U.S.A.).

In the text the following abbreviations for descriptive statistical measures are used: 'AIC' for Akaike's Information Criterion, 'ß' for regression coefficient, 's.d.' for standard deviation, 's.e' for standard error, 'x_m' for median, 'HRR' for hazard rate ratio, 'OR' for odds ratio and 'CI' for confidence interval. 'I' stands for index of dispersion and 'I_p' for standardized Morisita index of dispersion.

RESULTS

Meteorological Data

The distribution of observed measurements of total monthly rainfall, average monthly temperature and average ratio of minimum to maximum daily temperature was estimated for the time period from 1972 until 1990 (see tables 9a and 9b, figures 24a, 24b and 24c).

Table 9a: Total monthly rainfall summarized over the period 1972 until 1990

Month	n	x (mm)	s.d.	1989	1990
January	17	41.66	33.37	?	?
February	18	65.30	41.59	?	14
March	18	97.46	86.95	51	?
April	19	62.28	47.86	48	55
May	18	97.06	45.30	194	?
June	18	122.39	64.73	154	?
July	19	113.19	46.97	95	48
August	19	96.02	55.63	137	138
September	19	83.28	49.84	60	101
October	19	59.83	37.90	78	29
November	19	62.3	35.50	47	102
December	18	56.5	29.63	28	25

Figure 24a: Distribution of monthly total rainfall during the period 1972 - 1990

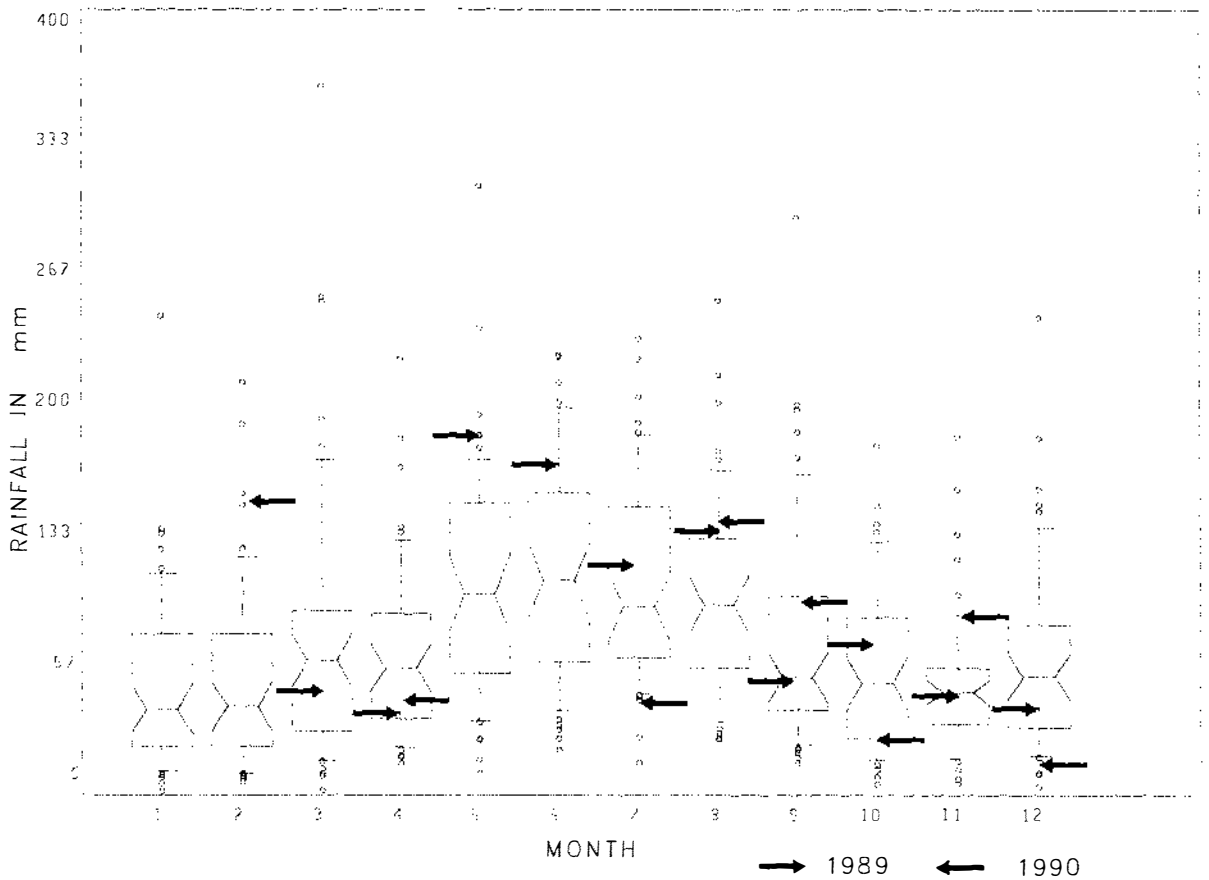


Table 9b: Average ratio of minimum and maximum daily temperature summarized over the period 1972 until 1990

Month	n	x	s.d.	1989	1990
January	18	0.663	0.0387	?	?
February	19	0.690	0.0047	?	0.718
March	18	0.694	0.0035	0.645	?
April	19	0.666	0.0043	0.649	0.589
May	15	0.633	0.0049	0.696	?
June	15	0.616	0.0037	0.574	?
July	17	0.577	0.0052	0.491	0.637
August	18	0.595	0.0498	0.685	0.659
September	18	0.609	0.0041	0.664	0.573
October	18	0.608	0.0034	0.653	0.637
November	19	0.636	0.0033	0.703	0.651
December	18	0.668	0.0030	0.648	0.659

Figure 24b: Distribution of monthly average ratio between minimum and maximum daily temperature during the period 1972 - 1990

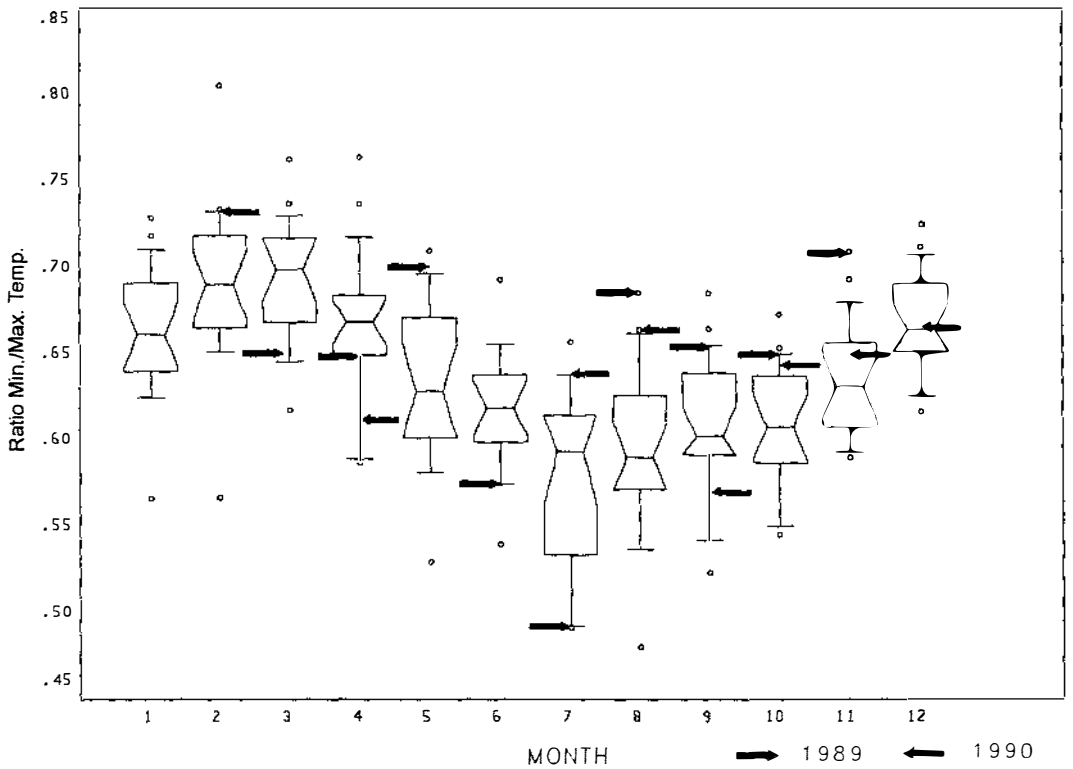
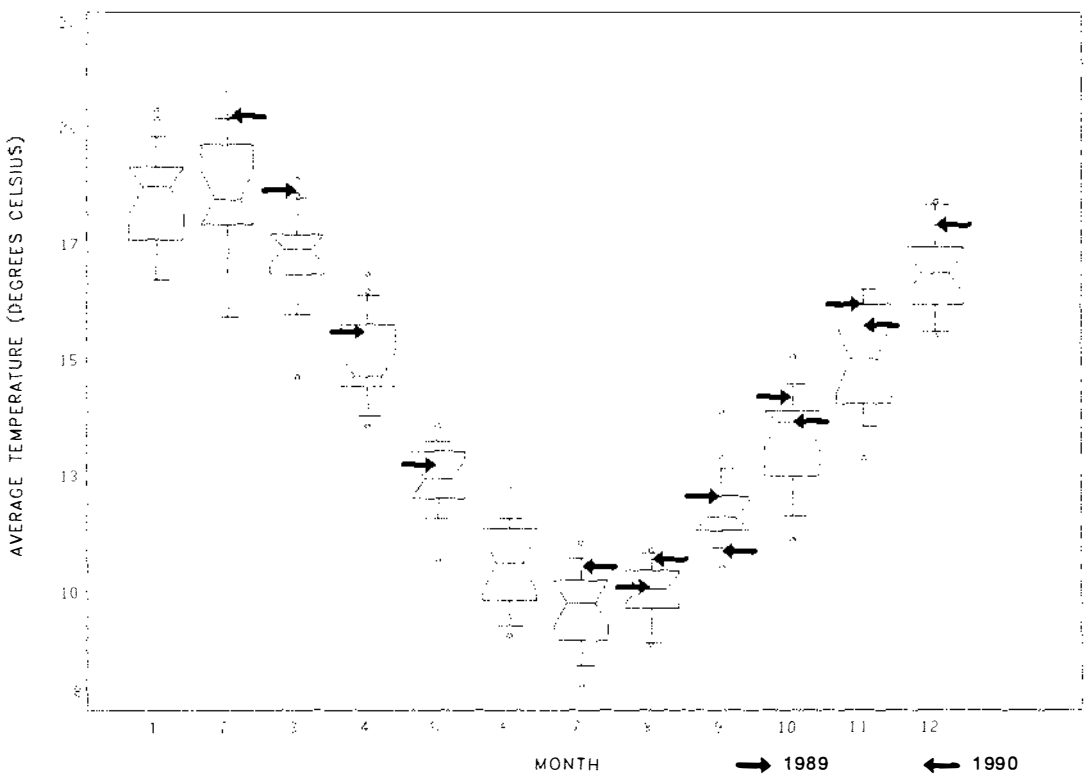


Figure 24c: Distribution of monthly average daily temperature during the period 1972 - 1990



POSSUM ECOLOGY

Trapping Statistics

The catch effort over the period under consideration totalled 26779 trap catch units over 108 trap nights and 22 visits, with an average catch success of 0.21 (see figure 25a). In total, 378 possums have been captured and tagged, or were found dead and untagged within the study area. On average 58.8 possums were clinically examined per visit with a range from 24 to 86 (see figure 25b).

Figure 25a: Trapcatch statistics

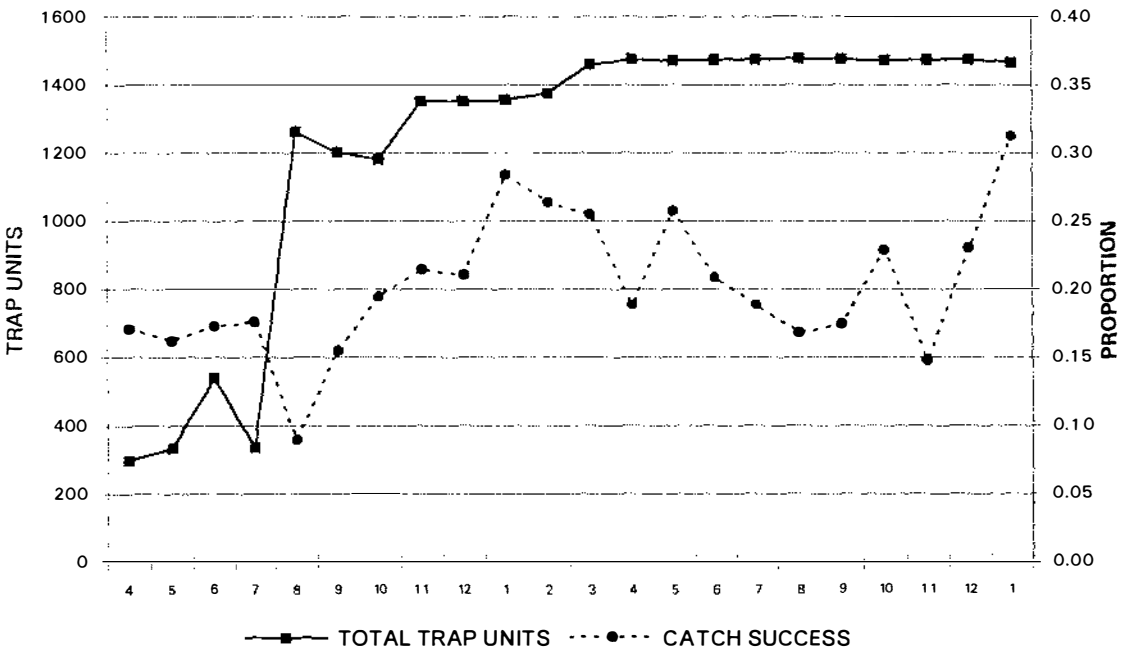
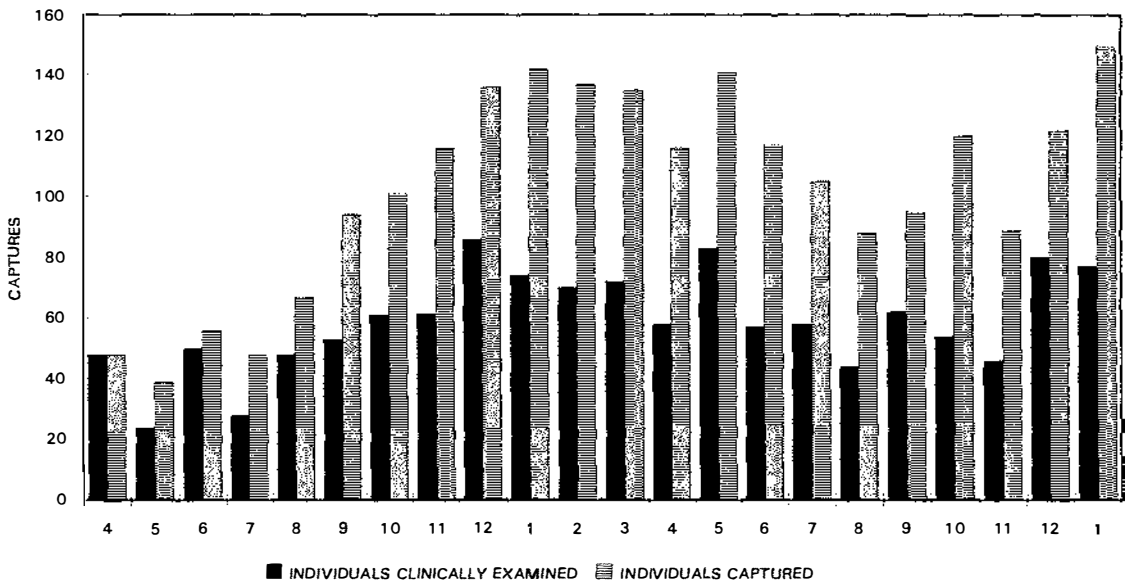


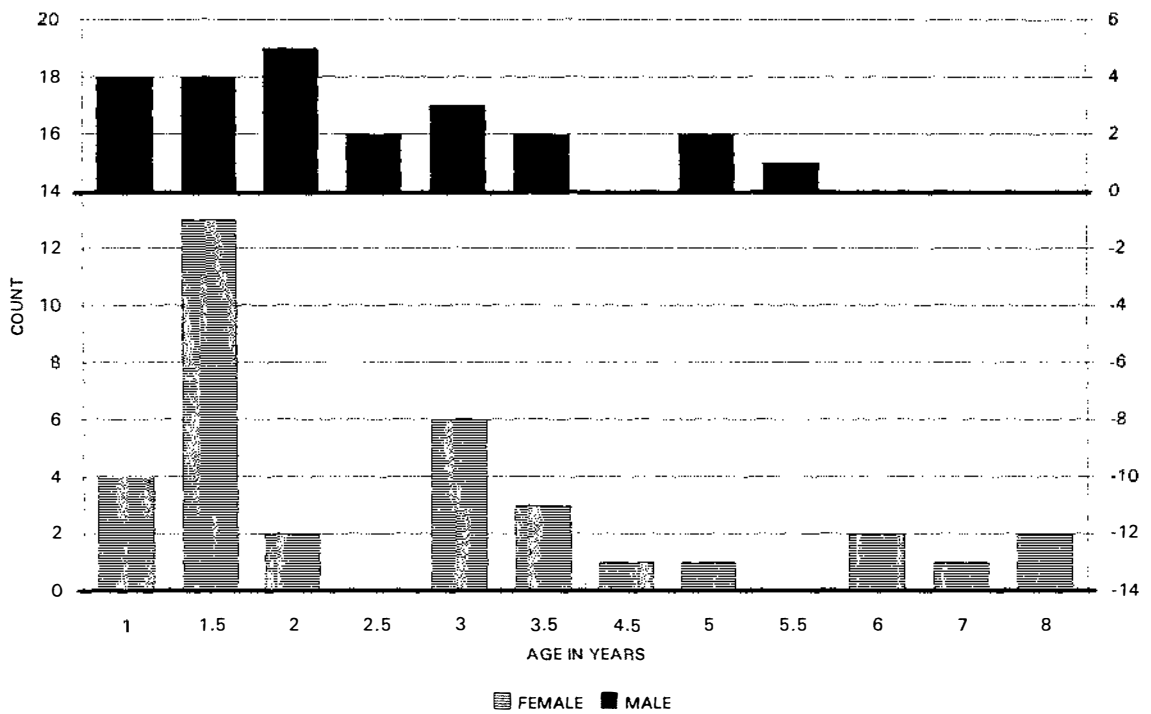
Figure 25b: Individual possum captures and clinical examinations



The proportion of female possums in the sample was 41%, 57% were males and 2% of unspecified sex. Using only data for the time span February 1990 until January 1991 21.4% of 84 female possums and 26.2% of 122 male possums were classified as sexually immature based on their last examination. During this same period 63% of a total of 51 immature possums were of male sex.

A sample of 61 possums which died during the study period were aged based on tooth cementum layer patterns. The average age of possums at post mortem was 2.7 years (s = 1.73). On average males (x = 2.46, s.d. = 1.32, x_m=2, n = 23) were younger than females (x = 2.87, s.d. = 2.01, x_m=2, n = 35; p = 0.42; see figure 26).

Figure 26: Distribution of ages in possums at post-mortem stratified by sex class

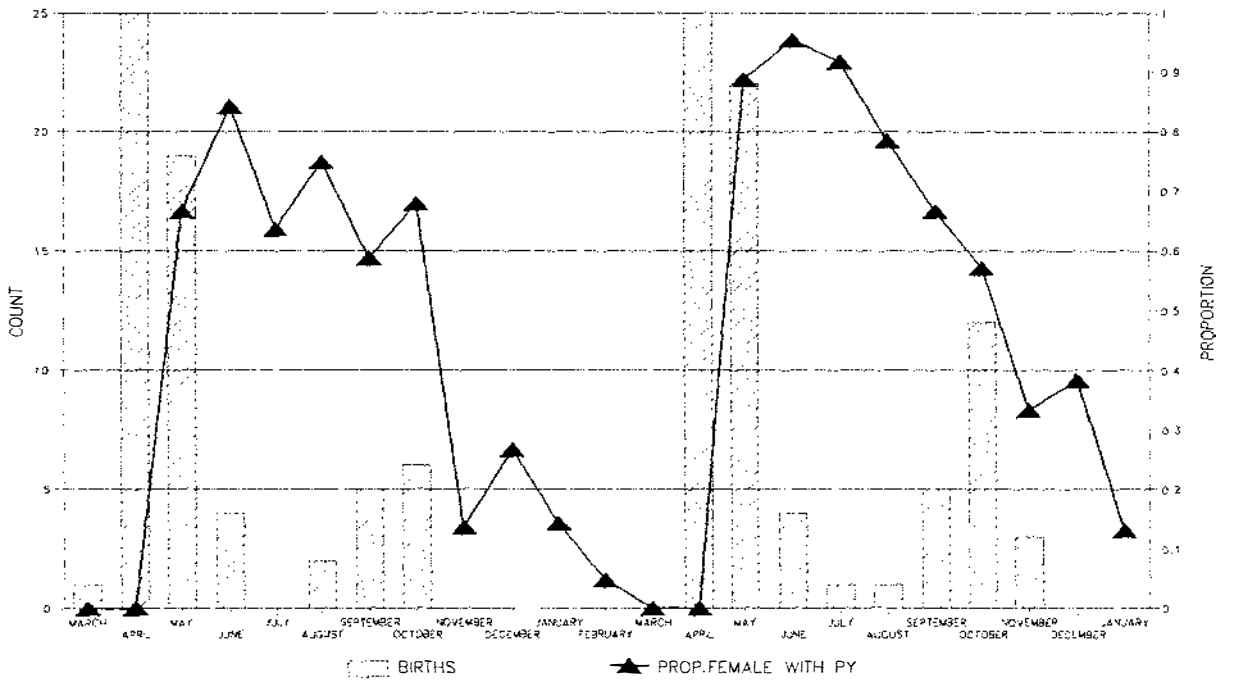


A comparison of the age index based on tooth wear and the age estimated from decalcified teeth was made for dead animals. There was a correlation of 0.68. The discrepancy appeared to be greatest for ages between 2 and 4 years.

Reproduction

The proportion of females with pouch young among total adult females was highest in June 1989 with 0.84 (N=19) and declined to 0.0 in March 1990. It peaked again in June 1990 with 0.95 (N=22). The proportion of juveniles within total new captures per visit increased from a low of 0.09 (n=23) in August 1989 to a high of 1.00 (N=8) in March 1990. This figure is of course influenced early in the study by the fact that in the first few monthly visits many resident possums were being captured for the first time. The proportion of juvenile possums contributing to total captures was lowest in October and November 1989 with 0.08 (N=230, N=290) and highest in May /June 1990 with 0.22 (N=379,N=308) and again in January 1991 with 0.21 (N=458). Figure 27 shows the temporal distribution of the proportion of pregnant animals in total number of adult females captured on a particular visit and the number of births as estimated from pouch young head lengths.

Figure 27: Fertility distribution of births and rearing periods in possums



During the study period pregnancy and rearing episodes were recorded for 87 of 119 adult female individuals. Of these, 22 consisted of less than 3 successive examinations. Forty-eight births appeared to have produced an independent offspring successfully, 31 of which were subsequently captured independently and permanently identified. During 5 parities the joey died before independence and in 82 cases the outcome was unknown. Two females reared 2 pouch young sequentially during the study period, and both progeny were recaptured subsequently. In five cases 4 and in 2 cases 5 pregnancy episodes were recorded. The average interval between births based on data from 45 episodes was 274 days with a minimum of 141 and a maximum of 560 days. Based on data from 30 parities where the joey appeared to have been reared successfully until independence, pouch young spent on average between 145 (s.d.=25.05; based on last capture with mother) and 197 (s.d.=22.2; based on first examination of mother without pouch young) days in close relationship with their mothers. Of the cohort of 19 female possums with known birth date (April-June 1989), so far (January 1991) 2 animals have had a first pouch young. Possum D3644 was born on 4.6.89 and had a joey when examined 16 months later on 14.10.90. Possum D3693 (born 21.4.89) had its first joey 14 months later when seen on 11.6.90. Males with known birth date reached sexual maturity as estimated by a testis width of at least 14mm on average at 493.7 (min=389, max=551, n=6) days after birth. The average weight at that time was 1.98 kg.

Analysis of survival of 30 known joeys revealed that 33% disappeared during the months following independence from their mother, on average at 322 days (s.d.=102.1) after birth. There was no statistically significant difference between sex classes.

Population Dynamics

Summary information over all visits was based on data beginning with visit number 5, when monthly trapping effort first exceeded 1000 trap units. Jolly-Seber estimates of survival probability between successive visits and population size averaged 0.93 (s.d. 0.04) and 148.8 (s.d. 16.4) respectively. Immigration plus births averaged 9.83 (s.d. 6.94) animals per visit. Figure 28a describes the temporal dynamics of these three estimates. In figure 28b the y-axis is scaled down to allow comparison of immigration/births with disappearance. Survival probabilities were converted into numbers of animals which had disappeared. Over the study period disappearance of possums exceeds immigration and births during most months. The population size based on Otis' closed population model m_h averaged 152.22, with a range of 87 to 185. Figure 28c shows a comparison of the Jolly-Seber and Jackknife estimates of population size over time. The results of the tests for population closure and of the statistical assumptions for the Jackknife estimator are listed in table 10.

Figure 28a: Temporal dynamics of Jolly-Seber population parameters for the possum population

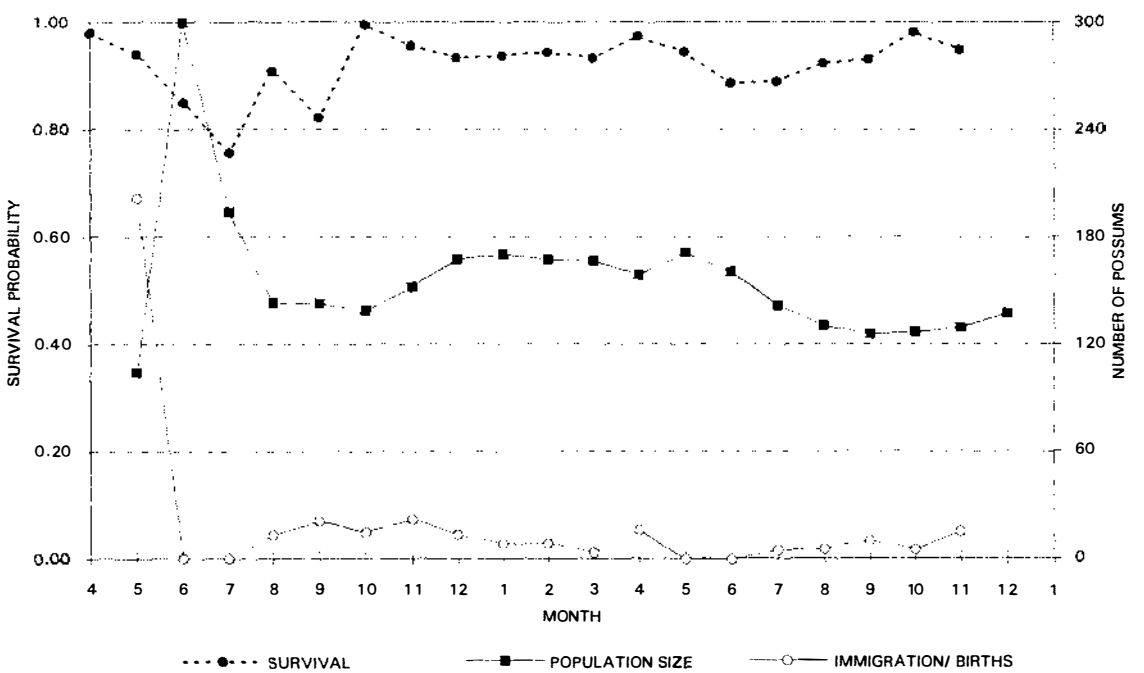


Figure 28b: Temporal dynamics of Jolly-Seber population parameters for the possum population emphasising relationship immigration/births and disappearance

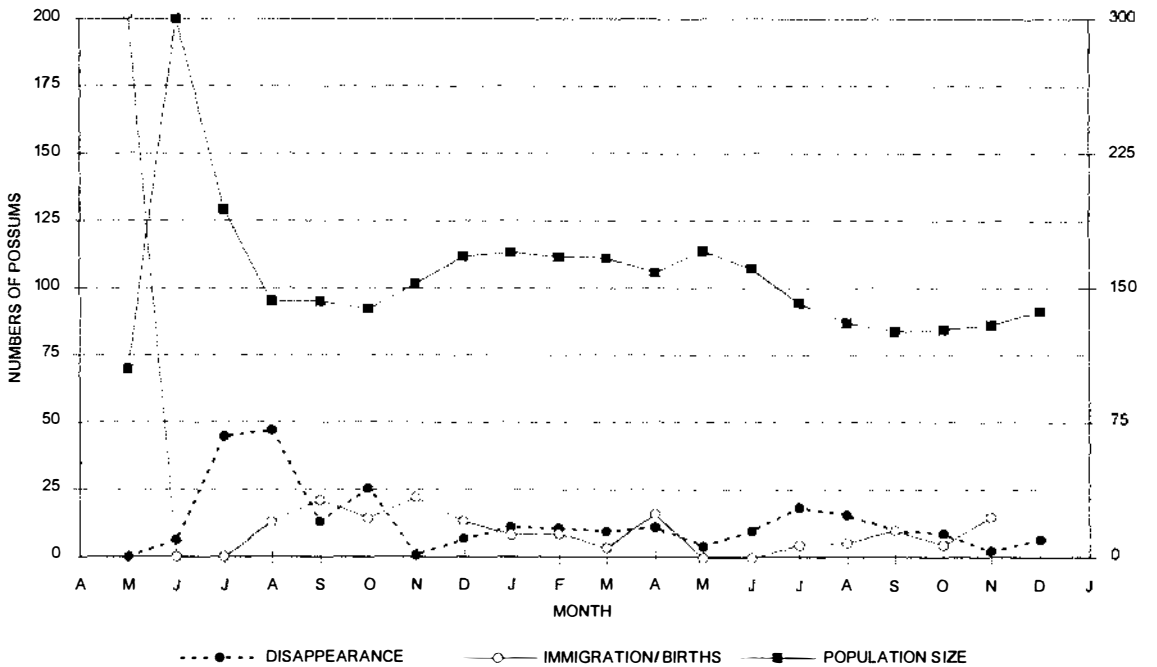


Figure 28c: Comparison of population size estimates based on jackknife (with 95% confidence limits) and Jolly-Seber estimator

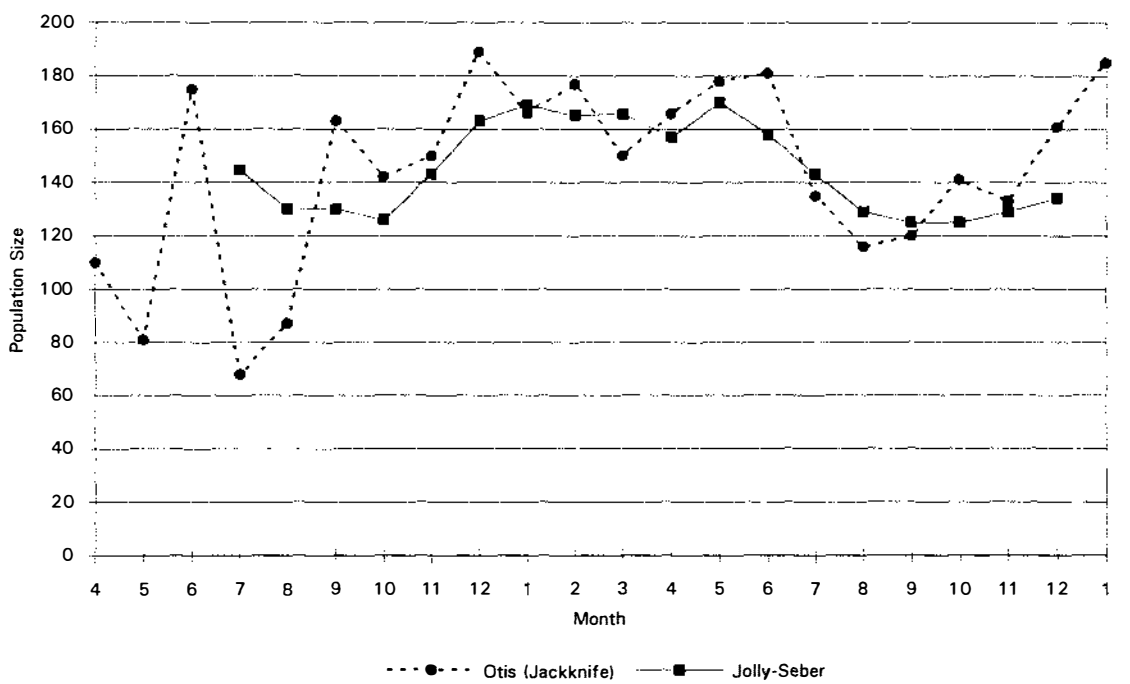


Table 10: Statistical tests of the assumptions for Jackknife estimator

Visit	Month	Test of Closure	Heterogeneity	Trap Response	Time Variation	Trap Response and/or Time Variation given Heterogeneity
1	4	0.186			0.000	0.000
2	5	0.153	0.365		0.001	0.027
3	6	0.028	0.103		0.000	0.007
4	7	0.999		0.086	0.640	0.000
5	8	0.513	0.251		0.226	0.000
6	9	1.000	0.000	0.019	0.029	0.000
7	10	1.000	0.002	0.025	0.084	0.000
8	11	1.000	0.000	0.156	0.014	0.000
9	12	1.000	0.000	0.281	0.406	0.000
10	1	1.000	0.000	0.066	0.019	0.000
11	2	1.000	0.000	0.431	0.044	0.000
12	3	1.000	0.000	0.694	0.003	0.000
13	4	1.000	0.000	0.000	0.000	0.000
14	5	0.987	0.000	0.001	0.083	0.000
15	6	0.999	0.000	0.000	0.000	0.000
16	7	1.000	0.000	0.210	0.005	0.000
17	8	1.000	0.000	0.000	0.000	0.000
18	9	1.000	0.000	0.000	0.000	0.000
19	10	1.000	0.000	0.001	0.001	0.000
20	11	0.847	0.000	0.370	0.016	0.000
21	12	1.000	0.000	0.000	0.000	0.000
22	1	0.901	0.000	0.845	0.182	0.000

General Body Condition

Body weight in adult possums ranged from 1.4 kg to 3.3 kg in females and 1.5 kg to 4 kg in males. Total length in adult possums ranged from 54 cm to 86 cm in females and 64 cm to 85 cm in males. A distinct seasonal pattern in average weight can be described in adult male possums which reach their best condition during the period January - March and their lowest body weight during the early winter months. Body weight in adult female possums does not show an apparent seasonality. Body weight in immatures increases gradually from independence in spring (autumn births) until they reach maturity a year later (see figure 29a). Average body weight in adult male possums was positively correlated with Jolly-Seber survival estimates (r = 0.751, N=18). The effect of sex and season on body weight was analyzed using repeated measures analysis of variance. Differences between sex classes were tested using possum number nested within sex class as the error term. The changes in weight between the four seasons of the year for the sex classes were tested for equality using the interaction between season and individual possum number nested within sex class as the error term. The results suggest that weights were different between seasons (F=45.90, p=0.0001) and the effect of season was different for each sex class (F=9.96, p=0.0001; see figure 29b). No statistically significant difference between sex classes was found when weight measurements were pooled over season (F=3.55, p=0.0614). A total of 171 possums (with 1091 observations between them) was used for the analysis.

Figure 29a: Temporal pattern of average body weights of possums

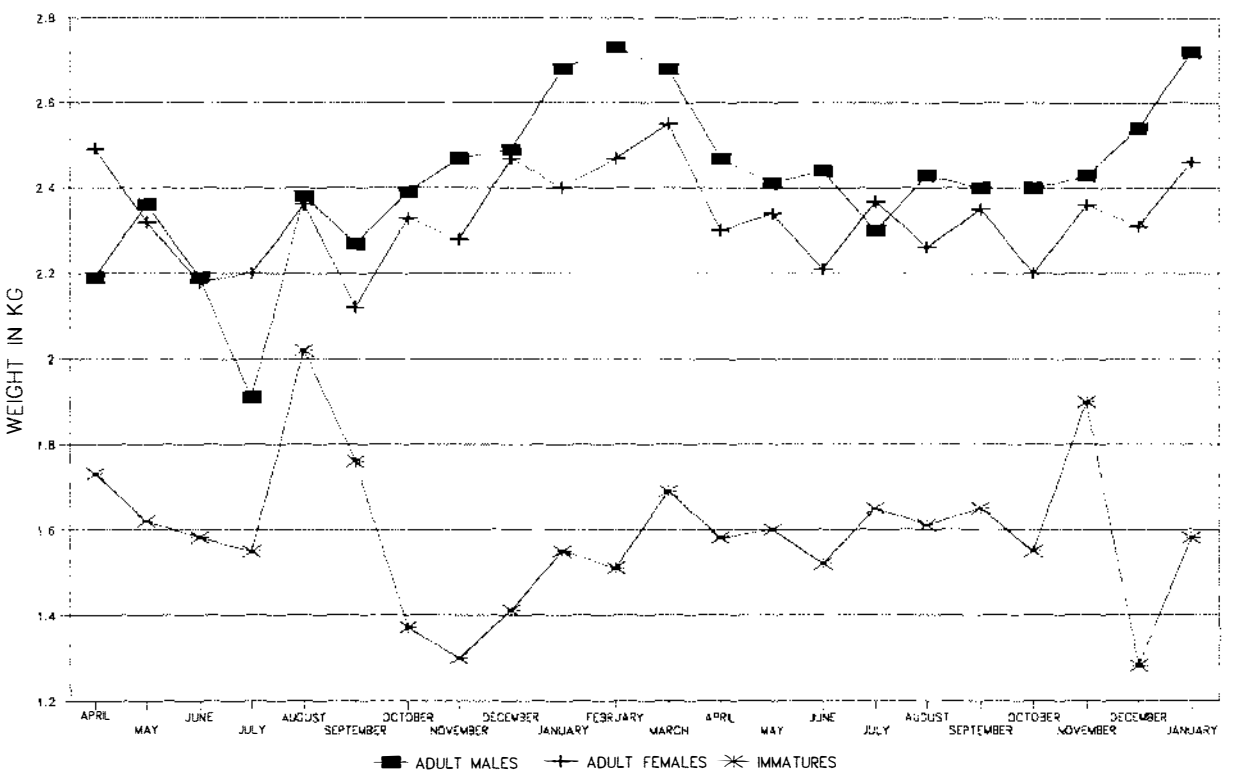
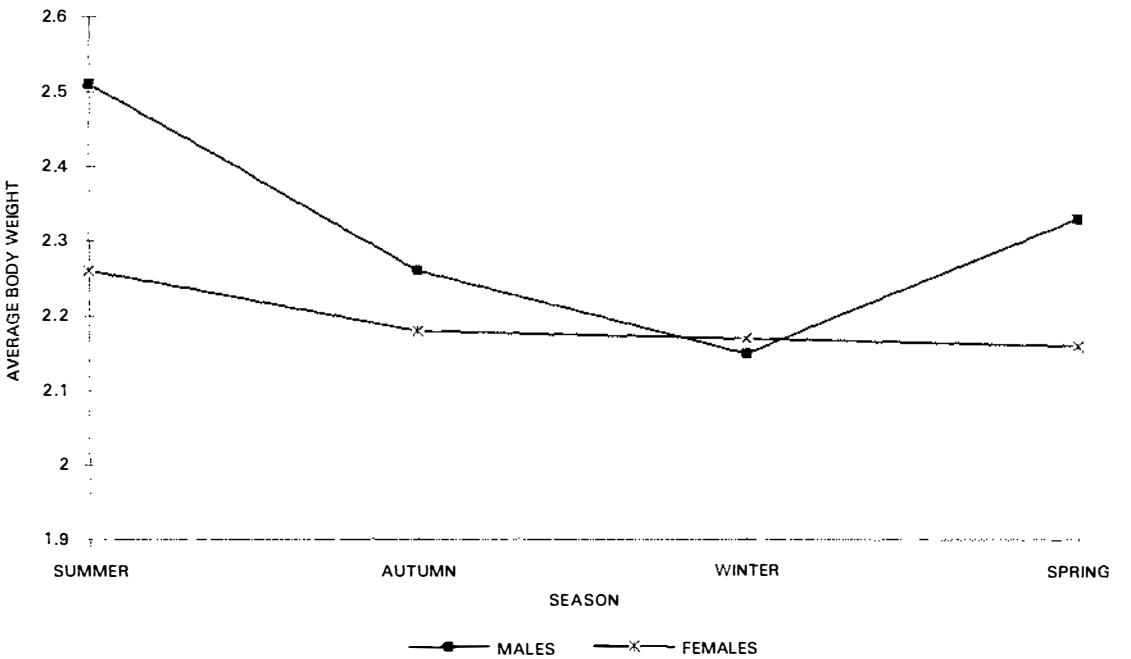


Figure 29b: Seasonal pattern of average body weights of adult possums stratified by sex class



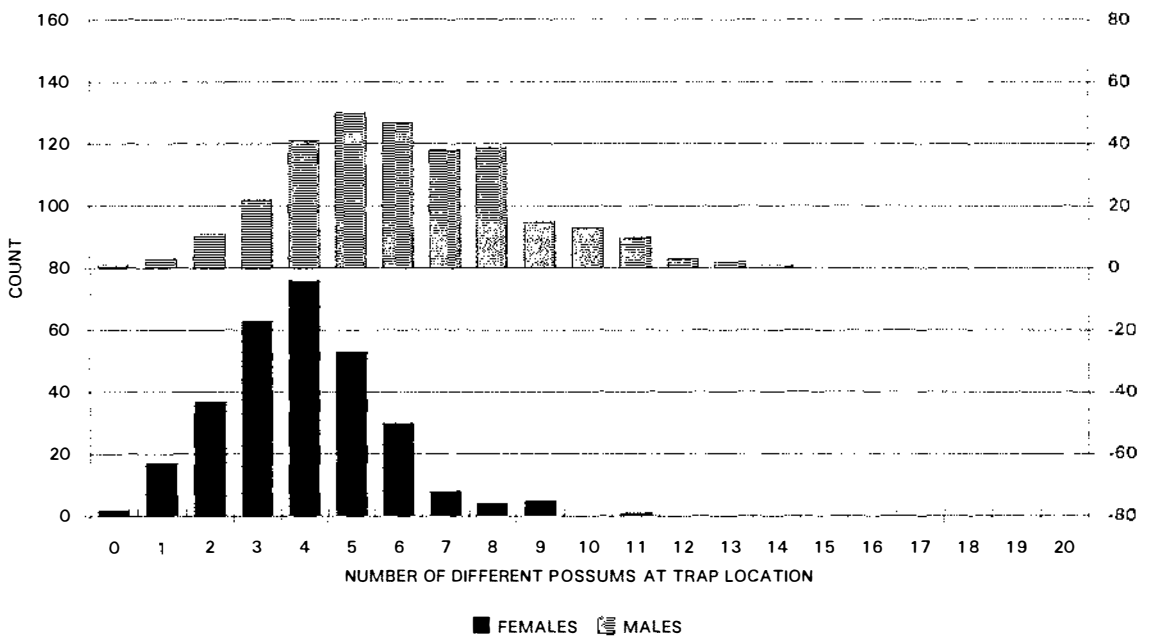
Home Range

Four different estimators of home range were calculated for the adult sex classes. For all methods the size of the female home range was significantly smaller than the area which was used by adult males (see table 11). Using the number of different individual possums captured per trap location both indices of dispersion suggest that males ($I=0.946$, $p > 0.10$, $I_p=-0.17$; $x=6.16$, $s.d.=2.41$, $N=295$) are randomly distributed and females have a tendency towards a uniform spatial distribution ($I=0.725$, $p < 0.025$, $I_p=-0.52$; $x=3.99$, $s.d.=1.70$, $N=294$; see figure 30).

Table 11: Home range estimates for adult males and female possums

	Females			Males			
Method	n	x (ha)	s.d.	n	x (ha)	s.d.	p
Convex	18	1.82	1.03	29	3.27	2.58	0.0099
95% ellipse	18	2.90	1.75	29	4.73	3.14	0.014
30% harmonic	18	0.14	0.09	29	0.25	0.20	0.0177
60% harmonic	18	0.55	0.35	29	0.89	0.59	0.0176
90% harmonic	18	1.83	0.98	29	2.91	2.08	0.0211
Activity radius (m)	18	56.69	26.27	29	68.80	21.78	0.0619

Figure 30: Distribution of number of different possums captured at individual trap locations stratified by sex class



Denning

During the study period radio collars were attached to 103 animals. Den site information is available for 91 individuals. On average possums were tracked to their dens 3.9 times (s.d.=3.47) and to 3.22 (s.d.=2.53) different dens. There was a positive correlation of 0.96 (p = 0.000) between den site tracking effort and the number of different den site locations which were identified for each possum (see figure 31a). Analysis of covariance revealed that there was no statistically significant difference in number of different den sites between sex and age groups. Of 252 individual den sites 221 dens were used by only one possum, 26 by 2, and 5 by 3 possums. Of 31 cases of den site sharing, in 4 situations possums shared a den simultaneously. Three of these were mothers sharing with their joey. Possums denned mainly

on the ground on hill sides and sides of gullies where suitable cover is available. 43% of den sites were in flax bushes, 17% in root rakings and 13% in gorse.

The maximum distance between den sites used was calculated for individual possums. Data was available for 61 animals. Using multiple least squares regression, maximum distance between den sites was regressed on individual den site tracking effort (x_{track}) and sex class (x_{sex}) as a dummy variable. The regression coefficients of both independent variables were statistically significantly different from zero (x_{track}=18.09, s.e.=3.54, p=0.000; x_{sex}=-50.84, s.e.=27.67, p=0.071; R^2=0.36; p=0.000). The average of the maximum distance between den sites was 175.3m (s.d.=153.77, x_m =124.95, n=33) for males and 102.7m (s.d.=85.15, x_m =75.78, n=28; p =0.0242; see figure 31b).

Figure 31a: Scatter plot of den site tracking effort and number of different den locations per possum

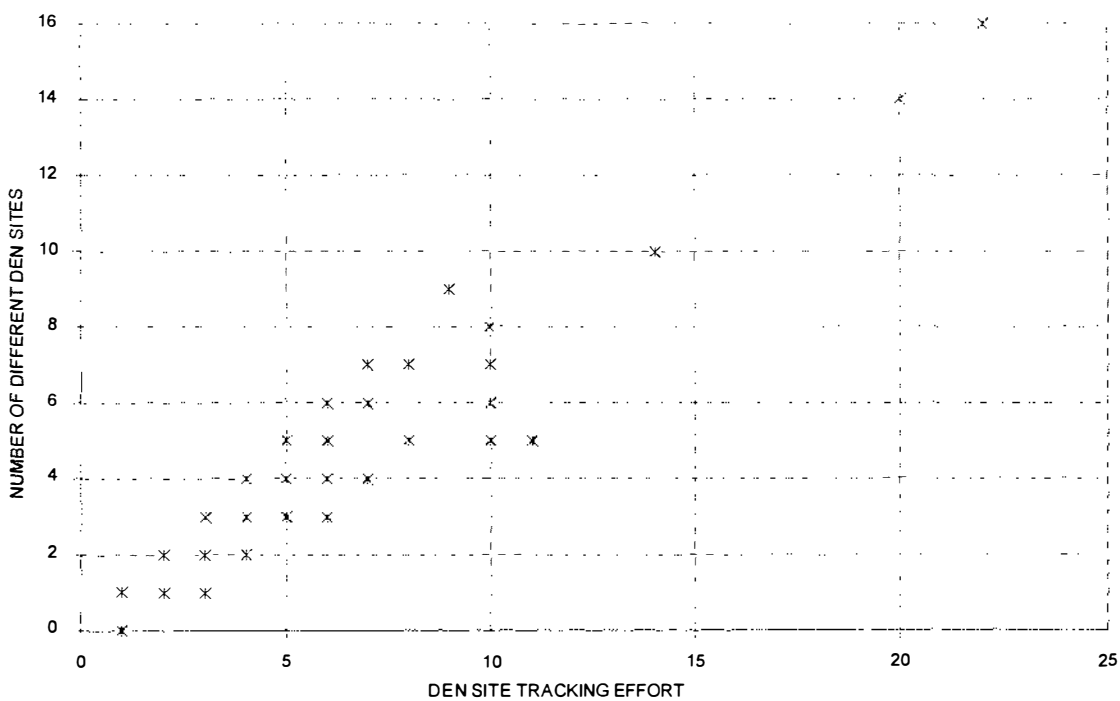
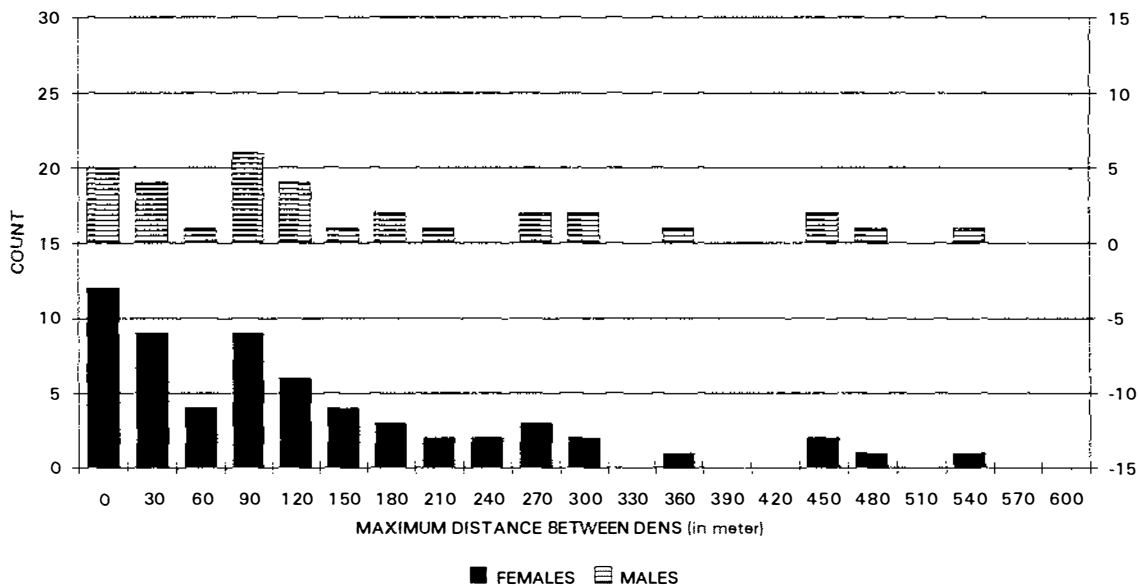


Figure 31b: Histogram of maximum distance between den sites stratified by sex class



Immigration

Estimates of immigration varied over time. During the last 12 months of the study period (December 1989 - January 1991), on average 6.3 new juvenile possums (known pouch young and apparent immigrants, ie previously unknown juveniles) and 3.7 new adults were captured per visit (see figures 32a). The proportion of males in juvenile immigrants was 0.77 (N=103), which was statistically significantly higher than the proportion of males in locally recruited juvenile possums, 0.49 (N=33; p = 0.002; see figure 32b). The proportion of total newly captured possums which were not subsequently recaptured was highest during the summer months and at a minimum during winter (see figure 32c).

Figure 32a: Captures of new possums stratified by age groups

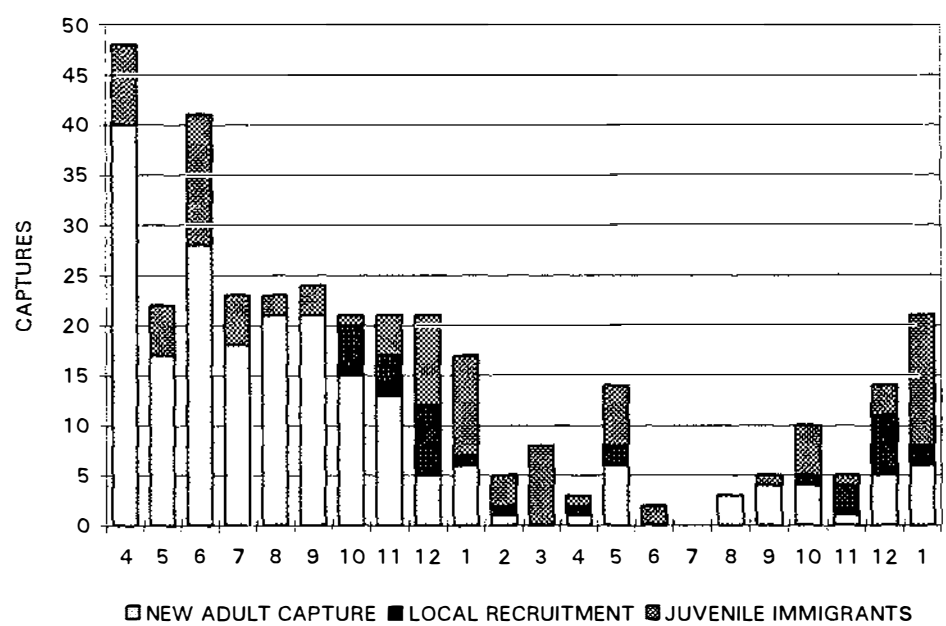


Figure 32b: Captures of new juvenile possums stratified into immigrants and locally recruited animals

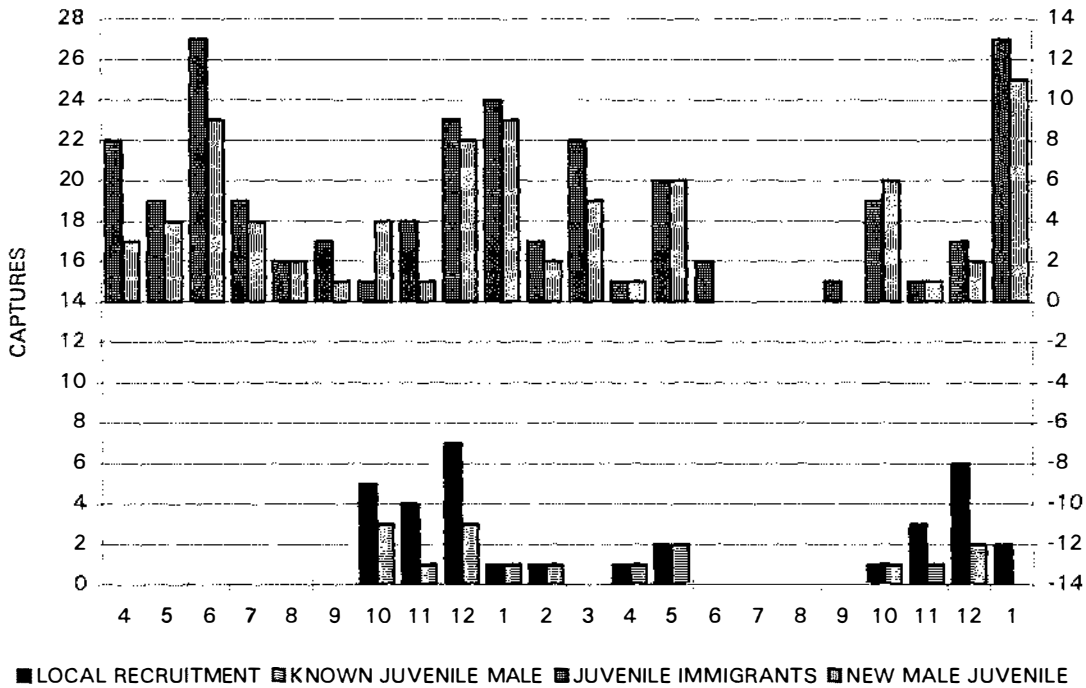
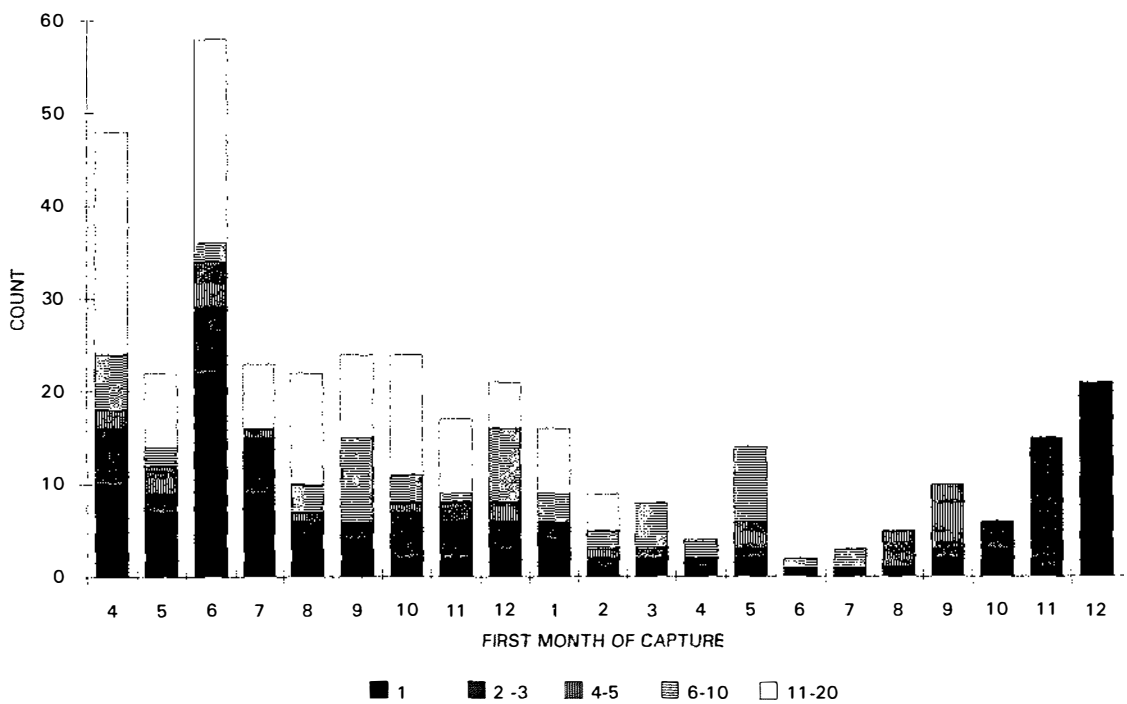


Figure 32c: Number of months for which possums were recaptured after initial capture depending on month of first capture



Dispersal

Four radiotagged possums were tracked to den sites which were distances of up to 1.7 km away from the main denning area (see figure 33a). Three of these possums were immature males and one of them travelled a distance of about 1678 meter. One mature male possum travelled 1362 meter and was shot in August 1990 by the manager of the neighbouring farm. It had been captured regularly (19 times) over a period of 14 months (May 1989 - July 1990) within the study area (see figure 33b).

Figure 33a: Distances of possum dispersal movements (dots represent trap site and den site locations)

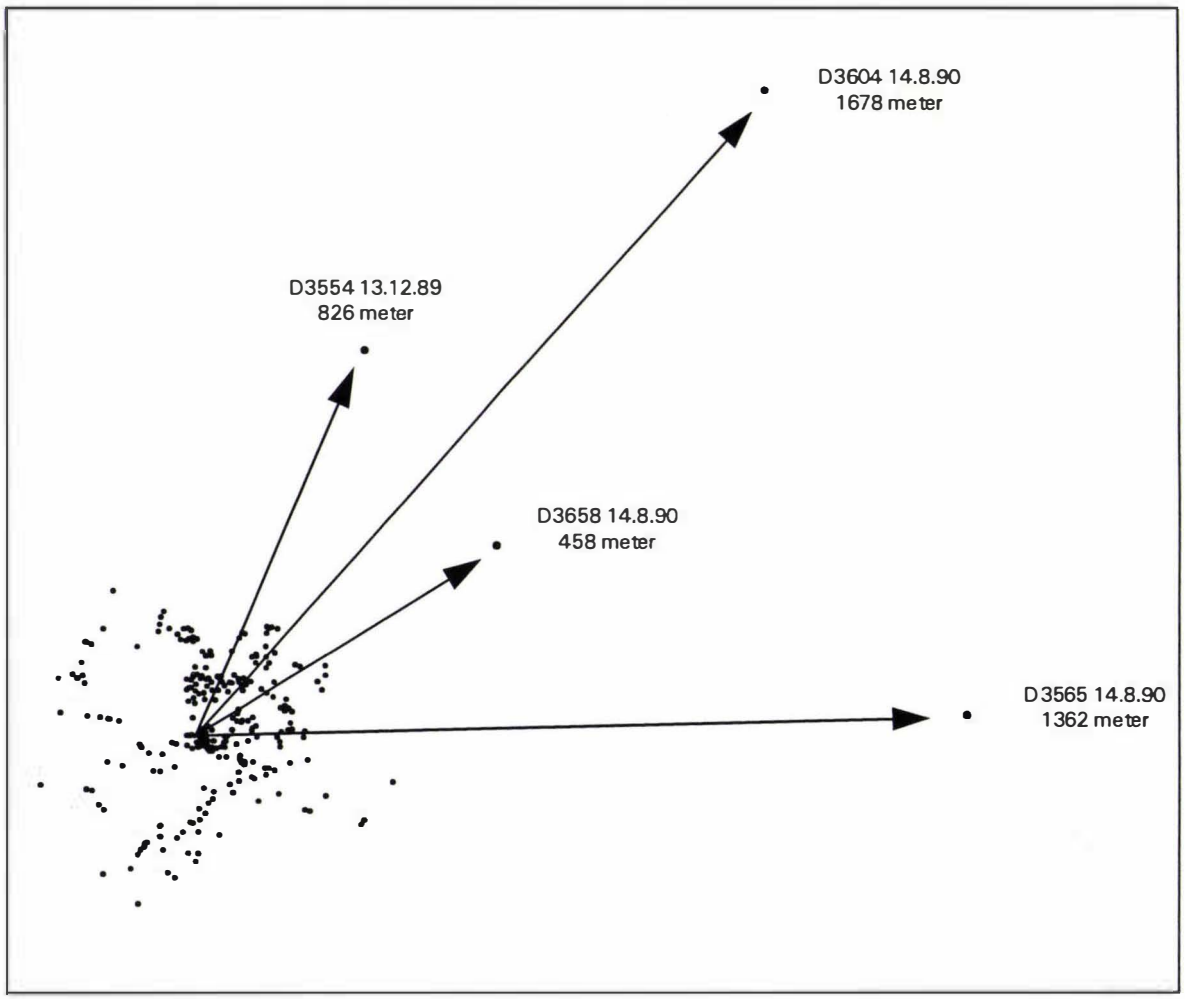
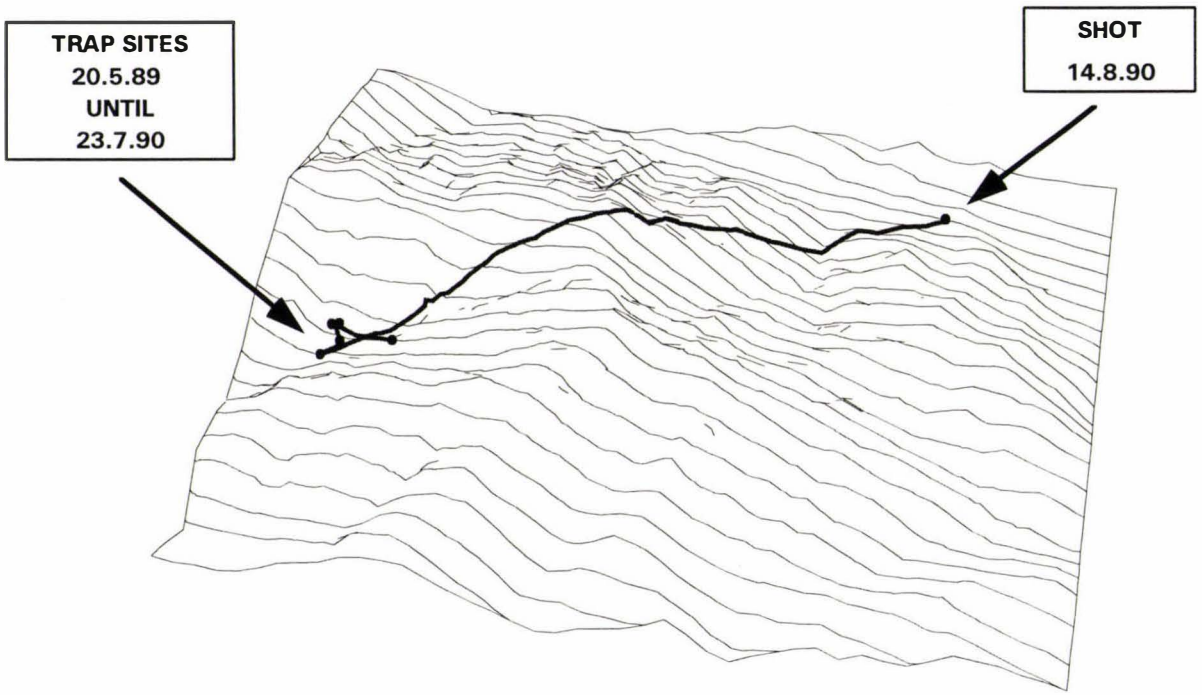


Figure 33b: Location data available for possum no. D3565

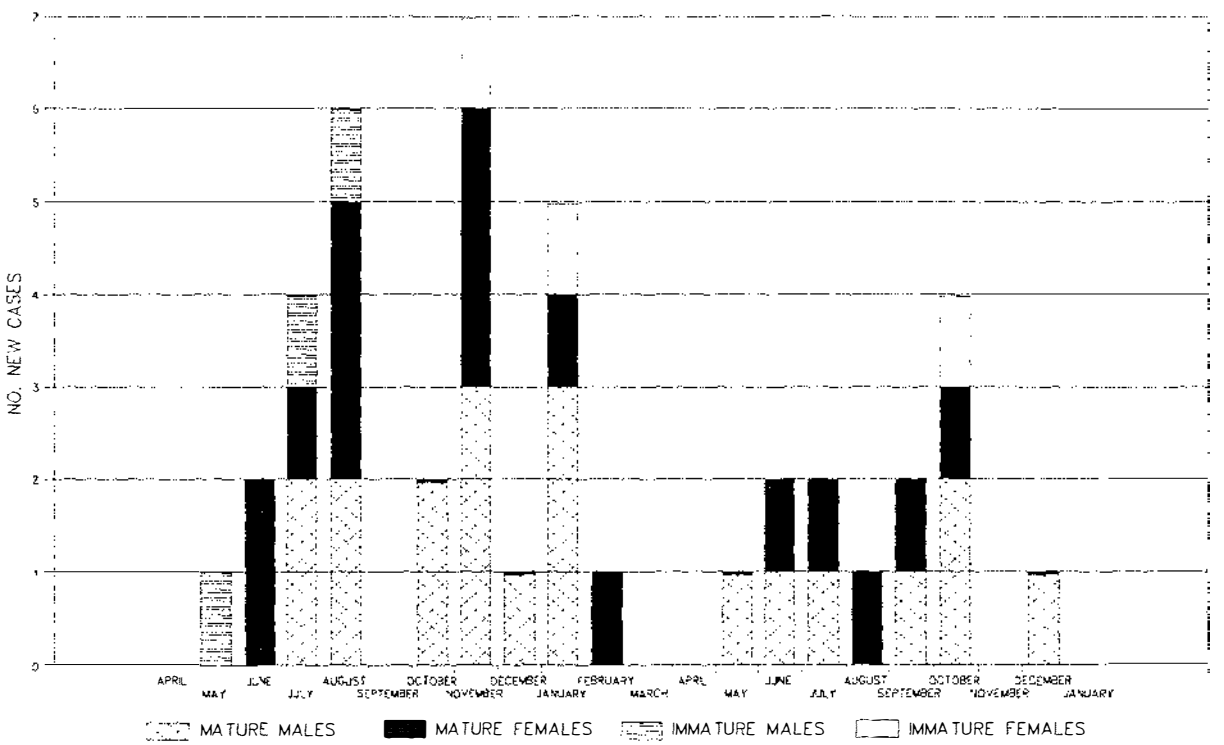


TUBERCULOSIS EPIDEMIOLOGY

Descriptive Epidemiology

To the end of the period studied (January 1991) 39 of 378 possums have been confirmed as infected with *Mycobacterium bovis* by bacteriological examination. Period prevalence based on these findings was 0.11 (95%CI 0.073 - 0.134) over 22 visits. Average monthly prevalence was 0.08 ranging from 0.02 to 0.18. Average monthly cumulative incidence was 0.03 ranging from 0 to 0.14. Monthly incidence density was estimated as 0.014. The proportion of animals which were found to be tuberculous was 0.05 in immatures and 0.12 in matures (OR=0.38, 95% CI=0.13-1.05, p = 0.0406; see figure 34). There was no difference between sex classes (OR=0.65, 95% CI = 0.32-1.32, p=0.197). Stratification on sex class showed that there was no difference in the risk of developing tuberculous lesions between age groups in female possums (OR=1.80, 95% CI=0.46-8.26, p=0.366). Mature male possums were 3.79 times as likely as immature males to develop clinical tuberculosis (95% CI=0.80-24.53, p = 0.062). The decomposing carcasses of 7 previously unknown possums were found on pasture. From 5 of these animals *Mycobacterium bovis* was successfully cultured.

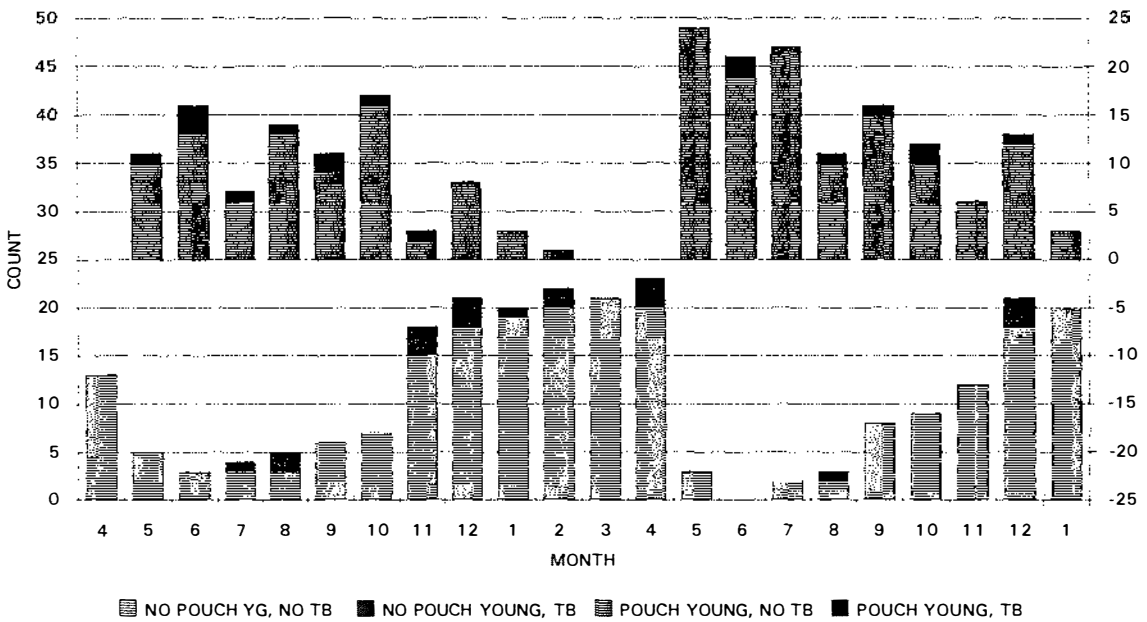
Figure 34: Temporal distribution of new tuberculosis cases in possums stratified by age and sex class



Mean ages of 59 possums which died during the study period did not differ significantly between *Mycobacterium bovis* infected (X_1 =2.96 years, s.d.$_1$=1.91, n_1=24) and non-infected animals (X_2 = 2.54, s.d.$_2$ = 1.61, n_2=35, p = 0.39). The mean age of infected female possums was 3.35 years (n=14) and of males was 2.5 years (n=9, p=0.25). There were no statistically significant differences between infected and uninfected animals of the same sex.

Analysis of tuberculosis incidence in adult female possums did not show a dependence on pregnancy status of the animals (see figure 35). Incidence density averaged 0.027 in adult females without pouch young and 0.023 in adult females with pouch young.

Figure 35: Temporal distribution of incident tuberculosis cases in adult female possums stratified by pregnancy status



Eleven adult female possums with pregnancy episodes were found to be infected with *Mycobacterium bovis*. In 3 cases the joey was recaptured after independence from its mother. Two of these were found to have tuberculosis, the other (D3578) disappeared 2 months after tagging at an age of about 8 months. The joey (D3694) born to the mother D3513 showed tuberculous lesions at an age of about 16 months after having been caught independently from its mother for about a year. The mother was found with lesions at about the same time while she had another (then about 6 months old) pouch young (D2910). She died at about 2 years of age after having weaned this second joey. In another case the mother (D3720) died after weaning the joey. The joey (D3692) was found dead a month later at the age of about 8 months. In another situation a grown up joey (D3644) showed tuberculous lesions at the age of about 15 months. It was found to be pregnant 2 months later. Its mother (D3728) had disappeared 7 months after she had last been caught together with D3644.

Possums suspected of having tuberculosis infection and a number of apparently non-tuberculous possums were tracked to their den sites. In one case, 2 male mature possums were sharing a den site simultaneously. One of them was later confirmed as infected with *Mycobacterium bovis*, but the carcass could not be discovered due to failure of the radio transmitter. The other possum was reported as weak and being in poor body condition when last seen, and the dry carcass was found 4 months later. A swab of the body cavity did not

reveal the presence of *Mycobacterium bovis* on culture. One den site was found which contained the carcasses of 3 possums (one of them eartagged) with the current occupant (radiotagged) resting on top of them. Laboratory examination of a swab taken from the carcasses revealed the presence of *M.bovis*.

Pathological Findings

Mycobacterium bovis was cultured from 28 animals which were identified as probably tuberculous during clinical examination on capture. These palpable or visible lesions in 78.6% (n=22) of cases were associated with the axillary lymphocenter (see figures 36a and b). Seven percent (n=2) each were related to the head lymphnodes, the inguinal and both the axillary and inguinal lymphocenter. Of the 28 animals, 39% (n=11) had open lesions; the others were identified based on enlarged lymph nodes. There was no statistically significant difference in the distribution of external lesion sites between sex classes. Lesions in the maxillary head lymph nodes were only found in 2 males. Open lesions were statistically significantly more common in adult females than in adult males (OR=8.75, $p = 0.02$). Seventy-five percent (n=3) of tuberculous adult females with a pouch young had lesions with open sinuses compared with 57% (n=4) in adult females without a pouch young (OR = 2.25, $p > 0.10$). Post mortems were conducted on 24 tuberculous animals. Of these, 54% (n=13) had external lesions, of which 4 were draining exudate. In 50% (n=14) of cases the disease process involved the lungs (see figure 36c). Ten cases showed lesions in the axillary lymphocenter, 2 in the inguinal lymphocenter, 4 had mediastinal lesions, 2 showed kidney lesions and 1 case had a spinal cord lesion. Two animals macroscopically showed only lung lesions and one appeared to have only lesions in the axillary lymphocenter. Macroscopic lesions in the lung were more common in mature animals than in immatures (OR = 2.2, $p > 0.10$).

Figure 36a: Possum with a draining lesion in axillary lymph center

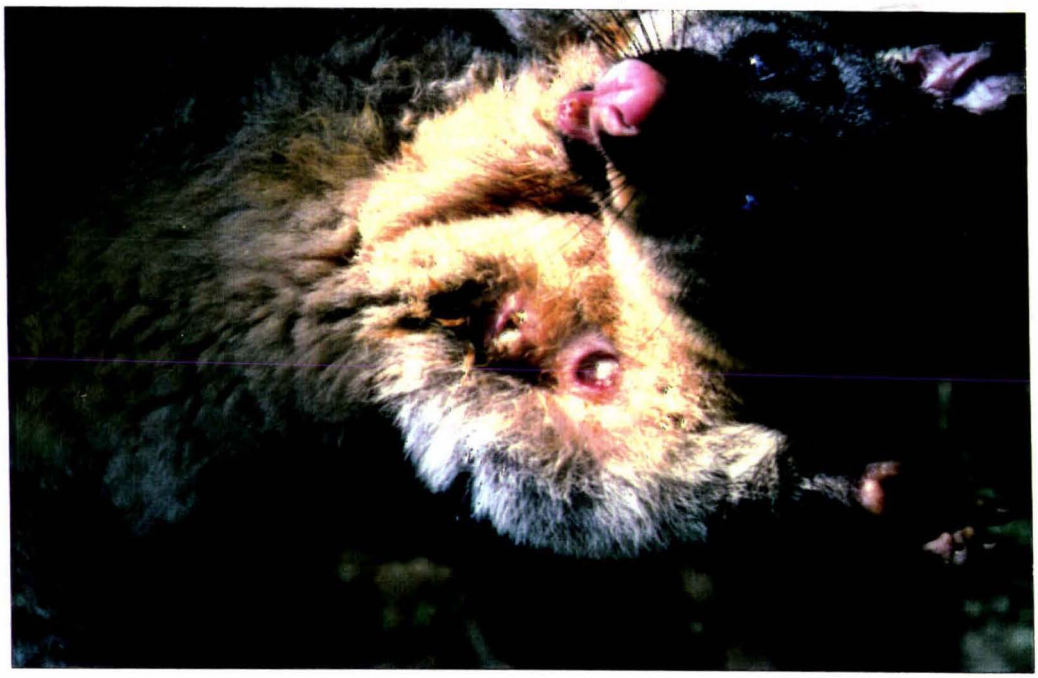


Figure 36b: Tuberculous lesion in axillary lymph node of a possum

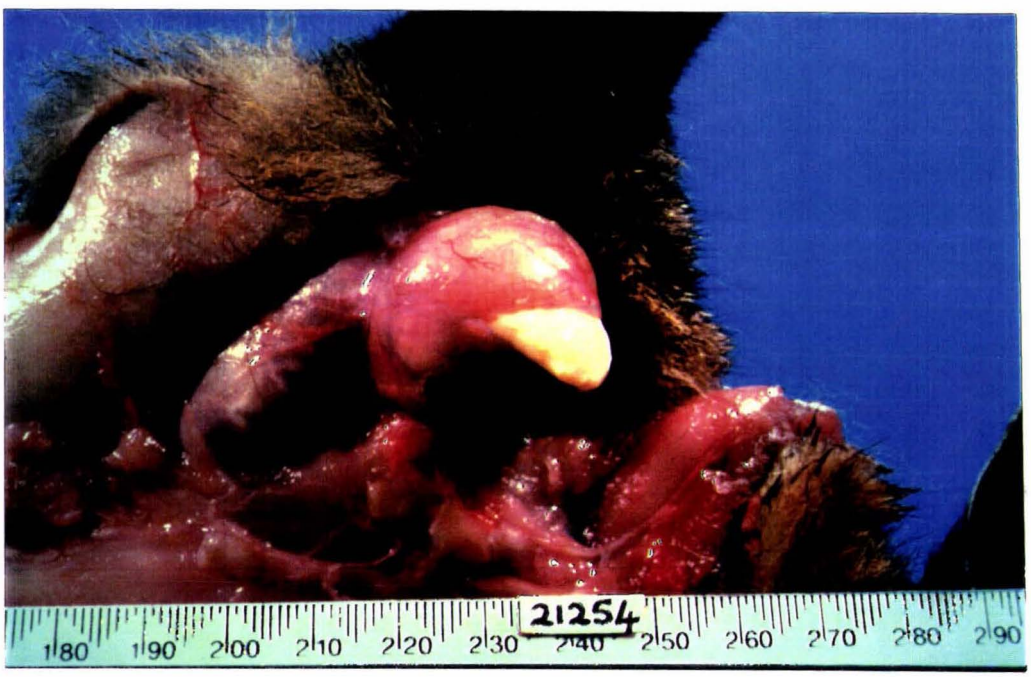
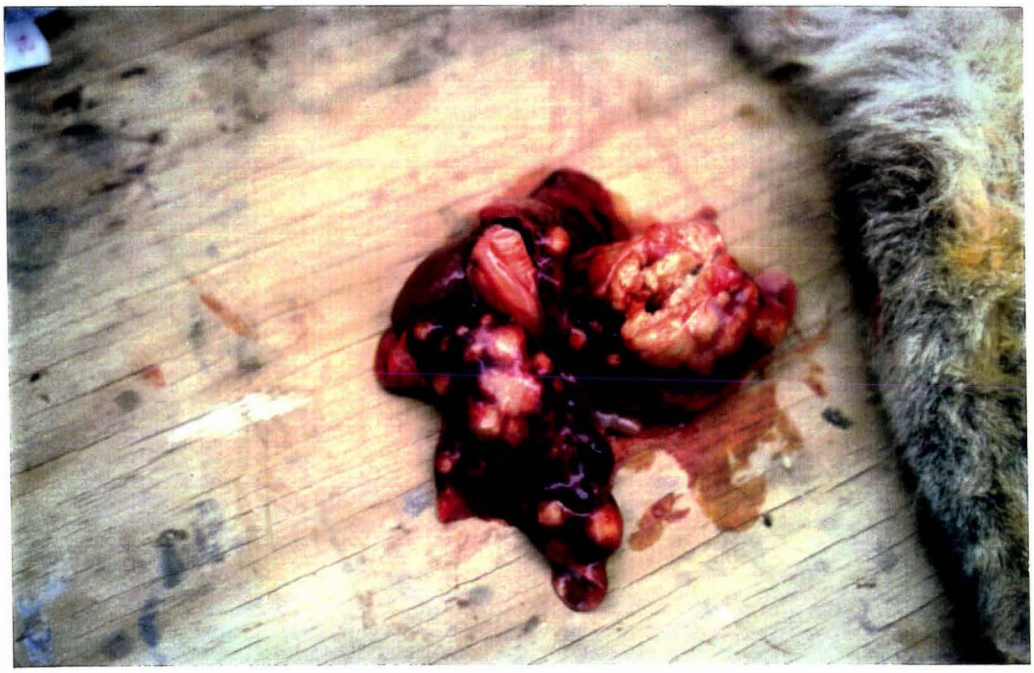


Figure 36c: Tuberculous lesions in the lung of a possum



Survival of Tuberculous Possums

Using the Kaplan-Meier estimator a survival probability for lesioned animals of 0.52 (95% CI: 0.32 - 0.69) for a period of 6 months after entry into the study and of 0.37 (0.2 - 0.55) for 12 months has been calculated. The survival estimates for the apparently noninfected population are based on confirmed deaths and the proportion of possums which have not been subsequently recaptured after a particular visit. The figure for known deaths is certainly an underestimate and the one for disappearance an overestimate. Survival probabilities over 12 months for confirmed deaths and disappearances are 0.96 (0.88 - 0.98) and 0.51 (0.37 - 0.64) respectively. The log-rank test has been used to test if these three survival functions come from the same survival curve. Chi-square tests indicate a statistically significant difference between survival curves at the 5% level (see figure 37). A discrete hazard regression approach was used to test for important covariates with regard to survival of lesioned and non-lesioned possums. Survival time was estimated as time from entry into the study until death or disappearance. Observations of animals surviving past the end of the study period were treated as censored (144 events, 130 censored observations). The final regression model included the time-invariant variable age group and the time-varying covariates lesion status, winter and summer season (see table 12). The time-constant risk factor sex class and the time-varying factors for the other two seasons of the year, presence of pouch young and body weight were not statistically important. No interaction effects were present between predictor variables. The proportional hazards assumption was violated for age group. Therefore the interaction term between log(time) and age group was included into the final model. A stepwise analysis stratified on age group was also conducted. The final model only included the time-varying risk factors, tuberculosis lesion status (HRR=7.25, 95% CI 4.16-12.65,

p=0.0001) and spring season (HRR=1.52, 95% CI 1.03-2.24, p=0.0319). The magnitude of the effects of these factors was similar to the results of the final model of the unstratified analysis. In another analysis possums which had never been recaptured were considered censored information. The final model included the same effects as in the analysis where disappearances were counted as events except for winter season. The magnitude of the effect of tthe time-varying factors tuberculosis infection and spring season increased.

Figure 37: Kaplan-Meier survival curves for infected and uninfected possums

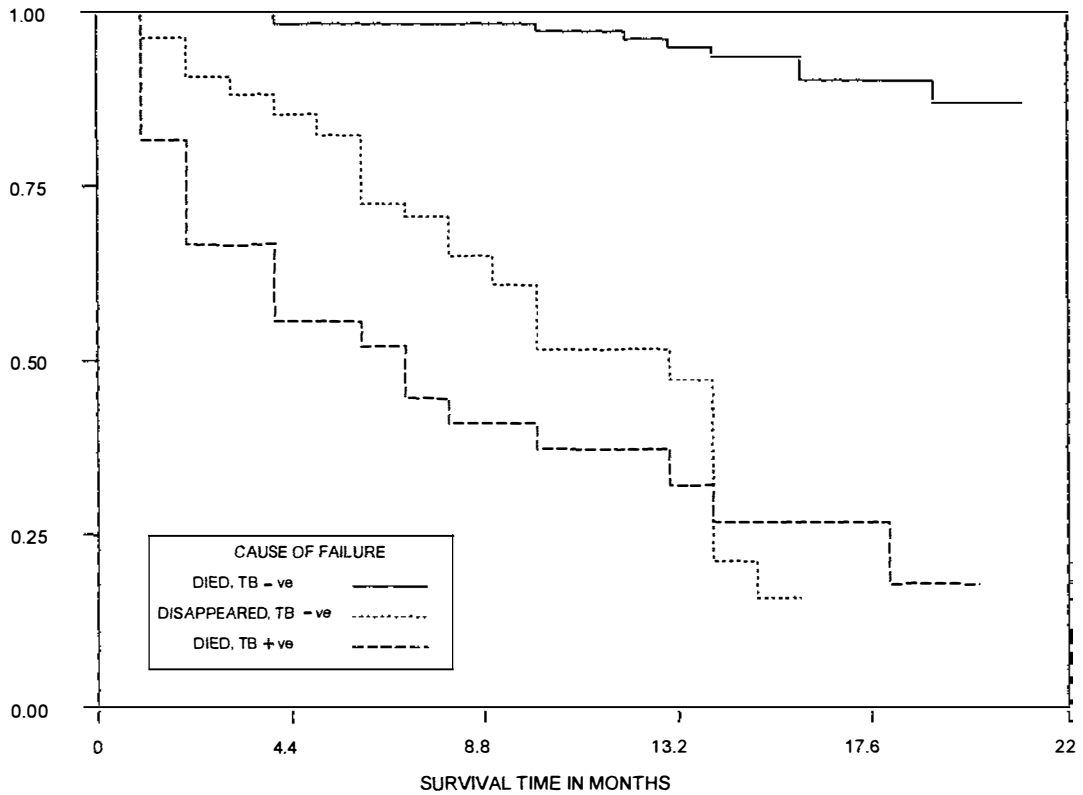
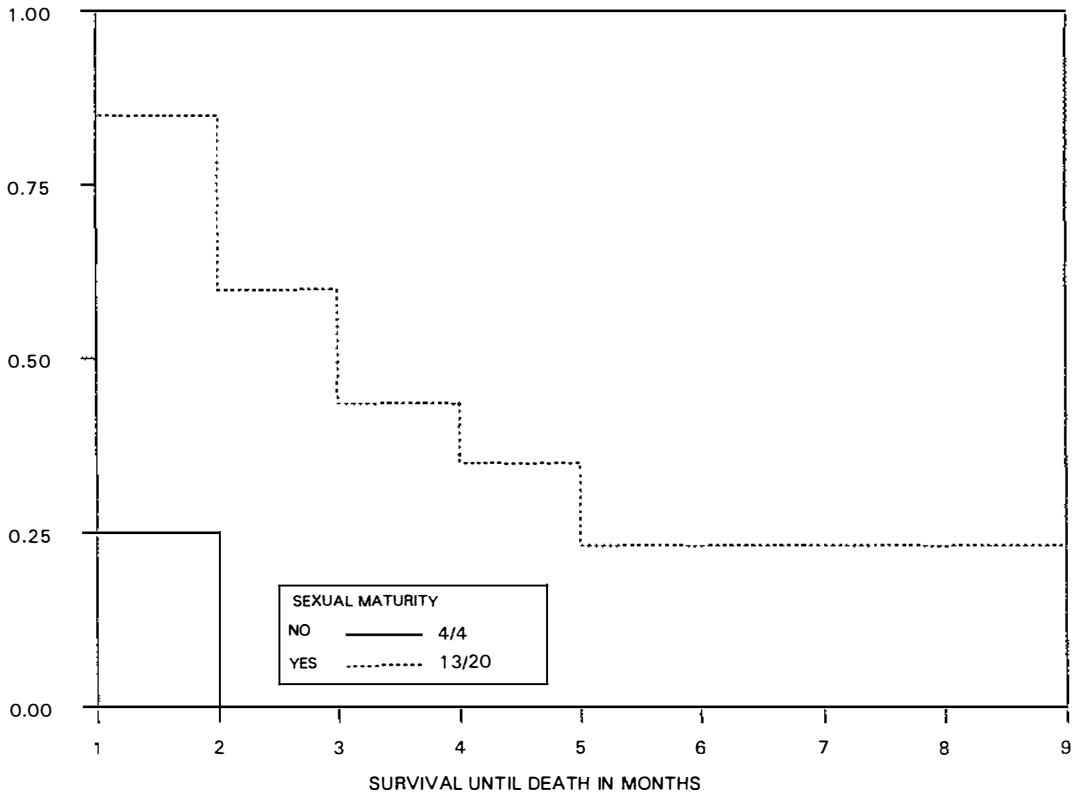


Table 12: Summary of stepwise selection procedure for the 'best' discrete hazard rate regression model of time until death or disappearance

	STEP 1			STEP 2			STEP 3			STEP 4			MODEL 4 INCLUDING AGE*TIME INTERACTION		
PARAMETERS	HRR	95% CI	p	HRR	95% CI	p	HRR	95% CI	p	HRR	95% CI	p	HRR	95% CI	p
AGE GROUP (1=IMMATURE, 2=MATURE)	0.16	0.10-0.25	0.0001	0.16	0.10 - 0.25	0.000	0.16	0.10 - 0.26	0.000	0.16	0.10-0.26	0.000	0.44	0.20-0.97	0.042
TB LESION (0=NO,1=YES)				6.95	3.99 - 12.1	0.000	7.35	4.18 - 12.9	0.000	7.62	4 31-13.5	0.000	7.98	4.57-13.9	0.000
WINTER SEASON (0=NO,1=YES)							1.60	1.09 - 2.33	0.016	1.41	0 94-2.12	0.100	1.38	0.92-2.08	0.124
SUMMER SEASON (0=NO, 1=YES)										0.69	0.43-1.12	0.133	0.72	0.45-1.17	0.191
AGE GROUP * LOG(TIME)													0.38	0.20-0.72	0.003
		CHI^2	p		CHI^2	p		CHI^2	p		CHI^2	p		CHI^2	p
-2 LOG LIKELIHOOD		54.28	0.000		91.31	0.000		96.91	0.000		99.24	0.000		110.3	0.000
SCORE		75.47	0.000		136.0	0 000		142.8	0.000		145.6	0.000		182.9	0.000

The Kaplan-Meier product limit estimate of the survival function was modelled for the time from lesion detection until death. The data fitted closest to a log normal distribution. 50% (95% CI: 0.29 - 0.68) of tuberculous possums survived about 2 months after lesion detection. After 5 months 81% of lesioned possums were dead. The survival distribution stratified by age group is summarised in figure 38. The survival curve plots the cumulative proportion of possums that have not died at the beginning of each time interval (adjusted for data censoring). A stepwise regression approach using the discrete hazard regression model was applied to 24 observations from tuberculous possums with complete data on the time-invariant factors sex class, age group and the time-varying factors for each season of the year, body weight and presence of pouch young. The time-varying covariate 'Spring Season' was the statistically most important risk factor. A HRR of 3.81 (95% CI: 0.78 - 18.6; p = 0.099) was estimated comparing survival in possums during spring with other seasons of the year using the discrete survival model.

Figure 38: Kaplan-Meier survival curve for tuberculous possums, by age group

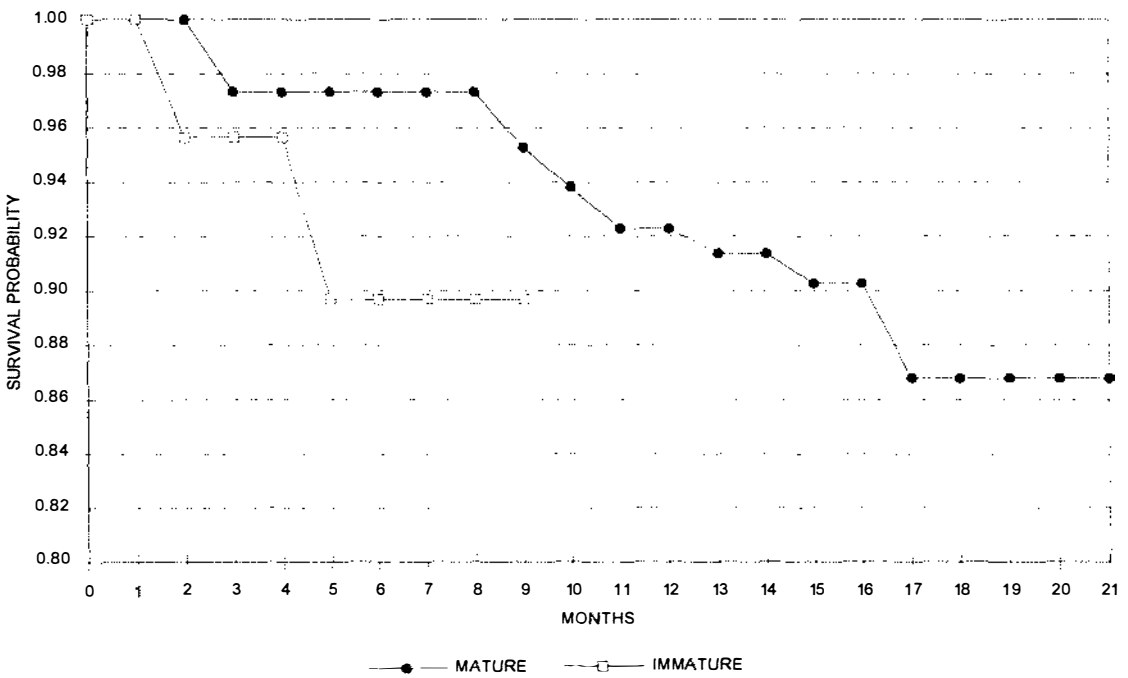


The probability of not developing a lesion for a possum was modelled using the Kaplan-Meier estimator stratified by age group. The cumulative probability after 6 months was 0.89 (95% CI: 0.77 - 1.00) for immature and 0.97 (95% CI: 0.94 - 1.00; see figure 39) for mature possums. The Wilcoxon test which was used to test if the two curves came from the same distribution was not statistically significant at a p-value of 0.1118. Hence, the probability of developing a lesion over a period of 6 months was not different between immature and mature possums in this analysis. The difference between findings from this analysis and the incidence data results in part from differences in the nature of the analytical approach adopted plus the fact that sample sizes available to calculate individual data points on the survival curve for the immature possums were relatively small. Time until the detection of lesions was tested for statistically significant covariates using the discrete hazard rate model including data from all possums with appropriate information (N=258, including 19 events and 239 censored observations). Individuals which did not develop tuberculous lesions were considered censored observations. The time-constant variables age group, sex class and the time-varying factors season (as four dummy variables), body weight and presence of a pouch young were specified for inclusion in the stepwise selection process. The 'best' model included age group, spring season and body weight (see table 13). When restricting the analysis to possums which developed tuberculous lesions the risk factors 'spring season' and 'age group' were the most important factors and showing similar effects. In this group of animals median time until lesion detection since first capture was 1 month in immatures (N=3) and 8.5 months in sexually mature possums (N=16; p = 0.0028).

Table 13: Summary of stepwise selection procedure for the 'best' discrete hazard rate regression model of time until development of detectable tuberculous lesions

	STEP 1			STEP 2			STEP 3		
PARAMETERS	HRR	95% CI	P-VALUE	HRR	95% CI	P-VALUE	HRR	95% CI	P-VALUE
SPRING SEASON (0=NO, 1=YES)	3.41	1.31 - 8.85	0.0118	3.88	1.47 - 10.2	0.0062	4.01	1.50 - 10.7	0.0057
AGE GROUP (1=IMMATURE, 2=MATURE)				0.25	0.06 - 1.02	0.0539	0.14	0.03 - 0.71	0.0175
BODY WEIGHT							2.21	0.79 - 6.20	0.1305
		CHI-SQUARE	P-VALUE		CHI-SQUARE	P-VALUE		CHI-SQUARE	P-VALUE
-2 LOG LIKELIHOO		5.973	0.0145		9.004	0.0111		11.34	0.0100
SCORE		6.970	0.0083		10.450	0.0054		12.36	0.0063

Figure 39: Kaplan-Meier curve of cumulative probability of not developing tuberculous lesions

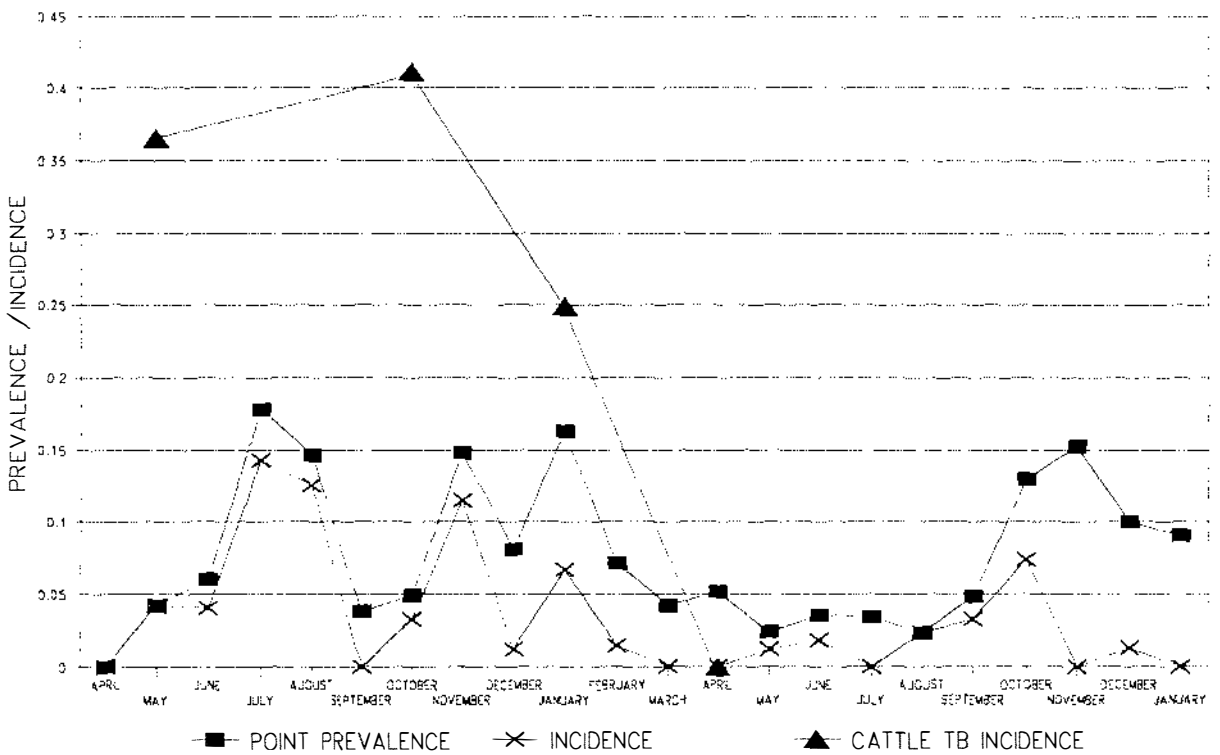


Temporal Dynamics of Tuberculosis Infection

Analysis of tuberculosis prevalence data over time reveals the presence of 3 distinct peaks in point prevalence, one in July 1989 with 0.18 (N=28), another in January 1990 with 0.16 (N=74) and a third in November 1990 with 0.15 (N=46). Monthly prevalence averaged 0.08. Cumulative monthly incidence reached peaks in July 1989 with 0.14 (N=28), in November 1989 with 0.11 (N=61) and in October 1990 with 0.07 (N=54) (see figure 40). Except for the initial field visit, tuberculous possums were known to be present throughout the entire study period.

Incident cases in adult possums stratified by sex were compared between winter and other seasons of the year. Adult female possums contributed 60% (N=9) of cases in sexually mature animals during the winter months and 35% (N=7) during rest of the year (OR=2.79, 95% CI 0.57 - 14.22; p = 0.14).

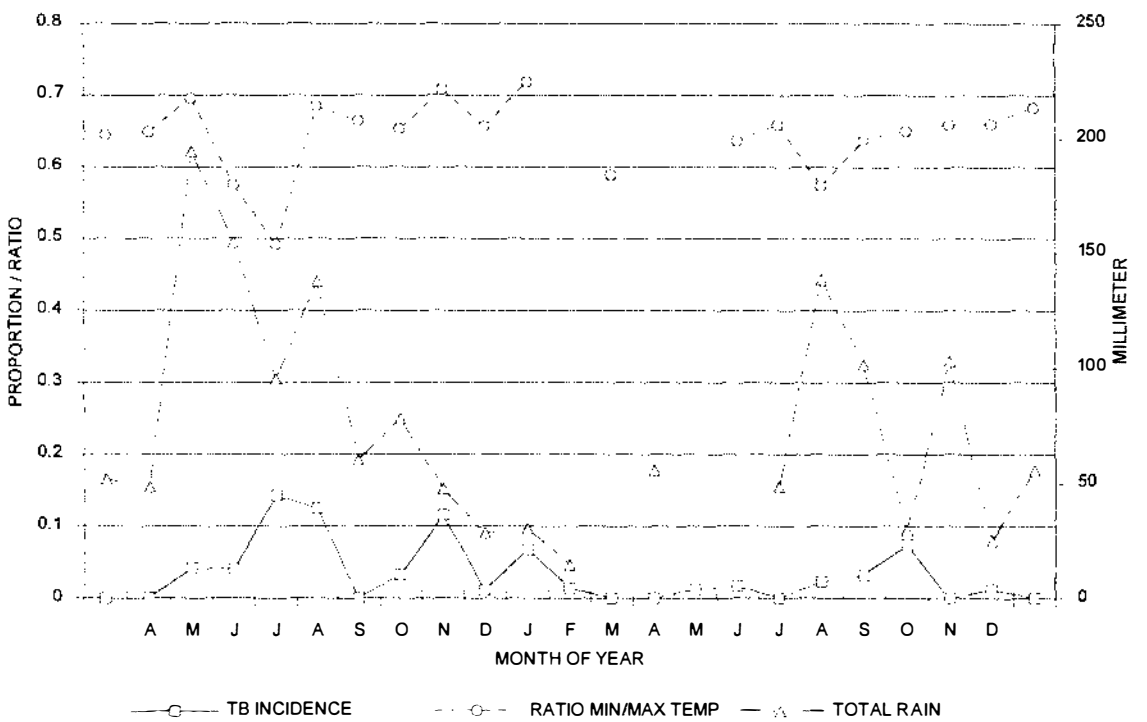
Figure 40: Incidence and prevalence of tuberculosis in possums and cattle



Using repeated-measures analysis of variance average body weights of individual possums were found to vary significantly over time (see under paragraph 'General Body Condition'), but there was no statistically significant difference between tuberculous and non-tuberculous possums. Further analyses were conducted comparing disease dynamics between visits using aggregated data for each month describing population and environmental characteristics. Monthly meteorological data was examined for the presence of a statistical association with cumulative incidence for lagged values of up to two months. In the multivariate analysis the most important meteorological factors were the variables "Ratio of Monthly Average Minimum and Average Maximum Daily Temperature (lag 1 month)" (ß=-

9.489, s.d.=2.533, p = 0.0002) and "Total Monthly Rainfall (lag 1 month)" (ß=0.007, s.d.=0.0037, p=0.0572; criteria for assessment of model fit: AIC=309.016 [intercept only: AIC=322.33], -2 LOG L Chi-Square=17.31 with 2df and p =0.0002). The result was based on a total of 18 observations. Hence, it was unlikely to detect any seasonal, cyclical or trend effects. Variables included in the analysis did not show the presence of autocorrelation (see figure 41).

Figure 41: Tuberculosis incidence in possums and weather conditions



The importance of population parameters with respect to tuberculosis incidence was tested using poisson regression. The factors "Immigration", "Population Size", "Survival between Visits", "Proportion of Adult Female Possums with Pouch Young", "Average Body Weight in Adult Males" and "Prevalence of Tuberculosis Infection" at monthly time lags of 0 to 2 were included in the analysis. The final model included the single factor "Average Body Weight in Adult Males (lag 1)" (ß = -3.6402, s.d. = 0.7682, p = 0.0001; criteria for assessment of model fit: AIC = 331.45 [intercept only: AIC=348.97], -2 LOG L Chi-Square= 19.5 with 1df, p=0.0001). The model was based on data from 21 observations.

In the next step population parameters and climatic variables were used as independent variables for stepwise poisson regression. The final model was the same as above with only including the independent variable "Average Body Weight in Adult Males".

During the study period blood samples were collected from 364 animals. Fourteen animals were found dead within the study area. More than 4 blood samples were obtained

from 70 animals. Possums were recaptured on average 9.4 times with a maximum of 91 times (N=1). A preliminary serological analysis using a monoclonal antibody blocking ELISA was conducted on 293 sera by the Central Veterinary Laboratory, Weybridge, England (Nolan 1991). Using culture results as the gold standard, which were available for 57 samples, a sensitivity of 22.5% and a specificity of 100% was calculated. Dissemination of lesions inside the body did not appear to increase sensitivity of the test.

Spatial Dynamics of Tuberculosis Infection

The average distance to the nearest tuberculous possum was calculated based on the locations of the arithmetic centers of activity for tuberculous and non-tuberculous possums. Distance to the nearest den site which had been used by a tuberculous possum were calculated for each den site which was located by radio tracking (see table 14, figures 42a and b).

Table 14: Distance to nearest tuberculous possum or den site utilized by infected possums

DISTANCE	TUBERCULOUS				NON-TUBERCULOUS				
TO NEAREST	n	x (m)	s.d.	Median	n	x (m)	s.d.	Median	p
DEN USED BY A TB POSSUM	73	24.86	28.05	15.09	179	37.47	30.66	30.91	0.000
TUBERCULOUS POSSUM	22	43.50	41.77	21.37	78	59.26	35.14	54.67	0.012

Figure 42a: Distribution of distances to the nearest den site utilized by tuberculous possums

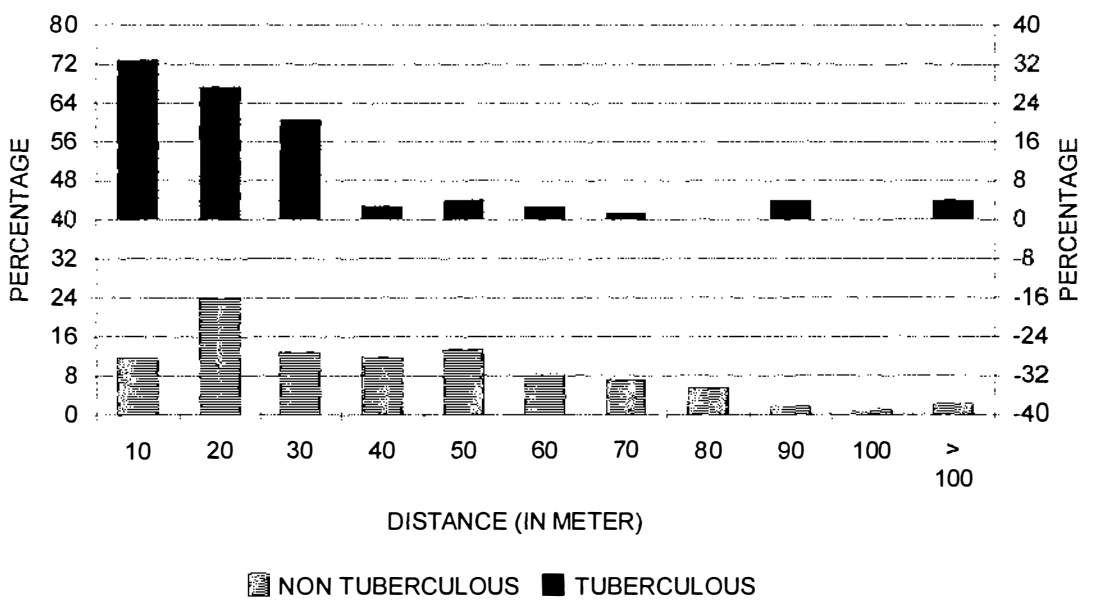
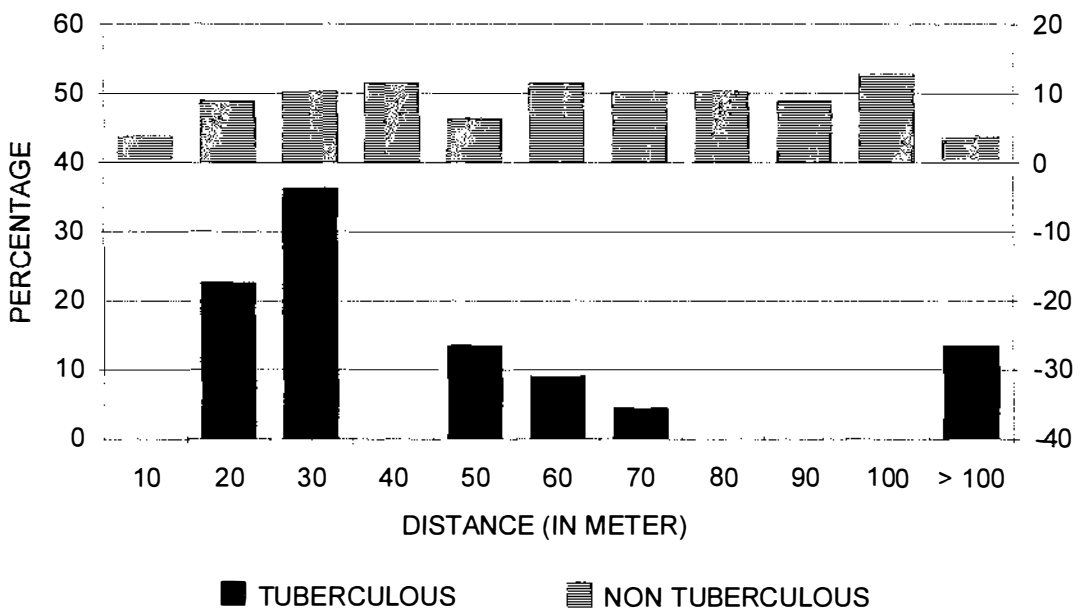


Figure 42b: Distribution of distances to the tuberculous possum with nearest arithmetic center of activity



Spatial analysis of the distribution of tuberculous possums based on cumulative catch location data shows a concentration of tuberculous possums in terms of proportion of TB cases in total cumulative capture in a single part of the study area. Figure 43 shows a contour map of proportion tuberculous captures.

Figure 43: Contour map of the spatial distribution of proportion tuberculous possums in total catch

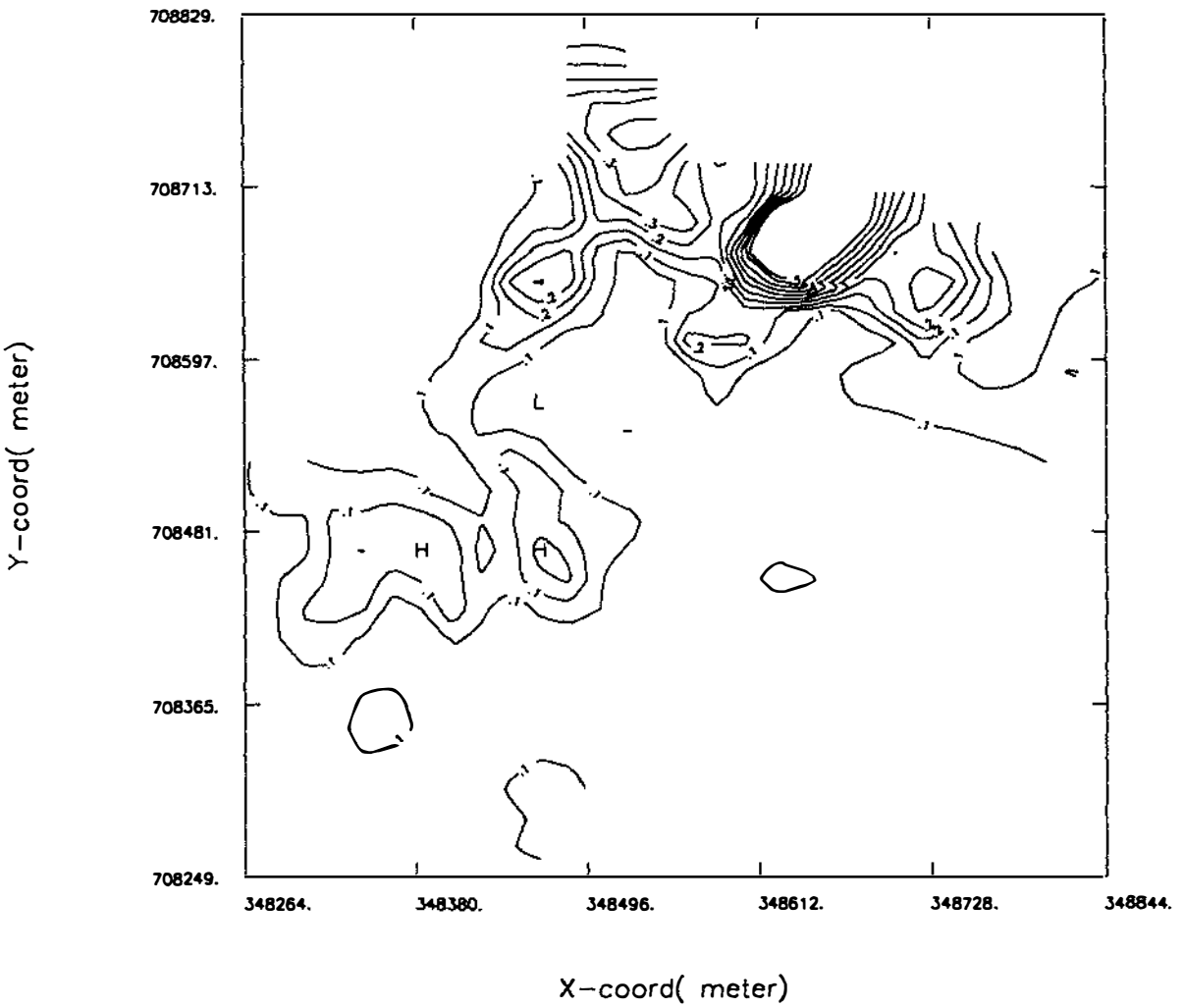
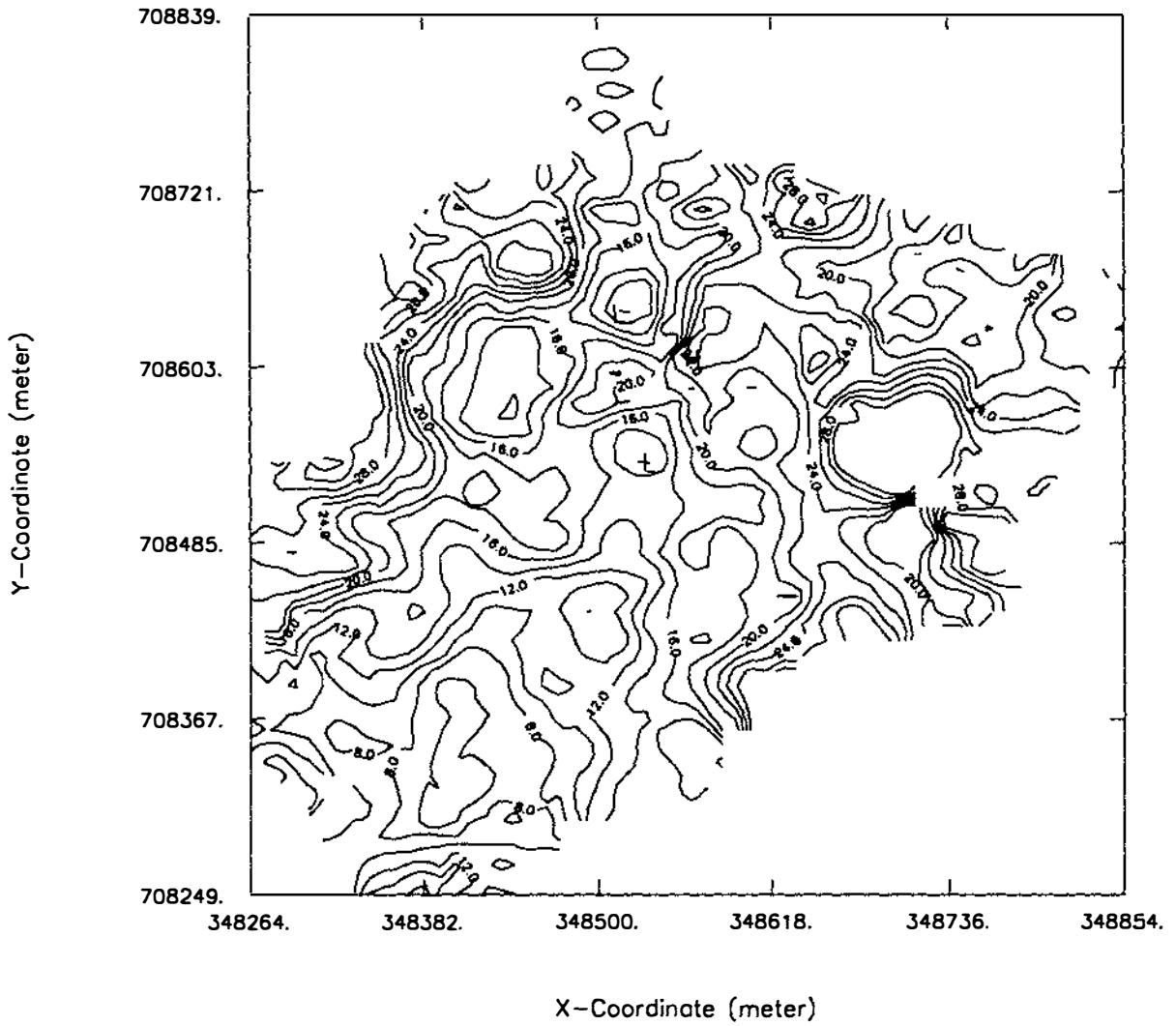


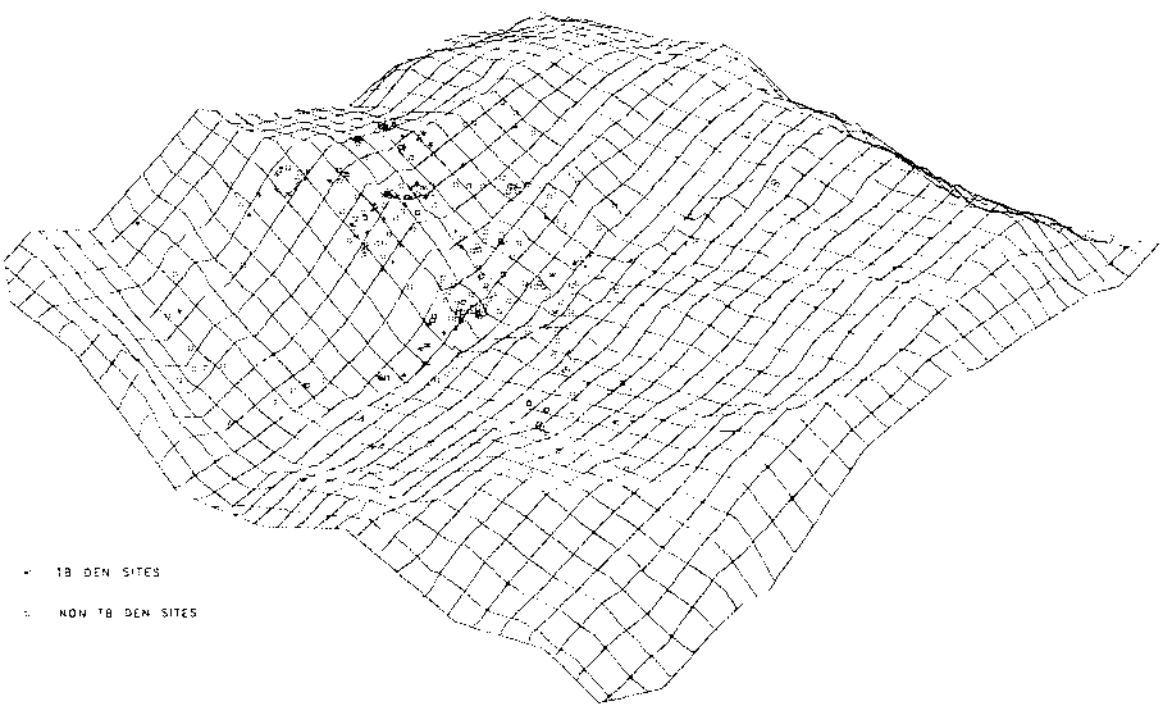
Figure 44 shows a contour map of the spatial distribution of total captures over the study area. It allows a comparison of local possum catch density and the respective local period prevalence catch estimate for tuberculous possums shown in the previous figure.

Figure 44: Contour map of the spatial distribution of total captures



A concentration of den sites which had been used by tuberculous possums was observed in one particular area (see figure 45). This infection associated cluster of dens was linked to new diagnoses of tuberculosis over an extended period of time.

Figure 45: Spatial distribution of den sites used by infected and non-infected possums



Average slope measured in percent was not statistically significantly different between den sites which had been used by tuberculous possums and those which had only been used by non-tuberculous possums ($p = 0.50$). Slope aspect (measured in degrees) was categorized into four groups according to exposure to sunlight. Slopes facing north were considered most favourable, those facing to the west came next, then the east and finally slopes which were facing south. Almost half of all den sites were located on slopes facing west. Aggregating north and east slopes into one group and south and west into another group gave the following results. 38% of den sites on north and west slopes had been used by tuberculous possums compared with 25.4% of den sites on south and east slopes ($p = 0.0447$; see figure 46a). Average height above sea level of 164.2m (s.d.=36.65) for den sites used by tuberculous possums was statistically significantly higher than for the den sites used by non-tuberculous possums, at 153.7m (s.d.=34.17; $p = 0.0284$; see figure 46b).

Figure 46a: Histogram of the distribution of slope aspect for den sites which had been used by tuberculous possum and which had only been used by non-tuberculous possums

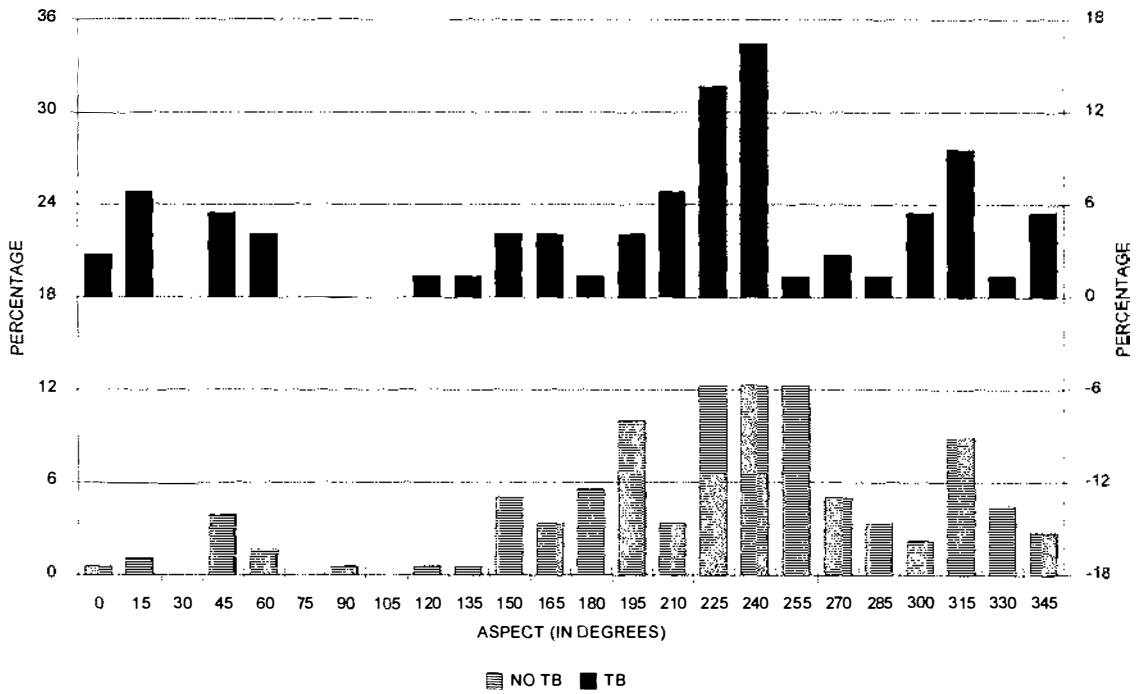
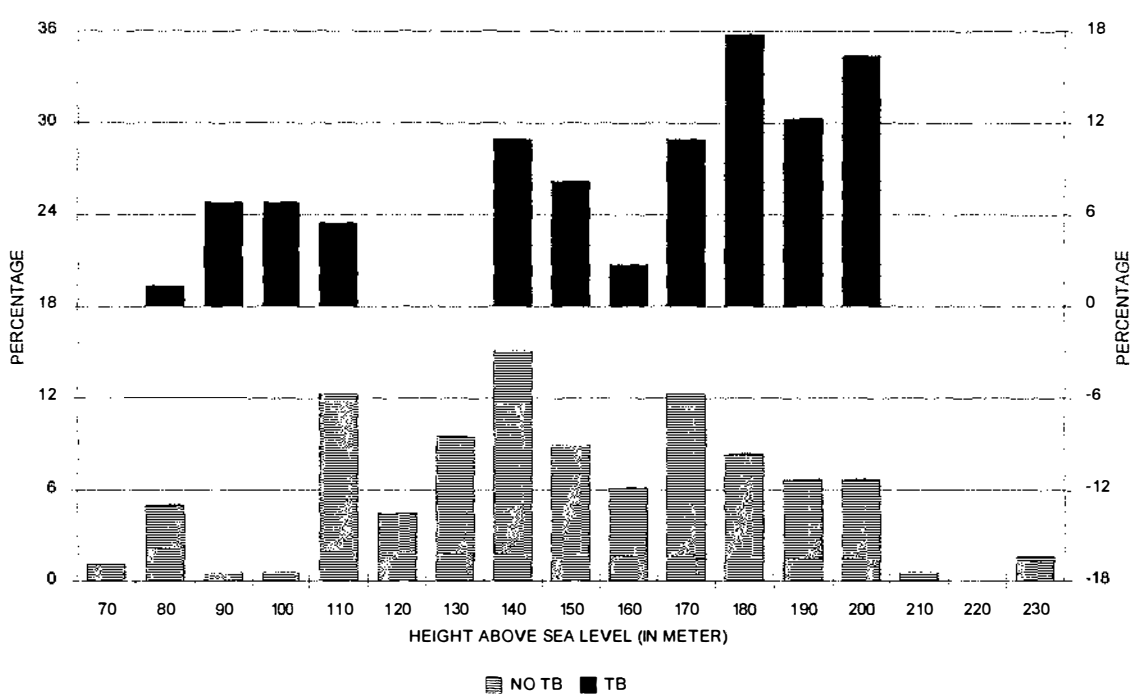


Figure 46b: Histogram of the distribution of height above sea level for den sites which had been used by tuberculous possum and which had only been used by non-tuberculous possums



Stepwise logistic regression was used to identify the statistically most important characteristics which differentiated den sites used by tuberculous and non-tuberculous possums. Distance to the nearest den site which had been used by a tuberculous possum, slope, height above sea level and categorized aspect were used as dependent variables. The final logistic regression model included the variables "Distance to the nearest Den Site", "Height above Sea Level", "Aspect Categories" and the interaction between "Height above Sea Level" and "Aspect Categories" (see table 15)

Table 15: Results of multivariate analysis of den site utilization by tuberculous possums

Variable	ß- coefficient	s.e.	p
Distance to next TB Den	-0.1700	0.00651	0.009
Height above Sea Level	0.0421	0.0141	0.0029
Aspect Category	2.1622	0.7825	0.0057
Interaction Aspect Category - Height above Sea Level	-0.0116	0.00451	0.0103
AIC Intercept Only	305.341	AIC Intercept + Covariates	288.948
-2 Log Likelihood Chi^2	24.39 with 4df (p=0.0001)		
Sensitivity	5.5%	Specificity	96.6%

Spatio-temporal Dynamics of Tuberculosis Infection

Time-space clustering was investigated using Mantel's regression approach. For untransformed distances a positive regression slope comparing distance and time measures was found in males (ß = 0.368, s.e.=0.053, p=0.0001). No time-space clustering was found in females or in a comparison between sex classes (see figure 46a). Using the inverse transformation of distance measurements there was a statistically significant positive regression slope for interaction between female possums (ß = 0.015, s.e.=0.007, p=0.04). There was no interaction within the male strata and between sex classes. The spatial and temporal distribution of possums which showed lesions during the study period is shown in figure 47b. Data points represent cumulative information on trap sites at which tuberculous possums were caught and include locations of den sites to which they were tracked. Point data was converted into area information by creating "Thiessen" polygons, which were shaded when used by tuberculous possums. The extent of the areal coverage provides an estimate of the areas covered by tuberculous possums during feeding and social activities.

Figure 47a: Time-space interaction between tuberculosis cases stratified by sex class

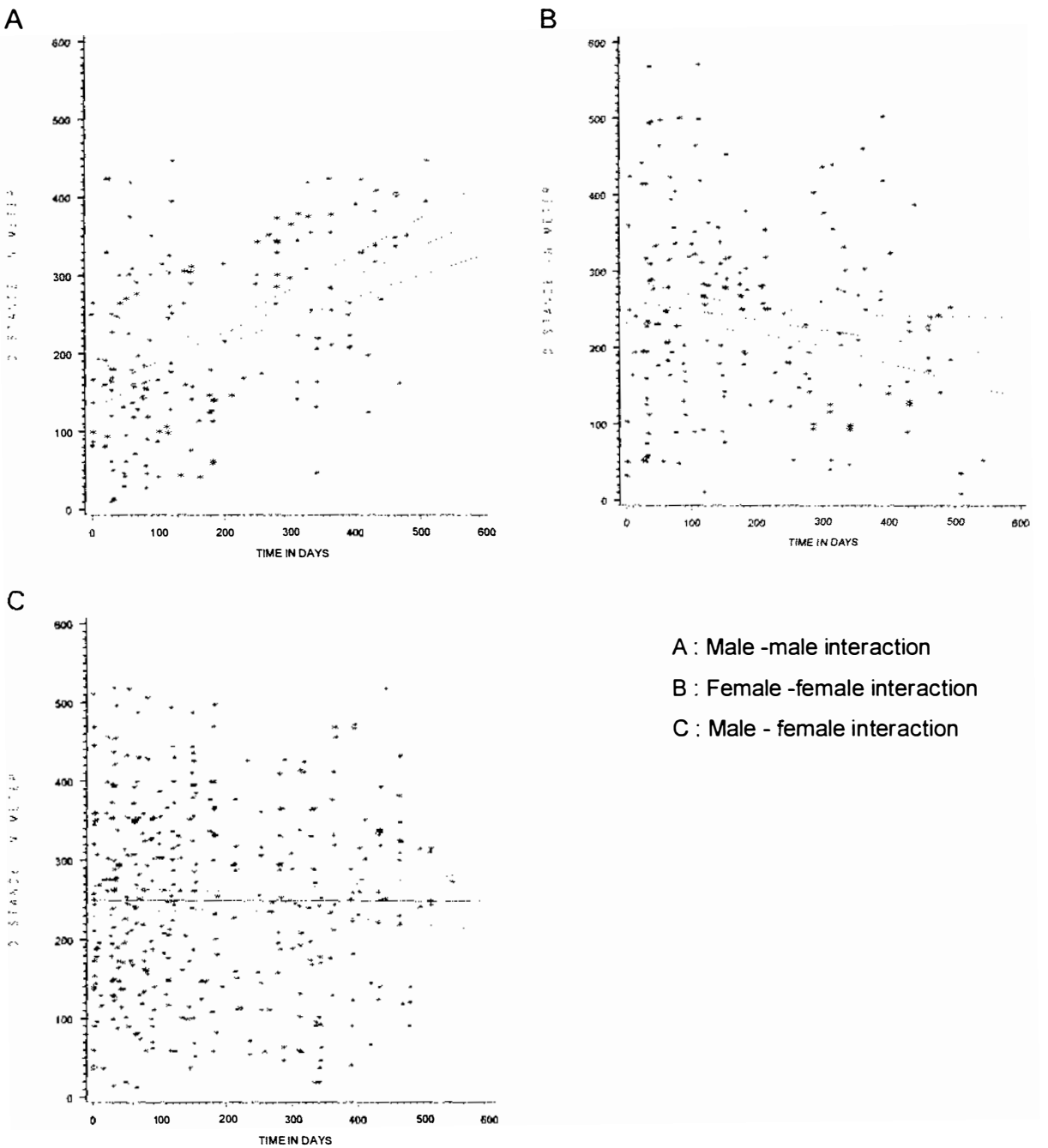
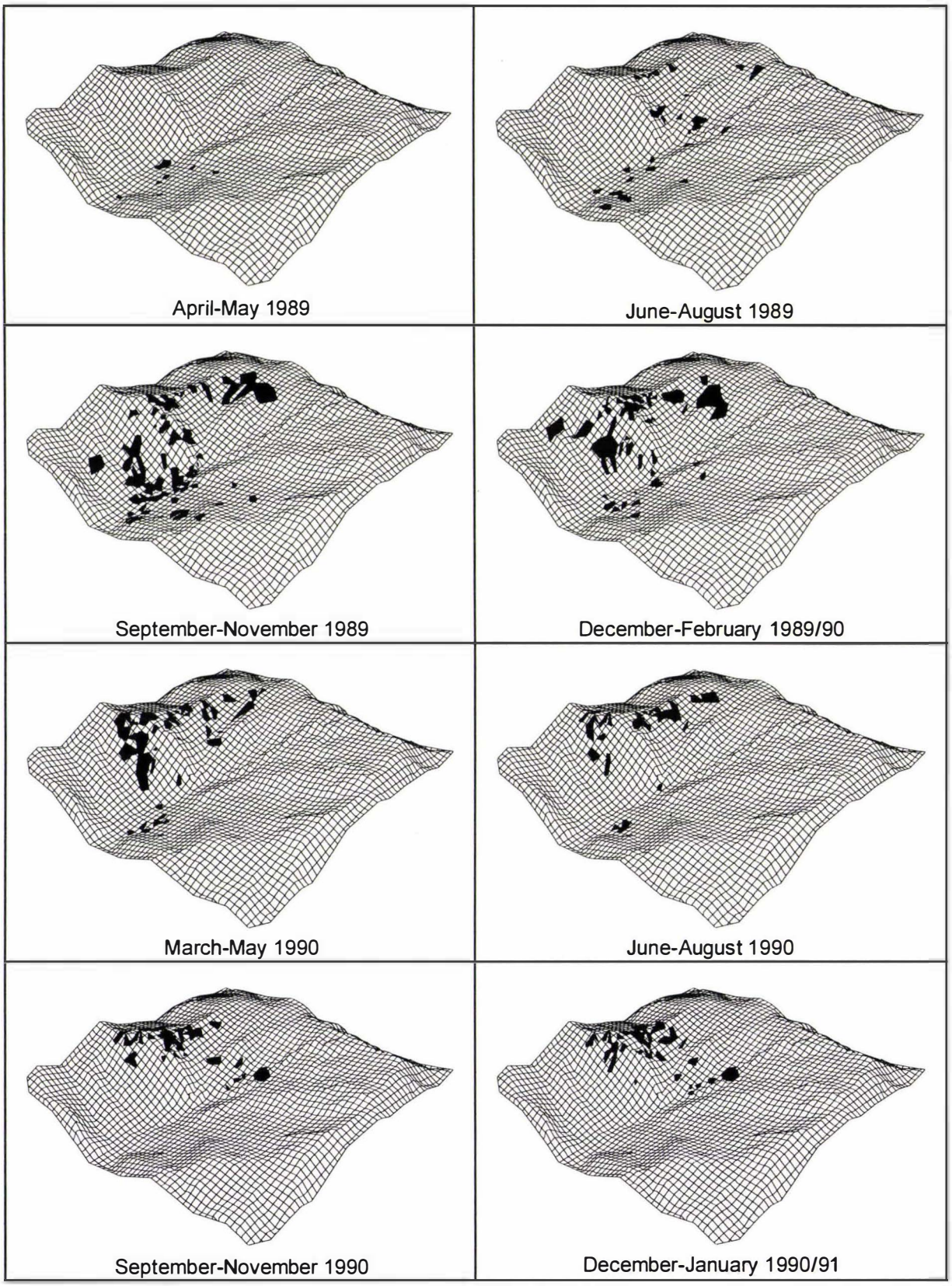


Figure 47b: Temporal and spatial distribution of tuberculosis infected possums stratified by season and year



Epidemiological Analysis based on Restriction Endonuclease Analysis Types of *Mycobacterium bovis*

Using DNA restriction endonuclease (REA) typing, 2 major and 2 minor strains of *Mycobacterium bovis* were identified among isolates originating from tuberculous animals in the study area. Thirty-eight individual samples from 4 different species were characterized. Three were classified as very similar to Wairarapa type 10 (REA type 10), 14 as Wairarapa type 4 (REA type 4), 21 as very similar to Wairarapa type 4 (REA type 4a) and the pattern for 1 isolate was considered similar to Wairarapa type 4 (REA type 4b). Figure 48a summarizes case histories of tuberculous possums and other species with REA type information. Possum D3574 was classified as REA type 4 based on a sample from October 19 and as type 4a when another sample was taken from the carcass in December 1989. Mature female possum D3720 and its offspring D3692 were both found to be infected with REA type 4 of *Mycobacterium bovis*. The following cases of sequential den site sharing between tuberculous possums with known REA types were recorded. Possum D2430 with REA type 4a was located on 3.5.90 in a den which was used by D3570 (REA type 4) on 21.6.90. On 15.2.90 Possum D3598 with REA type 4 used the same den site as D3671 which had REA type 4a on 31.1.91.

Multiple correspondence analysis was used to create a graphical display of the association between categories of REA types, sex class, group and season (see figure 48b). The first dimension explains 19.81%, the second 16.09% and the third 14.59% of total inertia. The plots show that REA type 4a was more common during winter. There was evidence of a close association between REA types 4 and 10 and occurrence of the disease in spring, summer, autumn. The plots do not suggest a clear difference between sex classes and age groups. Figure 48c describes the temporal distribution of cumulative incidence for each REA type of *Mycobacterium bovis*. REA type 4a dominated during both winter/spring periods of 1989 and 1990, whereas REA type 4 was most common during summer 1989/90. Capture and den site locations which had been used by tuberculous possums with known REA type were mapped to describe the temporally aggregated spatial dynamics of the different REA types (see figures 48d and e). It should be noted that the size of individual patches on the maps is exaggerated in areas with low trap density. Visual inspection of the maps which were draped over a digital terrain model of the study area reveals that there is only limited spatial overlap in terms of activity areas and den site locations between areas covered by possums with different REA types. The minor REA types 4b and 10 only occur in the bottom area of the spaddock which does not represent the main den site area, but is extensively used for feeding on pasture especially during spring-summer. Time-space clustering was investigated using Mantel's regression approach. Based on untransformed measurements of closeness there was evidence of time-space clustering between REA types 4a (ß= 0.177, s.e. = 0.054, p=0.001; see figure 48f). No time-space clustering was detected between REA types 4 and between different REA types. Analysis of inverse transformations of the closeness measurements did not show a statistical significant association between distance and time differences.

Figure 48a: Details from case histories and post mortem examinations of tuberculous possums with REA type data

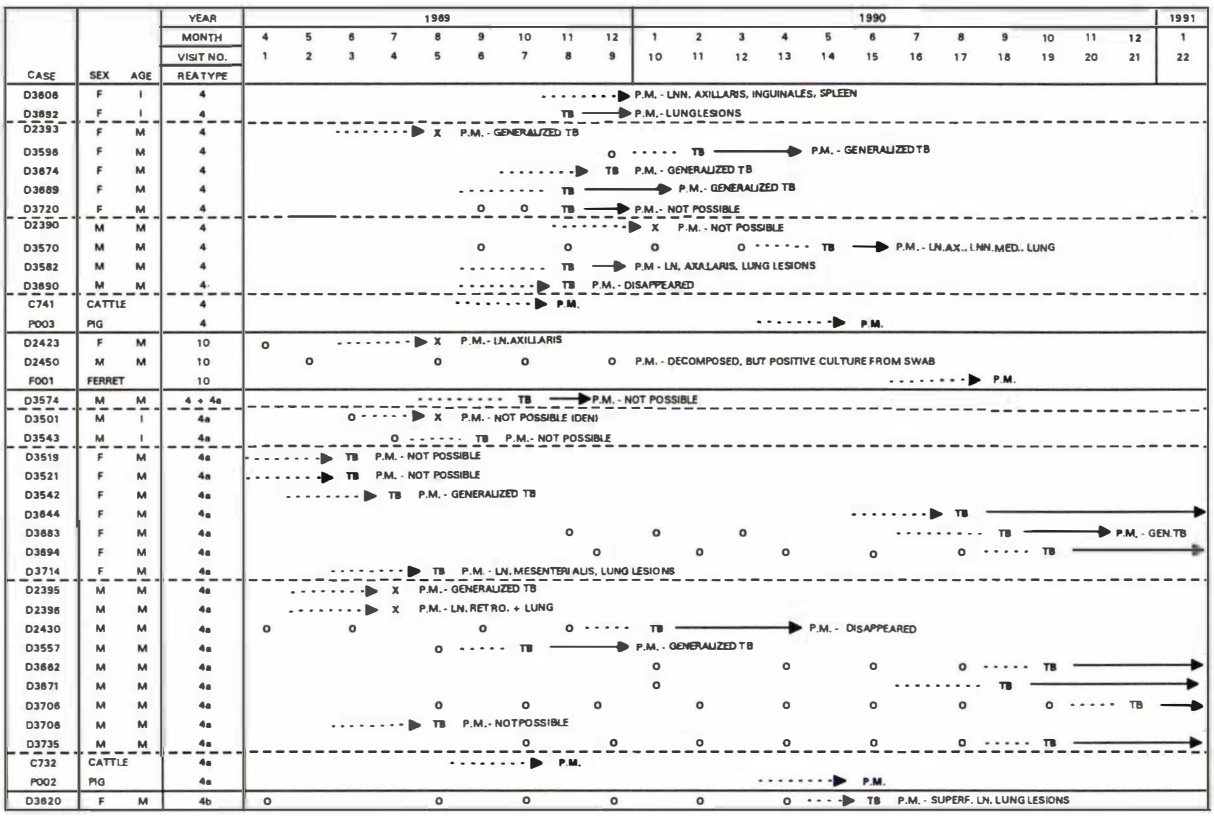


Figure 48b: Joint plots of the second and third against the first dimension of the result of multiple correspondence analysis describing the association between REA type, sex class, age group and season

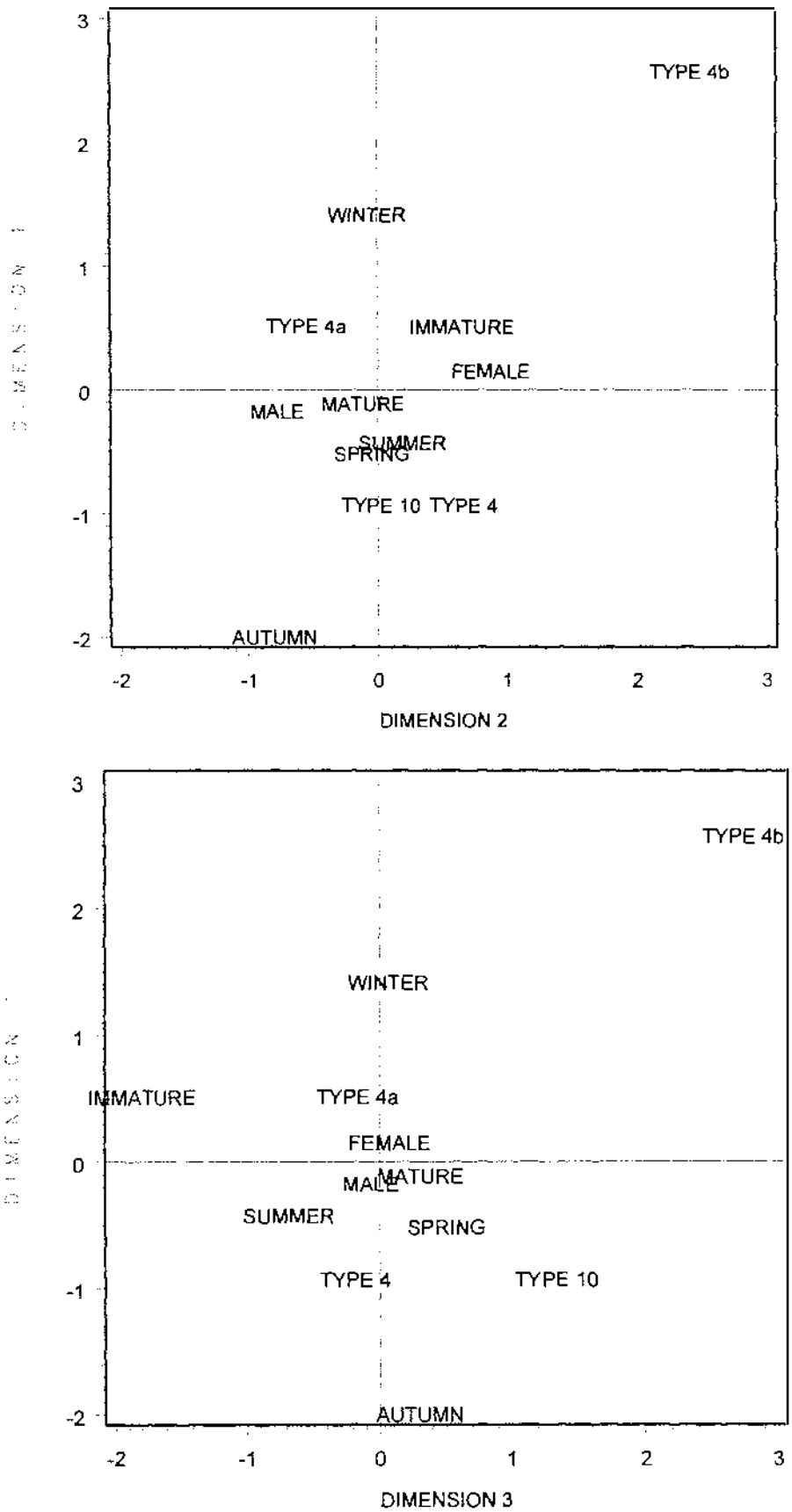


Figure 48c: Temporal distribution of restriction endonuclease types of *Mycobacterium bovis* isolates

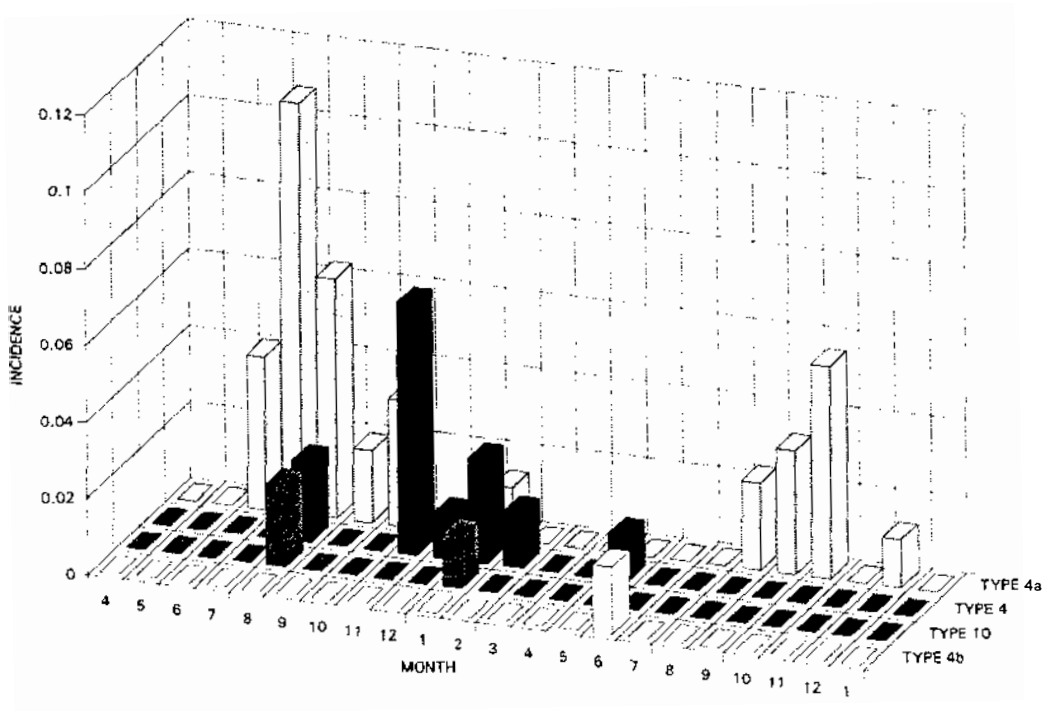


Figure 48d: Spatial distribution of restriction endonuclease types of *Mycobacterium bovis* isolates based on capture and den site locations used by tuberculous possums

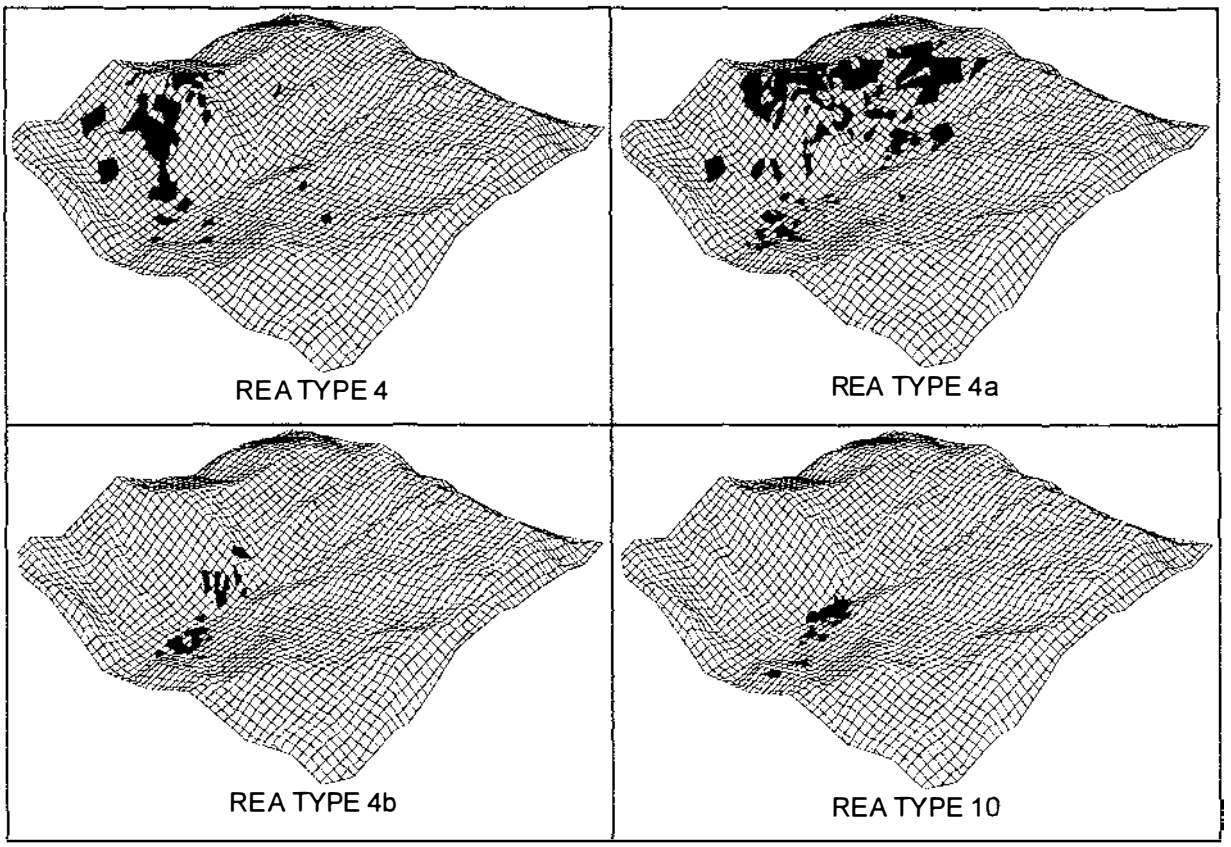


Figure 48e: Spatial distribution of restriction endonuclease types of *Mycobacterium bovis* isolates based on den site locations used by tuberculous possums

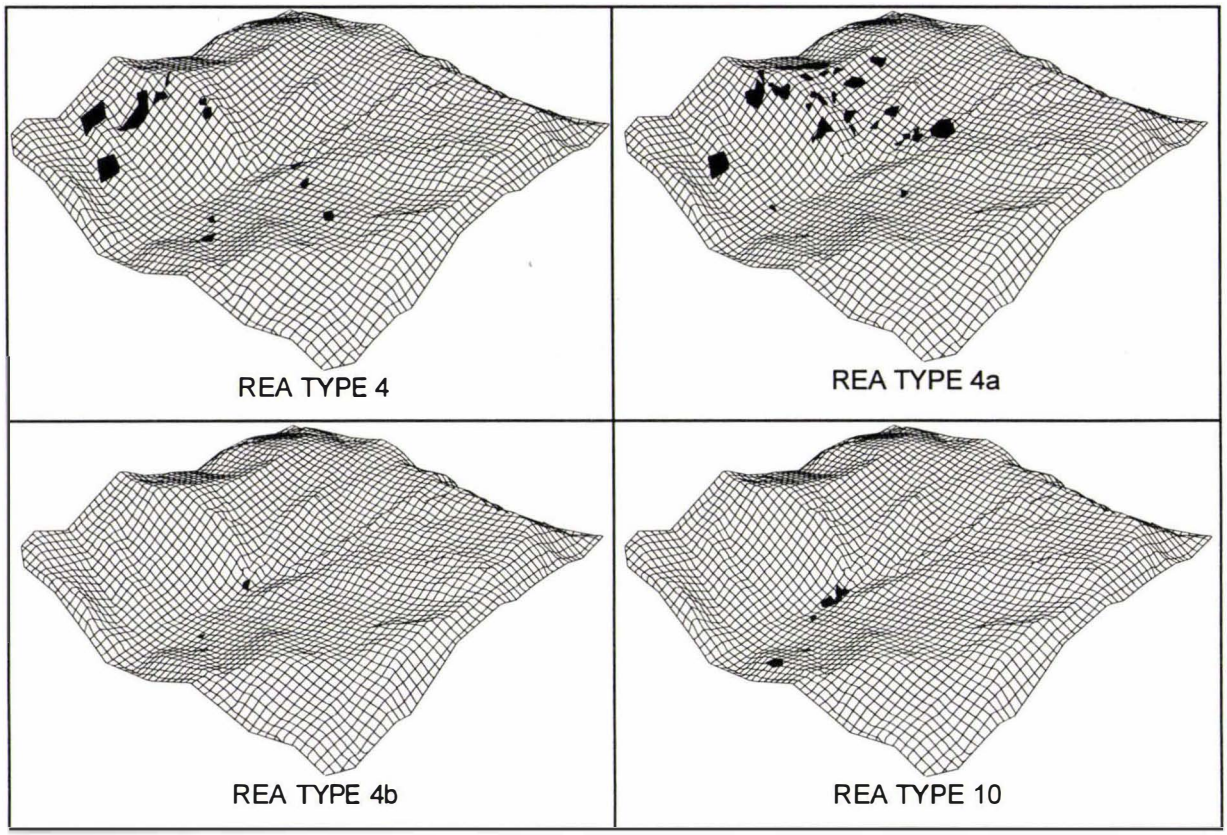
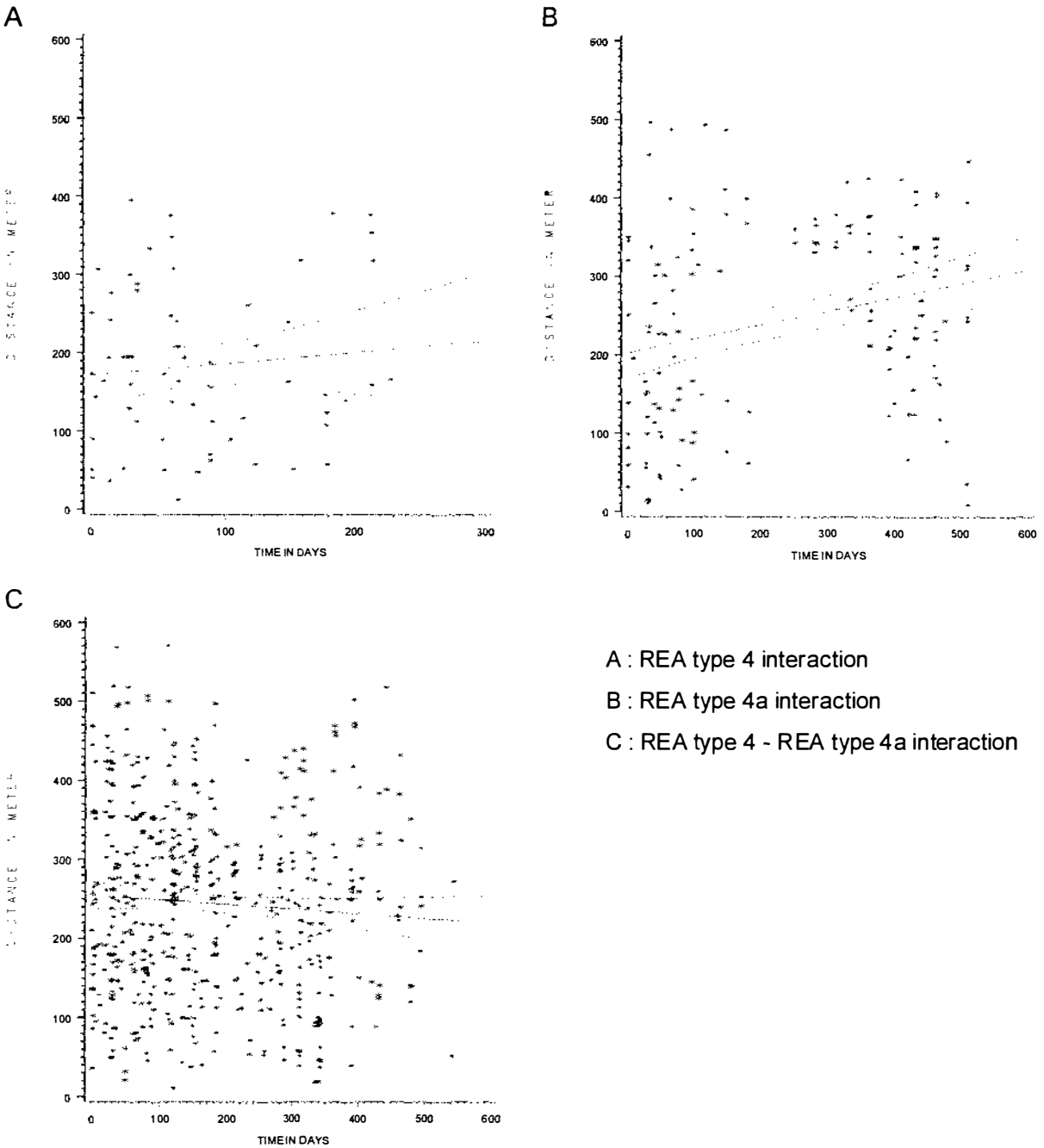


Figure 48f: Time-space interaction between tuberculosis cases stratified by combination of major REA types



Cattle Tuberculosis

At the start of the project in April 1989, a total of 31 tuberculin test negative cattle belonged to the study herd kept in or in the vicinity of "Back Drop" paddock. On the first tuberculin test in May 1989, 10 animals reacted positively and showed tuberculous lesions on slaughter. Twenty-one animals were tested again in November 1989, when 4 animals reacted to the tuberculin test and 2 of them had tuberculous lesions on slaughter. The remaining 17 cattle were tested in January and April 1990. One animal reacted on the first test, but not 3 months later in April. From this tuberculin test history a cumulative incidence of 0.48 and a incidence

density of 0.33 was derived (see figure 19) (Kleinbaum *et al* 1982). The initial study herd has been sold and replaced by a mob of about 30 steers. The new herd was included into the 3 monthly TB testing procedure beginning in November 1989 as yearlings, but was not introduced to the study site until July 1990. Two reactors were detected on the July 1990 tuberculin test and were subsequently removed from the herd. Neither had any visible lesions. Since then no other reactors had been found before the end of the period considered in this analysis. In 1990 a fence was built which divided the paddock into two. After the completion of the fence cattle no longer had access to the areas of the paddock where the majority of denning took place.

Catch Methods

During the visits of phase I, both cage traps and leg hold traps (type "Victor No.1.5 Softcatch") were used. The latter were tested for their applicability in capture/recapture studies. Leg hold traps were set on 302 occasions and 62 possums were caught, including 9 recaptures. Three of the possums suffered from fractures of the lower leg, one of the animals with fractures was repeatedly recaptured and developed a callus. Two animals disappeared with the trap and one animal died from exposure shortly after release from the trap. No direct adverse effects of cage traps were found.

DISCUSSION

Ecological Findings

Ecological findings for this possum population are broadly consistent with those for other comparable sites around New Zealand (Batcheler and Cowan 1988). When comparing results between studies it has to be taken into account that differences in the method of data collection can influence the results. The results reported here were based on a capture - mark - recapture design, with 5-day trapping sessions at monthly intervals on a trapping grid of 295 traps at a density of about 14 traps per hectare. This resulted in a very intensive trapping effort which was considered necessary to study the epidemiology of tuberculosis in sufficient detail. To reduce the stress which capture and examination impose on the possums the minimum interval between examinations was set at two months. This had the disadvantage that some pouch young reached independence before it was possible to tag them and new tuberculosis cases could have developed lesions up to two months before detection.

Trap catch success averaged 0.21 but was variable over time, reaching maxima during summer and minima during the winter months. Trap catch success is a reflection of population density and extent of foraging activity, and is influenced by the characteristics of the habitat. It is also influenced by trap density, which was very high in this study. Brockie *et al* (1987) had a mean success of 30.2% under similar habitat conditions. In their case, trap catch success was quite variable between open pasture areas and a swamp area with a particularly high population density. Efford (1991) reports an average trap catch success in 1990 of 52% in forest habitat in the Orongorongo Valley study.

Reproduction and Mortality

A bias in the sex ratio towards males has been found in some studies (Efford 1991), but balanced sex ratios or female bias (especially in the older age groups) are expected in long-established, stable populations (Batcheler and Cowan 1988, Brockie *et al* 1984). In this study females appeared to dominate slightly in the older age groups. A bias towards males which is common in younger age groups is influenced by the sex ratio at birth but increased by male immigration if there is a net inflow of possums. Males predominate especially in populations which are recolonizing a habitat after poisoning operations, or areas which are under intensive kill trapping (Clout and Efford 1984). About half of the possums which were post mortemed were under 2 years old. A similar age structure has been found in a number of other studies in New Zealand, but age structure can be influenced by a wide range of factors (Brockie *et al* 1990) and similar structures can be produced by different combinations of factors.

Births showed strong seasonal variation with a major peak in April-May (autumn) and a minor peak in September-October (spring), which agrees with the findings from other studies in New Zealand and Australia (Cowan 1990). Since pregnancy is 17 to 18 days, this indicates that there are mating seasons around March-April and August-September. The extent of spring births in possum populations varies according to habitat and population phase. In colonizing populations and in populations in exotic forest or pasture/scrub habitats the proportion of spring births has been reported to range between 2 and 33%. In established populations in indigenous forest, spring births were almost absent. It is believed that the level of spring births in New Zealand is indirectly affected by habitat and population density, mediated by body condition of the female animals (Green 1984). The majority of adult females (84% - 95%) captured after the autumn peak had given birth to a pouch young. In 36% of rearing episodes the pouch young survived successfully until independence. 5% died prematurely and in 59% the outcome was uncertain because insufficient capture records were available to make an accurate judgment. Due to the high proportion of uncertain outcomes it is difficult to draw any conclusions on pouch young mortality from these results. Bell (1981) recorded survival of pouch young in forest habitat varying between 42.2% and 58.2%. A second pregnancy episode in the same year appears to be common in females which lose a pouch young in the first episode, from results in this work.

On average, joeys spent between 5 and 6 months in close association with their mother. Sexual maturity in females occurred as little as 8 to 9 months later, and 11% of female offspring produced their first pouch young between 14 and 16 months of age. In this study area, males would appear to be sexually mature at an age of about 16 - 17 months according to testicle size measurements. Gilmore (1969) concluded from his study at Banks Peninsula that males would not mate until the spring or autumn in which they are at least 18 months old, and it is clear that in this population males were developmentally capable of breeding by 18 months.

Mortality in independent offspring could not be distinguished from emigration, and is best termed disappearance. One third of the offspring disappeared after independence, at an average age of 10-11 months. There did not appear to be a difference between males and females. It is generally believed that immature females are less likely to disperse to a distant site than males (Efford 1991).

Population Size and Demographic Characteristics

The Jackknife and the Jolly-Seber estimators were used to estimate total population sizes for each visit. From a biological point of view heterogeneity of capture probabilities and behavioural trap response would have to be expected in this kind of study. Heterogeneity of capture probabilities (certain individuals are persistently more likely to be caught than others for reasons other than deliberate behaviour) and a deliberately trap-happy response by some possums both result in a negative bias of the Jolly-Seber population size estimator and therefore an underestimation of true population size. A trap-shy response would overestimate population size. The relative magnitude of the bias is more severe if population turnover is substantial and if the marked proportion within the total population is small (Pollock *et al* 1990). The Jackknife is considered robust especially to heterogeneity of capture probabilities, but requires the study population to be closed. It has been said that the Jackknife provides an adequate estimate of population size in experiments in which many animals are caught a relatively large number of times (Otis *et al* 1978, Burnham and Overton 1979). The statistical tests provided by the program CAPTURE were applied to the data from this study and suggest that during most of the visits the assumptions of heterogeneity of capture probabilities, no behavioural trap response and no time variation in capture probabilities were violated to some extent, which is not surprising considering the relatively small size of the site in relation to its perimeter, and the diverse habitats within it. It appeared that except for the third visit the assumption of population closure was met. Keeping in mind these limitations on interpretation, the data show that population size estimates based on the Jolly-Seber model and jackknife estimates were different, especially at the beginning and at the end of the period, but they exhibited similar trends. There did not appear to be a consistent directional relationship between the two, as also reported by Efford (1991). For the purpose of this analysis it was considered appropriate to use the Jolly-Seber estimates of population size in order to minimize violations of model assumptions (Pollock *et al* 1990).

Population density (based on the Jolly-Seber estimation method) in the study area is about 6.9 possums per hectare over the 21 ha study area, with variation from a low of 5.7 in winter-early spring up to a summer peak of 7.9 per ha. The seasonal change in density is mainly accounted for by high mortality over the winter and local recruitment and immigration in spring/summer. Interpretation is complicated by the fact that the size of possum activity areas is likely to be smaller in winter than summer, altering capture probabilities. Also, the habitat of the study area is not homogeneous in that den sites and feeding areas are clustered in separate parts of the site. Hence, a population density estimate in a more homogeneous

habitat such as a forest would have to be interpreted differently from one in a study such as this. Other mark-recapture and removal studies on farmland habitat showed possum densities varying from 1.0 - 1.4 per ha (Jolly 1976) and 0.12 - 16 per ha (Brockie *et al* 1989) depending on season and local habitat characteristics (e.g. swamp, open pasture or riverbank). Items such as population size, survival, births, immigration and emigration (dispersal) are important parameters for describing the dynamics of populations using simulation models. Amongst ecologists, there is broad agreement that populations are regulated around a equilibrium density through density dependent factors (Sinclair 1989). Fairly reliable estimates of the five main population parameters are therefore important for the development of a computer simulation model. The field data can provide the estimates for population size. But it is not possible to differentiate mortality from emigration and births from immigration using data from a study period of only 22 months, and more comprehensive analysis of this data will have to await accumulation of results for a longer period.

Body weight can be interpreted as an indicator of nutritional status. It is influenced by sex, age, behavioural and environmental factors. Adult males were heavier than females during most months of the year. Male body weight was strongly seasonal, reaching a maximum in mid summer and minimum in winter. Similar results have been reported by Efford (1991) and Bell (1981) from the Orongorongo valley possum population. Brockie *et al* (1987) found in their study of farmland possums a seasonality in body weights in both adult sex classes, but it was less pronounced in females. In this study average weights were lower during winter 1989 compared with winter 1990. This difference was more pronounced in adult male possums than in females. The measurements at Castlepoint weather station show that winter 1989 had higher rainfall and larger daily temperature fluctuations. In fact, for New Zealand, winter 1990 was the 7th warmest since records began in 1853 (Burgess 1991). Adverse weather conditions with extended periods of rain would make it difficult for the possums to satisfy their energy requirements, and their energy deficiency would be exacerbated if environmental temperature was low. Jolly-Seber survival estimates were positively correlated with average body weight in adult males. Poor nutritional status is likely to reduce the individual's resistance to environmental and disease hazards. Survival as estimated by the Jolly-Seber model is affected by emigration and mortality. Seasonality of the latter would mainly be related to changes in the effects of environmental hazards. Emigration is more strongly related to the annual breeding cycle, which has its own seasonality. As discussed below in the paragraph dealing with immigration, it appears that emigration was uncommon during the winter months.

Home Ranges and Den Use

Home range calculations based on cumulative trap catch information over the study period indicate that adult males ranged over larger areas than adult females. The actual home range estimates depend on the method of calculation, and although the use of 90% or 95% confidence ranges provide a way of estimating the full area, they are nevertheless likely to be

an underestimate of the true values as they were based solely on trap catch and den location information, without any radio-tracking data (Trevor-Deutsch and Hackett 1980). Because it is acknowledged that the home range estimates derived here are biased low by the method of data collection, the term "activity area" is used in cases where it is desired to avoid the suggestion that the information relates to true home range. This is being investigated separately in a later stage of the longitudinal study. Ward (1984) found that such home range figures were underestimates when comparing trap- and radio-revealed home ranges of four possums which were tracked hourly on 3 nights per month. In forest habitat he measured annual home range sizes varying between 0.29 and 4.75 hectares. In scrubland/pasture habitat Brockie *et al* (1987) used radiotracking to determine that males and females both ranged over an area averaging about 30 ha. Brockie *et al* write that possums from the study area whose home ranges stretched across open pasture exploited areas more than 10 times larger than their average counterparts living in forest habitats. Jolly (1973) also studied possums in a mixed scrub and pasture habitat and found trap-revealed home ranges of 0.8 ha for males and 0.3 ha for females. Dunnet (1977) conducted the first longitudinal study of a possum population in Australian scrub/pasture habitat. He reported average trap-revealed areas of 1.08 ha for adult females and 3.03 ha for adult males. In New Zealand forest habitat Clout (1977) estimated trap-revealed range areas of 1.1 to 1.4 ha for males and 0.93 to 0.94 ha in females.

Comparing these figures with the results from this study obtained by various estimation methods, it seems reasonable to conclude that average trap-revealed home range sizes in the study area are about 2 to 3 ha for adult females and about 3 to 4 ha for adult males. Taking into account the problems involved with using trap catch information for home range estimation it was decided to take the analysis of the data no further than this, since the radio-tracking information required for a full analysis is being collected in later parts of the study. Trap catch information was used to get an indication of overlap of activity areas of possums, comparing adult males and females. The results suggest that female ranges are less randomly distributed (with a tendency towards a uniform distribution) than males. This can be interpreted as less overlap between female activity areas than between males. Winter (1976) found in his study area that adult females occupied mutually exclusive areas whereas males did not. In contrast, Clout (1977) came to the conclusion that in his study the distribution of adult male captures differed significantly from randomness, whereas for females it did not. He interpreted his findings as an indication for a tendency towards mutual exclusiveness between the ranges of adult male resident possums. Differences between habitats and measurement circumtances may partly explain these differences in territorial behaviour, but Clout's findings demonstrate the degree of uncertainty which remains about various aspects of behavioural patterns in possums. Further investigations have to be directed towards clarification of the issue of territoriality in possums, to assist in clarifying behavioural aspects of tuberculosis transmission.

Possums in this study used up to 20 different den sites. As the individual den site tracking effort was relatively low, it was not possible to determine how many different den sites a possum might potentially use. Cowan (1987) found that possums in forest habitat used up to 18 different den sites over a period of 2 years. In similar habitat other authors estimated a yearly total of 10 -15 dens for individual possums from a fitted regression curve (Green and Coleman 1987). In contrast, possums in Australia were reported to use from 2-5 dens per year (Winter 1976). Preferential use of a few den sites has been observed in the forest habitat of Orongorongo valley (Cowan *et al* 1987). The same author also reported that males used significantly more dens than females. This was not corroborated in this study. But the data suggests that female possums tend to select den sites over time which are relatively close together whereas males choose dens which are spread out. This supports the findings from the home range calculations where female possums also had a smaller activity area. Den site sharing occurred infrequently and simultaneous den sharing was rare, except for mothers and their pouch young. Green *et al* (1987) and Cowan (1976) came to similar conclusions when studying den site sharing in New Zealand forest habitat, as did Winter (1976) in Australian eucalypt forest. However den sharing does occur in some environments. In a New Zealand study on open farmland with a significant proportion of swamp area and streamside willows four den sites in hollow trees were examined regularly. On five occasions 2 of the sites were found to be used by up to at least 3 possums simultaneously and by a total of six possums over a period of one month. Such sites may be atypical because they are far superior to alternatives in this environment. The carcasses of two dead possums were found in one of the dens while it was being inhabited by live possums (Fairweather *et al* 1987). In the present study one possum was found to live in a den on top of the carcasses of two dead possums. On one occasion the carcass of a possum was found in the same pile of root rakings which was used by another possum as a den site.

Kean (1967) and other authors (Green 1984, Clout 1977) discussed population strategies of possums in various habitats. They stressed the importance of the abundance of key resources, such as dens, for regulation of population density. It was suggested that the possum population density regulating mechanisms which evolved in a more "uniform" space setting (e.g. Australian eucalypt forest with limited numbers of suitable den sites) are ineffective in the more complexly structured New Zealand habitats. With ample den sites available (and adequate feed), possum densities reach figures rarely recorded in Australia. Under these conditions physical separation of territories breaks down and den sharing may become a more common event.

Home Range and Dispersal

Analysis of immigration can only be done using data beginning in late 1989, when the trapping grid first reached its full size and trap density. Within the time period of the present study it was difficult to distinguish immigration from local recruitment. Most of the locally born juveniles which were captured independently were first found between October and

December during both years. Significant numbers of untagged juveniles (which must be considered as immigrants) were observed between December and March. Juvenile immigration was almost nil during the winter months. Efford (1991) came to similar conclusions in his analysis of data from the Orongorongo valley study. This suggests that the survival estimates based on the Jolly-Seber model did in fact mainly measure mortality during the winter period. A few untagged adult possums were captured during most months of the year. Males clearly dominated among juvenile immigrants, whereas the sex ratio in locally recruited animals was not different from unity. This supports the view which has been expressed by a number of authors that juvenile male possums are more likely to disperse than are their female counterparts (Dunnet *et al* 1976, Winter 1976, Clout and Efford 1984). In an analysis of 10 years of data from the Orongorongo valley, Efford (1991) found that this possum population can be considered a 'dispersal sink'. He notes the somewhat surprising conclusion that although possums in the Orongorongo Valley are living in an 'optimal' habitat, local reproduction on the site seems to be inadequate to replace animals lost through mortality and thus it is not possible to maintain population density without immigration. In the present study it was found that over the 22 months net disappearance exceeded net inflow (births and immigration), as calculated using Jolly-Seber population estimates. The duration covered by the available data is not long enough to draw firm conclusions about the stability of the population.

The issue of what movement distance represents dispersal is difficult to resolve definitively. Some authors have considered movements of distances between 0.5 km (Keber 1988) and 3 km (Brockie *et al* 1989) as long-range movements, whereas Efford (1991) restricted his review of possum dispersal to movements of at least 2 km as the crow flies. In the present study taking into account topographic characteristics and capture statistics a threshold dispersal distance of about 1 km was considered appropriate. Only two animals satisfied this criterion, although two other immature males appeared to make a definite shift in home range by a smaller distance. One possum which moved over 1 km was an immature male, the other was a mature male. The data is generally insufficient as yet to do more than report individual cases. The mature male possum suddenly left the study area after it had been captured regularly. It wandered over a distance of about 1.4 km, crossing a number of farm paddocks and ridges.

For a understanding of the dynamics of spatial spread of tuberculosis infection in possums detailed information describing the statistical distribution of both dispersal occurrence and distance for the individual sex and age groups is required. For the spread of rabies in foxes the dispersal of sub-adult male foxes is believed to be a very important factor (MacDonald 1980). Mollison (1986,1987) emphasizes the importance of the shape of a dispersal distribution for the success of an invasion, as well as merely the value of its mean. Long-distance infectious contacts, though individually occurring with low probability, can contribute significantly to the advance of an epidemic.

Tuberculosis Epidemiology

Research Design

Previous studies of the epidemiology of bovine tuberculosis in possums have used a cross-sectional design (Cook 1975, Cook undated, Coleman 1988, Hickling 1989, Hickling *et al* 1991). The use of a prospective longitudinal study design in aetiological research has substantial advantages over the cross-sectional approach. It permits measurement of potential risk factors which are varying over time, and allows for a time interval between exposure and the onset of disease (Rothman 1986). It also avoids the serious biases to which cross-sectional studies are susceptible in studying time-varying diseases. They depend on when the cross-sectional samples are taken in relation to the peaks and troughs of annual reproductive cycles and of changes in disease transmission and duration. Martin *et al* (1987) define prospective longitudinal studies as investigations where the original sample was obtained by cross-sectional methods and the sampling units were then observed over a period of time. This type of epidemiological study combines the advantages of cohort and cross-sectional study methods in elucidating the epidemiology of a disease. Kleinbaum *et al* (1982) classify such studies as hybrid designs. The strength of the longitudinal study is its ability to define temporal and micro-scale spatial patterns in relation to factors which may be influencing them, while the strength of the cross-sectional studies is in giving low cost spatial information at the risk of distorting temporal and age/sex patterns by the nature of the sampling process.

In a situation where a wildlife population is being studied special problems may be present such as losses to follow-up, varying intervals between individual examinations and continuous recruitment of new individuals during the follow-up period. In this study a further complication was caused by the lack of a sufficiently sensitive method for diagnosis of infection with *Mycobacterium bovis* and/or onset of clinical tuberculosis in possums. Currently it is not possible to diagnose *Mycobacterium bovis* infection reliably in the possum without sacrificing the individual. Diagnosis has to be based on the detection of palpable or visible lesions with subsequent confirmation of bacteriological samples, and evidence from cross-sectional studies suggests that at most about 50% of grossly affected animals at autopsy have lesions likely to be detectable by palpation and visual appraisal.

Prevalence and Incidence

Over 22 months, 11% of 378 possums under study developed tuberculous lesions. This crude "period prevalence" estimate is only of limited value for describing the disease situation. The denominator is inflated by the high number of new entrants to the population, which means that the population at risk is larger than the standing population size at any particular time. The numerator is deflated, since a substantial number of cases cannot be diagnosed. As clinical examinations of individual animals were conducted at minimum intervals of two months, incident cases which were diagnosed at a particular visit could have developed their lesions up to two months prior to examination, even assuming that they were actually caught at each monthly trapping session. For this analysis it was decided to use the month of

diagnosis as the onset of "disease" or more accurately the development of visible or palpable lesions. An estimate of cumulative incidence for the whole study period was not calculated, as it was not possible to derive a valid denominator representing the population at risk from the beginning until the end of the study.

Monthly incidence density was calculated using the average population at risk multiplied by the study period in months as the denominator and the total number of cases as the numerator. Population at risk was approximated using the minimum number of tagged animals which were assumed to be alive (Pollock *et al* 1990). This results in an average monthly incidence density of 0.014. Summary information based on monthly prevalence and cumulative incidence estimates was considered more appropriate for further analyses. These figures indicate that on average 8% of animals in the population were identifiably tuberculous during each month. This is the lower limit of possible values for true prevalence. Among the tuberculous animals examined at autopsy, 54% had superficial lesions which were detectable on clinical examination. This agrees with findings from other studies (Julian 1981), and suggests that the true average monthly prevalence in this population over the study may well have been about twice as high as the recorded figure, about 16%. Although infected possums were present on the site throughout the study period, monthly point prevalence estimates showed marked temporal peaks and troughs between the extremes of 0.02 and 0.18, demonstrating the extreme care which must be used in interpreting cross-sectional study data obtained from kill-trapping.

Age and Sex Distribution of Infection

Sexually mature animals were 2.25 times as likely as immatures to show tuberculous lesions, but within each sex group there was no significant difference between age groups. This lack of significance is probably an artifact, since stratification by sex class for the data from the present study resulted in a loss of statistical power, because the numbers of cases dropped significantly. An increased risk of tuberculosis for mature possums was also found in two major cross-sectional surveys in the Buller/Inangahua counties and the Hauhungaroa Ranges (Cook 1975, Hickling *et al* 1991). As mentioned in the discussion of the latter study such a difference might be expected in an area with endemic tuberculosis, as individuals are under continuous exposure to infection and remain fully susceptible throughout their lives. However the validity of such a conclusion depends on which of the potential mechanisms of spread are operating. In a cross-sectional study in the Hohonu ranges both age groups appeared to be at equal risk (Coleman 1988). If this finding is not biased by the nature and timing of the investigational method, then the results would suggest that the disease had not been in the particular area for very long, that the total population of tuberculous animals included a substantial proportion of infected sexually immature immigrants or that in this particular case infection mechanisms affecting immature animals were more important than the ones between adults.

In the present study prevalence was higher in females than males. Stratification on age group reduced the number of cases in immatures to 5, which was considered too small a sample size for further analysis. In mature possums prevalence was 0.094 in males and 0.15 in females. A higher risk of infection in female possums across age groups is a rather surprising result. Considering that the level of significance was p=0.083, it has to be interpreted cautiously. But taking into account the finding that on average infected female possums were almost a year older than infected males and that the population age structure was slightly skewed towards older females, it seems reasonable to attribute this result to females being exposed to risk of infection during a longer life time provided that one or more mechanisms of spread result in risk of transmission being cumulative over adult life.

Another issue with regard to females is that it seems reasonable to expect that they are under increased stress when raising pouch young, which makes them more susceptible to establishment of infection and/or development of lesions. Pregnancy is generally considered as exerting an unfavourable influence upon tuberculosis cases (Rich *et al* 1951). Mims (1987) points out that increased susceptibility to infection in pregnant women, especially from developing countries, is likely to be the result of multiple factors interacting, such as pregnancy and malnutrition. If transmission were proportional to the amount of direct or indirect contact within their activity areas, prevalence should be higher in adult male possums, because adult female possums were found to have a tendency towards more exclusive activity areas. Females also had smaller activity areas and the den sites they used over time were more tightly clustered. One explanation would be that the males were responsible for the spatial dissemination of infection, as they had larger and more overlapping activity areas with den sites being further apart; but that females maintained infection within clusters. This could produce both higher prevalence in females and dissemination of infection among clusters.

Results from the large scale surveys in the Hohonu Ranges and the Hauhungaroa Ranges suggest that tuberculosis prevalence was higher in male immature possums compared with their female counterparts, but there was no such sex difference among mature possums (Coleman 1988). In contrast the survey in the Buller/Inangahua counties did not show a difference in tuberculosis prevalence between age or sex classes (Cook 1975). An increased risk of tuberculosis infection in immature males compared with their female counterparts would suggest either that males are subject to some special risk or that a higher proportion of male immigrants is infected than the proportion of locally born immature animals. The latter explanation would suggest that the particular area is subject to an infection gradient. Age and sex distribution of infection can provide important clues about transmission processes, but caution is required in interpreting this information from cross-sectional studies because of the susceptibility of the method to biased sampling. In contrast, the longitudinal study gives a more reliable assessment, but takes considerable time (as seen here) to accumulate sufficient evidence to clarify differences in incidence.

Time to Death or Disease for Different Categories of Possums

Survival analysis was used to estimate probabilities of the occurrence of time-dependent events such as death or tuberculosis lesion development in possums. When analyzing the probability of survival it was assumed that disappearance from the study site implied death of the animal. Interpreting the results it has to be kept in mind that disappearance is likely to include emigration as well as death. The results of the analysis suggest that possums which develop tuberculous lesions at some time during follow-up have a cumulative survival probability (= true survival) of 37% over twelve months. Possums without tuberculous lesions have a 51% probability of surviving (= survival or staying within the study area) for the same period. These results based on the Kaplan-Meier estimator are subject to potential bias as the method does not take account of time-varying confounding factors. Brockie (1991) reported from a study of farmland possums that the population turned over very quickly with an annual mortality rate varying from 22 to 77% between 1984 and 1987.

The discrete hazard rate model is a better way of handling survival data because it allows for inclusion of time-varying covariates in order to calculate hazard risk ratios which are controlled for seasonal effects. The final hazard regression model shows that once an animal develops tuberculous lesions its risk of dying becomes about 8 times the baseline risk. Although no conclusion can be drawn about the interval from infection to development of clinical disease since no suitable test is yet available, the analysis shows that once animals develop clinical disease they are likely to die fairly quickly. The model also suggests that possums are at increased risk of dying or disappearing during the winter period and that the risk is reduced in summer. This result emphasizes the importance of environmental hazards in controlling population size. This was pointed out during the discussion of the ecological findings, and has been found in other studies of small mammals (Krebs and Myers 1974). The difference between age groups shows that over the time under observation the risk of death or disappearance increases faster in sexually immature possums than in mature possums. Sinclair (1989) reviewed factors which may be involved in population regulation of animals. He argues that in species with intermediate reproductive rates (such as birds and small mammals) regulation through late juvenile mortality is of importance. While mortality in the juvenile group has been shown in possums and other species to be high, interpretation is complicated by the fact that dispersal occurs in the same age group, and in most cases cannot be differentiated from mortality. Maturing juvenile possums are in the difficult position of having to establish themselves in their own home range. This probably involves aggressive interactions with other possums and could also result in use of less well protected den sites. Thus only a proportion of the dispersing animals successfully establish themselves as breeding animals in a new home range.

The period of time between development of clinical disease and death is important for the transmission dynamics as it is reasonable to assume that this period coincides with the time of maximum infectiousness of the tuberculous animal. During this time the tuberculous

individual represents a spatially dynamic source of infection, which has multiple opportunities of infecting other possums within the population. After death the carcass will represent a static source of infection with its infectiousness limited by the characteristics of the location (den site or open pasture) and environmental weather conditions. Time from lesion detection to death follows a lognormal distribution, in which there is an initial increase in the hazard function but the distribution then tails off towards infinity. The shape of the curve represents a high disease hazard shortly after lesion development, but shows that occasional cases can survive for extended periods of time. Diseased possums are more likely to die during spring than during other seasons of the year. Hence, adverse environmental conditions reduce the survival probability of clinically tuberculous possums.

A survival analysis approach was used to analyze risk of developing tuberculous lesions, taking into account the duration of follow-up. Not knowing the length of the period between infection and clinical disease complicates interpretation of the results from this analysis. Two analyses were conducted, one assuming that all animals were potentially likely to develop lesions and a second only including animals which are known to have eventually developed tuberculosis and which can therefore be assumed to have been infected for varying periods of time. These analyses are meaningful in the sense that the outcome variable marks the beginning of infectiousness for a particular tuberculous animal. Both showed similar results with regard to inclusion of spring season and age group into the model. There was a 4-fold increase in risk of developing tuberculous lesions during spring compared with any other season of the year. The possible reasons for this are discussed below. The final model does not include winter as a risk factor, probably because environmental conditions during winter 1989 were unusually harsh, whereas rather favourable conditions were prevailing during winter 1990. Using this analytical approach, individual sexually mature animals were less likely to develop tuberculous lesions than were sexually immature possums. This is despite the fact that the incidence was higher in adults, and reflects the different ways in which incidence measurements and survival analysis measurements define their denominators for populations at risk. As outlined before, immature animals from independence to sexual maturity are under increased social stress which makes them more likely to develop clinical disease.

Insights from Epidemiological Differentiation of Strain Variants

Differentiation of *Mycobacterium bovis* isolates into restriction endonuclease (REA) types was used in an attempt to refine a set of hypotheses which could explain the observed spatial and temporal disease patterns. Based on major and minor differences of gel patterns it was possible to discriminate four REA types, two of which (4 and 4a) comprised the majority of isolates. At this stage of the investigation only a limited number of isolates have been REA typed, and in only a few cases could links between individual cases be investigated. Strong evidence for a specific form of transmission was found in the case of a joey which was infected with the same REA type as its mother, thus providing an apparent example of

pseudo-vertical transmission. This is likely to be a common situation for mother-joey pairs in which the mother has tuberculous lesions. The only other two cases in which links between infected individuals could be identified were where sequential den sharing had been documented, and in both cases the observed REA types were found to be different. Investigation of the temporal incidence of clinical tuberculosis shows that REA type 4a showed strong peaks during both winter-spring periods, whereas REA type 4 was found only over a single period from August 1989 to May 1990, with a much less pronounced peak. REA type 4a exhibits time-space clustering with a bi-modal distribution of time differences as had been observed for tuberculosis in males. In fact, males contributed 61% of tuberculosis cases with REA type 4a and 36% of REA type 4 isolates. Males also showed greater reduction in body condition in winter than did females. A plausible explanation for the time and space distribution of type 4a would be that infection was being disseminated by a number of males through male-male as well as male-female contacts, thus producing a less tight geographical clustering than for type 4, and producing winter-spring peaks in incidence at a time when body weights were reduced by adverse environmental conditions. There was no statistical evidence of time-space clustering for REA type 4, keeping in mind the low sensitivity of the Mantel statistic and that this strain was only prevalent for a relatively short period of time. The total number of isolates of this type was not much below that for 4a, but it was predominantly in females and when the infected animals died the strain appeared from the data to die out on the site. Given that type 4a was the predominant strain on the site among the isolates for which typing results were available, the findings for it may best represent the "endemic" situation for a particular strain (and, by implication, for all strains in the endemic situation). Type 4 may represent the situation where there are few males disseminating infection to new clusters of females, and persistence is dependent on pseudo-vertical transmission.

Inspection of the spatial distribution of the different REA types reveals that activity areas used by tuberculous possums with REA types 4 and 4a overlapped to a limited extent and even less if only locations of dens were mapped. This finding further emphasizes the importance of transmission factors which are related to the location of areas in which particular possums find their den sites.

Analysis of the involvement of other species at the site in the epidemiology of *Mycobacterium bovis* infection allows some interesting inferential conclusions with regard to transmission paths. All four REA types were found in isolates from other species. Hence, even though only 3 possums with REA types 4b and 10 were found within the area, the same strain was found in a ferret and probably in one heifer, which was found with a tuberculous lesion at the meat works. It is interesting to note that types 4 and 4a were each found in a cattle beast and a wild pig with tuberculous lesions, and that the date of detection suggested that the animals had become infected when the particular type was prevalent on the site.

Whereas cattle can become infected either by close contact with live tuberculous possums or from contact with carcases, wild pigs are behaviourally more likely to become infected by eating tuberculous possum carcases. Five of 7 possum carcases from previously unrecorded animals found on pasture were found to be tuberculous, thus reinforcing these carcases as a possible source for other species. Terminally ill tuberculous possums tended to move their denning location from high on the hillside down to the valley area as they apparently became incapable of climbing the hill, and hence are more likely to come in contact with cattle during foraging and more likely to die where cattle could find the carcase. The manager of the study farm has seen cattle investigate dead possums in that area.

Although infection appears to move fairly readily from possums to cattle, the evidence does not favour transfer of infection from cattle to possums as a significant part of the epidemiology of the disease. If infection was regularly seeded into the possum population by cattle, a temporally and spatially random distribution of REA types in possums would have to be expected. It also would have been likely that REA type 4 would have appeared again.

Temporal Course of the Disease Process

An understanding of the time course of the development of the disease from establishment of infection to death (or perhaps recovery) is basic to establishing a coherent understanding of the epidemiology of the disease. Yet in the absence of a sensitive and specific diagnostic method for the disease, the time course can only be inferred from indirect evidence.

In order to understand the disease process, it is necessary to consider the steps from pathogenesis through to death from the disease. The three main sources of bacteria from possums are exhaled organisms from the respiratory tract, liquid pus from discharging superficial lesions, and particles of dried pus on fomites (which for a time may still contain infectious organisms). Evidence from studies in other species show that organisms can be carried on dust particles, on exhaled water droplets and on the much smaller droplet nuclei which remain when droplets dessicate. It has been shown that large bacteria-bearing dust is too large to penetrate to the lung, and that large droplets are deposited in the upper respiratory tract, carried by the cilia to the larynx and then swallowed. In contrast, droplet nuclei carrying organisms penetrated the entire bronchial tree, became implanted on the alveolar walls and developed visible tubercles within 5 - 6 weeks (Wells 1955). Aerosolized droplet nuclei as a mode of infection would appear to be limited to situations where possums are in close proximity to each other. Dustborne infection would be more likely in contaminated den sites. Resuspension of contaminated particles may be possible (Houk *et al* 1968). However in relation to human tuberculosis the view is generally held that tubercle bacilli lodged on fomites do not constitute a significant infection hazard, both because most of them die quickly through action of drying, heat and sunlight, and also because dried secretions are very difficult to fragment and suspend in air, and airborne particles which do arise from surfaces are too large to penetrate the lung (Anon. 1967).

Possum carcasses would provide favourable conditions for extended longevity of bacteria. They would be relatively protected from the effects of ultraviolet radiation and dehydrate slower than out in the open. In some cases they would contain sufficient organisms to provide the much larger dose required for infection by the oral route. However such persistence is irrelevant unless there is a mechanism for these bacteria to then initiate infection in a further host. Scavenging may also make them unavailable to species other than scavengers.

During the discussion of the results of the cross-sectional study in the Hauhungaroa Ranges the distribution of primary lesions was used as an indicator for potential transmission paths. The longitudinal study design does not lend itself to these kinds of inferences, as animals were not necropsied until they died of some natural cause. Hence, tuberculous animals commonly had multiple lesion sites when examined. The importance of the axillary lymph center for lymph drainage in the possum was supported by the fact that in 75% of cases where diagnosis was based on clinical examination during capture, this particular site among the superficial lymph nodes was involved in the disease process. The lung was also strongly confirmed as a predilection site of infection. Lesions associated with either the lung or the axillary lymph center can be considered as of special significance for the transmission of infection between possums. About 40% of lesions in superficial lymph nodes were draining exudate. Based on autopsy data from all tuberculous possums examined, it appears that the main routes of excretion of *Mycobacterium bovis* organisms are from lung lesions and from open sinuses. These sinuses discharge mainly from lymph nodes in the axillary and inguinal regions. This contrasts with the badger, where excretion in urine from kidney infection is a common finding (Wilesmith 1991). Open lesions were found more often in adult females than males, and among the females those with pouch young were more likely to have discharging sinuses than those without pouch young. As outlined previously the offspring of tuberculous mothers would be extremely unlikely to evade infection.

Once infection is initiated by one of the mechanisms described above, it would appear that stress factors strongly influence the progression of the disease in the possum, much as they have been recognized to do in man. Breazile (1988) discusses stress as a factor influencing the pathogenesis of disease, and terms those forms of stress likely to influence disease pathogenesis as "distress". With regard to *Mycobacterium bovis* infection, it is of particular importance that as a reaction to stress, adrenal corticoid hormones are released which suppress immune-mediated cellular responses. As quoted in a review by Peterson *et al* (1991), it has been shown that mortality increased in *Mycobacterium tuberculosis* infected rats and mice under the influence of a stressor such as crowding or forced exercise. The marsupial species *Antechinus stuartii* provides an example of a natural situation where stress-induced mortality is considered to be a major factor in population regulation. The males of this species experience a total postmating mortality caused by a number of diseases. This phenomenon has been attributed to an increase in plasma free glucocorticoid concentration

with associated immunosuppression (McDonald 1980). Kelley (1985) showed that in calves cold exposure initially resulted in immunoenhancement, but after 2 weeks cell-mediated immune response as measured by the tuberculin test was depressed. Mims (1987) reports that tuberculosis in man is often activated or made worse by corticosteroid administration and that stress acts in the same way probably through increased secretion of corticosteroids. With regard to the present analysis, environmental (e.g. adverse weather conditions), physiological (undernutrition, modified exercise levels, pregnancy and lactation) and psychological influences (crowding, mating) could be considered as potential stressors in possums. These stress factors commonly act jointly in complex ways, where the outcome in any single case may depend on a number of other factors such as sex and immune status of the host; and type, timing and duration of the stressor relative to the onset of infection (Peterson *et al* 1991).

Spatial Aspects of the Disease Process

A notable feature of the epidemiology of tuberculosis in possums which must be explained by any theory of transmission, is the extreme spatial heterogeneity in prevalence. This spatial heterogeneity amounts to local clustering of disease with intervening populations which have negligible prevalence. Rothman (1990) considers the study of disease clusters as one of the main purposes of epidemiological research. He writes that in a very general sense any aggregation of cases by location, season, year, sex, age or any other environmental circumstance can be considered a disease cluster. The occurrence of clusters may be the consequence of clustering of the causal mechanisms of disease. Marshall (1991) defines clusters of disease as occurring in space, in time or in both. He adds that spatial clustering over a short time span may be due to infectiousness, but may also be the result of transient environmental hazards. Clusters can occur by chance alone representing spatial heterogeneity. Marshall writes that the duality between spatial dependence and spatial heterogeneity can only be unravelled by repeated temporal data. The evidence so far on the study site shows that infected sub-populations are co-existing with persistently negative ones, in clusters which are identified by the fact that animals which share the same infection status, den in close proximity to each other. Moreover in the case of REA type 4, it seems possible for a previously common REA type to disappear for an extended period when the animals which produced the infection cluster all died without apparently leaving any infected progeny on the site.

Evaluation of Potential Transmission Mechanisms

The dynamics of tuberculosis infection can be divided into two major components, one being the temporal and spatial dynamics *within* a population and the other spatial spread *between* populations. The longitudinal study described here was mainly concerned with identification of the most important transmission paths for *Mycobacterium bovis* infection *within* a possum population, and to its associated population of domestic livestock and wild animals sharing the same habitat. It is postulated that disease transmission occurs through a number of

different mechanisms, which are related to social interaction between the sex and age strata of the possum population. These processes vary temporally in their importance during the course of a year, and contain features which produce both the spatial clustering of infected animals found in this and other studies, and the temporal variation over the course of the year which has been investigated more fully in this study than in any previous investigation.

The following putative mechanisms were identified as requiring consideration and the evidence to date from the longitudinal study will be used to examine the extent of support for each mechanism and to suggest information which will be required to resolve uncertain issues.

- *Sharing of grazing area* may produce indirect transmission through consumption of infected pasture.
- Both adult sex classes may transmit infection to each other during *courting* and *mating*.
- Adult and probably immature males are considered likely to get infected during *fights and other agonistic behaviour*.
- Young possums while dependent upon their mothers are at risk of maternal infection by one or more routes (*pseudo-vertical*).
- Transmission during *simultaneous den site sharing* and *mutual grooming* provides an opportunity to transmit the disease between all groups of the population.
- All animals may be at risk from indirect transmission through bacterial contamination of *sequentially shared den sites*.
- *Territorial marking activities* (which are more common in adult male possums) represent another potential mode of indirect transmission.

In thinking about transmission mechanisms, these seven possibilities cannot be treated as mutually exclusive of each other. The way in which the dynamics of transmission must be considered can be illustrated with the data from the longitudinal study, using figures purely to explain the concept towards which epidemiological analysis of the disease should be directed.

During a year there is some number of transmission events per unit of population. Monthly clinical incidence density for the 21 hectare study site was 0.014, so annual incidence density would be 0.17. If the measured incidence is only 50% of the true figure, then the true incidence density might be as high as 0.34, although the true figure is not important to the argument.

If at any one time there are 7 possums per hectare and annual mortality is 30 to 50%, then in the course of a year there are likely to be in total at least 10 possums per hectare because of population turnover. Thus for a 100 ha area a conservative estimate is that there will be 1,000 possums in the population at risk, but (at 8% apparent and 16% true clinical prevalence) an average of 160 of these would be infected, making the population at risk 840.

This would mean that 143 successful transmission events would occur per year. If two of the seven transmission mechanisms described above accounted for 60% of incident cases, two more accounted for another 30% and the remaining three accounted between them for the remaining 10%, then reducing the first two mechanisms by 80% would reduce the number of transmission events from 143 to 74 (48% reduction) whereas equivalent reductions in the second pair would reduce the number from 143 to 109 (24% reduction) and in the final three from 143 to 132 (8% reduction), making a total of 80% reduction. It is therefore important to move towards an understanding of the relative importance of the seven putative transmission pathways in order to identify where the greatest gains could be made. It will later also be necessary to know the effect of control measures on each of the pathways, since reducing one mechanism by 80% will change the relative contributions of the mechanism to the remaining incidents which occur, and the greatest additional gain may then come through adding a different control action to the policy, rather than intensifying the same control measure further. For example, if the 80% reduction had been achieved in the first pair of transmission mechanisms above, then instead of 143 transmission events there would now only be 74. Complete elimination of these two main pathways would lower the number of events by only a further 17 to 57 (probably at very high cost) whereas 80% reduction in the second pair of transmission mechanisms would eliminate 34 events and reduce the total number of transmission events to 40.

As a practical illustration, a poison operation might substantially reduce transmission between adults because of the reduced total population and reduced social interaction, but may exert proportionately less influence on the number of mother-joey pseudo-vertical transmissions because mothers with pouch young which survive will have an unaltered probability of infecting the joeys and such mothers may perhaps be less likely to take a bait in a winter poison operation because of different foraging patterns. They would also gain in survival probability from reduced population density. However they may be quite susceptible to poisoning from bait stations, which have the bait available continuously.

This manner of thinking sees disease transmission as a dynamic process in which there are various different flows of infection within the possum population which can be separately manipulated by control actions, rather than a static situation in which all transmission events are seen as equivalent both in their importance and their susceptibility to particular control measures. As a first step towards being able to precisely target particular control measures, it is necessary to attempt to rank the seven transmission mechanisms in estimated quantitative contribution to total flow of infection. However in the absence of a diagnostic test to detect transmission events, this becomes a difficult process which must be assessed indirectly.

At this stage of the study the evidence is quite preliminary, but an assessment of the evidence supporting each transmission pathway can be carried out to guide later data collection, and hence to provide evidence to test each of the hypotheses put forward for evaluation.

Sharing of Grazing Area

It has been widely assumed that an important mechanism of spread of infection both among possums and between possums and domestic livestock must be the deposition of organisms on pasture and their subsequent ingestion or inhalation by susceptible animals. If this were an important transmission mechanism, it would be a particularly difficult one to control because of the inability to influence the movement of possums on pasture. Many farmers believe that this is a major transmission mechanism, and hence that they can do little or nothing to influence the disease.

However evidence to support it is quite limited. The finding that prevalence in possums is highest close to the forest-pasture margin (Coleman 1988) would seem to suggest that pasture is somehow a risk factor, but most other transmission mechanisms would tend to produce the same distribution for other reasons, so this is not a strong point. Available evidence from various sources would favour a short rather than a long survival period for organisms on pasture, and would also indicate that the more realistic the circumstances in such studies, the shorter the period of infectivity of organisms for natural hosts (Morris *et al* 1994). More detailed evidence is required on organism survival since the existing material is generally old and techniques used are mostly archaic, but it seems doubtful that organisms would survive on open pasture long enough to produce a significant proportion of total transmission events.

Data from the longitudinal study shows no support for indirect transmission on pasture. If transmission commonly occurred by this route, there should be little evidence of clustering of infection in groups of possums denning within the same vicinity. Mapping of possum movements shows that infected and uninfected animals regularly share the same grazing areas, yet infection is heavily concentrated in groups of possums which den in proximity to each other, while other nearby groups show low or zero prevalence. Evidence from the REA typing further reinforces this, by showing that different REA types were found in different parts of the study site, and that REA types which were only found in the grazing area rather than the denning area did not figure prominently among the strains circulating on the site.

Cattle readily became infected during the early part of the study when prevalence in possums was high and a substantial number of terminally ill possums was present. This supports the role of density of infectious possums as a risk factor for transmission to cattle. However it says nothing about how infection could have been transmitted. The fact that there was subsequently little further transmission despite the continuous presence of tuberculous possums casts considerable doubt on transmission through mere sharing of pasture. The fact that cessation of transmission coincided with completion of a fence which separated the cattle from the possum denning areas raises questions which could not be answered within this phase of the study.

Transmission through Behavioural Interaction of Possums

It is not possible to definitively distinguish different forms of transmission by behavioural interaction as listed above, but some indications of important factors can be deduced. It appeared that possums which denned in particular areas of the study site showed a higher incidence of infection than those denning in other areas, and a comparison of estimated possum density with incidence showed that areas of higher tuberculosis incidence were not the same as areas of high possum density. The clustering of infection was not therefore simply a density-dependent transmission process.

The evidence shows that tuberculous possums den closer to each other than to non-tuberculous animals, and also tend to have activity areas which overlap more with each other than do tuberculous and non-tuberculous animals. This could be explained quite adequately by various forms of behavioural interaction, although it could also be explained at least in part by indirect transmission in and around dens.

The time period covered by this analysis is too short to allow firm conclusions to be drawn about temporal variability in incidence, but there are preliminary indications that there may be a peak of new clinical cases in late winter or spring each year. This could be explained either by the prior occurrence of a peak in transmission, or by a peak in conversion of infected animals from incubating to clinical as a result of environmental and nutritional stress, which is usually most severe in late winter. If there are annual peaks of transmission between adults (which cannot be decided in the absence of a diagnostic test), then these might reasonably be expected to occur in association with the breeding seasons in February-March and August-September. A plausible explanation for a winter-spring peak in clinical incidence would be a peak in transmission at mating, followed by a peak in conversion of these incubating animals to clinical cases during the most stressful time of the year. However at most this can be a hypothesis on the data presented, which can be further investigated in the field but requires a diagnostic test for definitive assessment. The only apparent alternative explanation for seasonality of transmission (if it genuinely exists) would be that transmission peaks in winter and early spring when the clinical prevalence reaches a peak, and possums are likely to be competing more intensely for favoured den sites and scarce feed resources. During this time they may reach contact frequencies with other possums which approach those which occur during the mating seasons.

The fact that sexually mature animals had clinical incidence 2.3 times that of immatures shows either than *infection* accumulates with increasing length of exposure, or that *disease* accumulates with increasing age, because there is a pool of subclinically infected animals accumulated from earlier transmission events (or both of these). The tendency for tuberculosis in males to cluster in space/time (but not male-female or female-female) suggests that males which develop the disease are likely to transmit it directly to other males which have their home ranges in the same vicinity, but that the other sex-pair transmissions are not determined by denning proximity. This in turn would be consistent with the view that males interact with

females over a broader geographical area through courting and mating and hence that temporo-spatial clustering will be less apparent, but it seems surprising that female-female transmission does not show the tightest clustering of all in space-time. If this evidence for space-time clustering of tuberculosis in females continues to emerge as more data becomes available, it would suggest that there is little female-female transmission, with most adult transmission being male-male and male-female. This would then emphasize transmission through courting and mating rather than through mutual grooming or sequential den sharing. The temporo-spatial association of male-male transmission would emphasize the importance of agonistic behaviour and territorial marking behaviour and give it a higher ranking among the possible transmission mechanisms.

It is unclear from previous studies to what extent male possums mate with a segment of the breeding females within their home range which comprise a cluster or non-random sub-sample (whether determined spatially or on some other grounds). It may well be that this is quite variable among different habitats, which may in turn have implications for producing differences in tuberculosis transmission in different habitats. The initial den site data for this site is limited in quantity but suggests that female possums tend to den repeatedly in the same vicinity though not in the same den, and that because of the geographical nature of the study site, there is limited "social" mixing of possums which den in physically distinct though spatially close parts of the area, other than the mixing which occurs on pasture and which appears to involve relatively little interaction of a nature which could spread infection. Males showed the same tendency to den repeatedly in the same vicinity but (as might be expected) ranged more widely over the area and were more likely to den opportunistically in areas not normally considered denning areas, especially in the breeding season. Thus while if seen in terms of population genetics there may be only limited deviation from random mating, in terms of disease transmission there may be clustering of males with sub-groups of females within the study site, thus contributing to the spatial clustering of infection seen in this and other sites examined. Therefore in this restricted sense it is suggested that mating even within a study site as small as 21 ha may deviate from randomness as far as probability of transmitting infection is concerned.

Pseudo-vertical Transmission

Transmission from mother to joey which arises from their close contact after the birth of the joey, but does not occur through gametes or during intra-uterine development (which would be vertical transmission). Pseudo-vertical transmission could be a important path of transmission between possums, much as it appears to be for tuberculosis in the badger. It has been suggested that this particular mode of transmission may be important for maintenance of infection in a badger population (Cheeseman *et al* 1989). As pointed out in the discussion concerning the cross-sectional survey in the Hauhungaroa Ranges, the probability of successful transmission from mother to young during the prolonged nursing periods which are usual in possums, can be considered as high. To have an impact on tuberculosis infection

dynamics within and/or between populations it is necessary for an infected pouch young to survive for a significant period of time after independence. During the present study eleven cases of clinical tuberculosis were identified in adult females which had a pouch young present. Given the intimate contact between mother and joey, it is unlikely that the joey would escape infection if the mother was excreting organisms at any time over the rearing period. In four of these cases mother and joey did not survive long enough for the joey to become independent. In two instances the joey probably survived the nursing period and the mother died after the joey's independence. In three cases the joey was recaptured after independence, and in the remaining two cases the outcome is unknown. Hence, in at least 33% and as high as 55% of cases with known outcomes, the offspring of a tuberculous mother was potentially capable of spreading the disease. Because assessments had to be based purely on clinical examination of the mothers, another group of undetected but infected adult females would also have infected their offspring.

The possibility of a compromised immune response in females during pregnancy and the nursing period resulting in an increased risk of infection and/or development of clinical disease was mentioned above and in the discussion of the cross-sectional survey in the Hauhungaroa Ranges. The data from the present study does not so far support this, to the extent that there was not a statistically significant difference in the risk of developing tuberculous lesions between adult females with and without pouch young, for the small number of cases in each category so far. However there may be an important time influence on the epidemiology of the disease, associated with breeding. In winter and early spring when most females have dependent joeys and the incidence of new clinical cases reaches a peak, pseudo-vertical transmission is highly likely to occur from almost all the tuberculous mothers. Whether there is any tendency for infected females to develop clinical signs earlier than they would have done in the absence of a joey (and hence to increase the probability of infecting the joey) cannot be deduced from the data reported here, because there are various confounding factors inherent in the evidence which cannot yet be separated. However such a time shift is possible, and would assist in disease transmission. Certainly a substantial number of mothers with joeys become clinically tuberculous over winter.

Even if there is no specific interaction between reproduction and disease transmission, the hypothesis can be tentatively put forward that adult females are the most important reservoir group for maintenance of the disease on the study site. Incidence was highest in adult females (although from first principles of contagious disease epidemiology males should have higher incidence because they mix with other possums more widely). Females were detected as infected at an older age than males, and also died at an older age. Thus they may act as a source of infection for longer, although in the absence of a diagnostic test this must remain largely conjecture. Thirdly, they are the source of pseudo-vertically transmitted infections in joeys, which comprise an important component of total transmissions.

In fact, the possibility cannot be ruled out that the majority of infections are acquired pseudo-vertically during rearing, but that these infections then have very long and variable incubation periods, producing disease between about one and four years later. This would explain why there is no increased risk of infection in males despite the fact that they appear to have more opportunity of contact with potential sources of infection.

Den Sharing

Tuberculosis could be transmitted directly between possums which use a den together during the same day (simultaneous den sharing) or indirectly from an infected possum which deposits organisms in and around a den to a susceptible possum which uses the same den on some later occasion and is exposed to organisms persisting on fomites (sequential den sharing).

Simultaneous den sharing would have all the ingredients for effective transmission, since the animals would be in close contact for some hours under circumstances which would ensure that the susceptible animal was exposed to organisms exhaled or otherwise excreted by the source animal. Mutual grooming would be likely to occur, facilitating transmission from discharging superficial sinuses. However although simultaneous den sharing has been recorded regularly at a location where favoured den sites are in very limited supply (Fairweather *et al* 1987), both in the Orongorongo Valley study site and in this study site, simultaneous sharing appears to be very uncommon, other than between a mother and her joey, which is better considered as a form of pseudo-vertical transmission. Over the period of time reported here there was only one case in which two animals were identified as simultaneously sharing a den site, even though over 350 den site trackings were carried out and in most cases it was possible to determine definitively how many animals were present in the den. Thus simultaneous den sharing could account for almost none of the transmission which was occurring on this study site over the period considered.

The possibility of sequential den site sharing as a transmission path of tuberculosis infection between possums was definitively demonstrated when *Mycobacterium bovis* was isolated from a decomposing possum carcass in a den site. Work on human tuberculosis has shown that dust, droplets and droplet nuclei can transmit infection by the respiratory route. Den sites contaminated with *M. bovis* would present favourable conditions for all three modes. However as discussed above, only droplet nuclei are likely to commonly initiate infection by the respiratory route, and these will occur only in the vicinity of an excreting animal, not after it has used the den on some previous occasion. Therefore realistically, infection from fomites is only likely to occur by ingestion, due to organisms contaminating the fur and being licked off during grooming, or organisms contaminating den materials which are chewed or licked by the at-risk animal. Since at least for other species which have been studied, the infective dose by the oral route is far higher than that required for infection by the respiratory route, it seems doubtful that animals could come in contact with enough

viable organisms to make this a common mechanism of infection. A further crucial point is that in the data from this study, 221 of 252 den sites investigated were used by only a single possum, while 26 were used by 2 possums on different occasions and 5 sites were used by 3 possums. Four of these 31 were simultaneous sharing as discussed above, leaving only 27 of over 350 den site trackings where sequential sharing was demonstrated, and even then not necessarily within days or weeks. While the nature of den site tracking means that only a proportion of actual cases of sequential sharing will be recorded, the proportion found here is extremely low for an environment where it appeared that population equilibrium had been reached and presumably the animals were making best use of favoured den sites.

Thus it is difficult to explain much of the quite high rate of transmission on the study site in terms purely of simultaneous or sequential sharing of dens. Yet the evidence from the geographical analysis of risk factors for infection showed that distance to the nearest den site which had been used by a tuberculous possum, height above sea level (reflecting harshness of environmental exposure on this site), aspect of the den site and interaction between aspect and height all influenced the likelihood that a particular den site would be one which had been used by a tuberculous possum. Thus physical factors associated with the den site appear to be important in their association with tuberculosis. Moreover mapping of both den site use and activity area use showed strong spatial clustering of tuberculous possums on both measures, apparently independent of total possum density. Thus it would appear that development of clinical tuberculosis is concentrated in and around certain groups of den sites, even though it is difficult to explain transmission as occurring specifically through den sites. This is one of the important enigmas in epidemiological understanding of the disease, an explanation for which will be put forward below.

Transmission through Interactions between Males

Adult male possums have larger home ranges, and are much more involved in competitive activities and territorial marking than are females, especially during the breeding seasons. Fighting between males would provide an obvious mechanism of spread both through respiratory transmission and creation of superficial injuries, but an even more common event among male possums is the use of threat displays without overt aggression. Such behaviour in possums incorporates violent and noisy exhalation accompanied by loud sounds. Such respiratory movements are exceptionally effective ways of producing droplets which could carry organisms to the other animal.

In addition, male possums carry out marking behaviour involving rubbing the axillary glands located between the front legs against suitable surfaces for what appears to be territorial marking. Although male possums do not defend exclusive territories, they do exhibit territorial behaviour patterns. The axillary lymph nodes lie approximately below the axillary scent glands, and these lymph nodes are the ones most commonly affected by tuberculosis, of all the superficial lymph nodes. Thus possums which have discharging sinuses

from these lymph nodes could deposit large numbers of organisms on the marked surface, which could then be over-marked by a second male which may become infected through later grooming or even through a skin lesion of some kind in the vicinity.

Of these three specific behaviours, threat displays seem likely to contribute most to male-male transmissions because they are common and have a high probability of exposing the at-risk animal to an infective dose.

These mechanisms would explain why male-male transmissions appear to cluster in space-time, but leave unanswered the issue of why females in the study site had a higher incidence than males. At this stage such a finding must be regarded as provisional, but if it continues to be the case, it would be explained by the relatively high transmission ability of each infected male during the breeding season, when contacts capable of transmission are likely to occur between each infected male and a group of females with which he mates.

A Tentative Hypothesis for Transmission of Tuberculosis on the Study Site

Based on this analysis of 22 months out of the proposed 60 months for which the study will run, a set of hypotheses are put forward to synthesize the evidence accumulated so far, and to propose some ideas for testing in the remaining stages of the research program. Not only are the hypotheses provisional, but they must be interpreted in the light of the earlier explanation that there is almost certainly no single identifiable transmission pathway, but rather a combination of mechanisms which are operating at different levels of importance.

The hypotheses are:

1. Maintenance of infection in a local possum population is principally dependent on breeding females and their female progeny (which commonly establish home ranges in close proximity to those of their mothers). Of the female progeny of tuberculous mothers, a high proportion will themselves be infected. At least one third to one half of these will die before independence, and the remainder will develop clinical tuberculosis at quite variable times over a period which probably extends for two to three years, if they do not die earlier of other causes. Whether and when infection becomes clinical disease in such progeny will be determined principally by the degree of physiological stress which they suffer. Clinical disease can be precipitated in such animals by environmental stress (particularly high total rainfall over a period, fluctuating temperature and feed shortage), and will be more marked in animals which den in an area which is more exposed to an adverse environment. In addition, the stress of producing and especialy rearing a pouch young can precipitate clinical disease in an infected mother earlier than would otherwise be the case.

2. Direct horizontal transmission within groups of females which share the same denning area is infrequent. Where it occurs it results principally from social interaction among females rather than from simultaneous or sequential sharing of dens.

3. Transmission between mature males occurs principally among those which have substantially overlapping home ranges and share a higher than random proportion of the same female mates. Such transmission occurs through threat displays and other agonistic behaviour and through such display mechanisms as axillary gland marking. It thus should show marked temporal variation and occur predominantly in association with the mating season.

4. Transmission of infection between mature animals of opposite sex occurs largely during courting and mating, and hence is confined to the two mating seasons in February-March and August-September each year.

5. Spatial clustering arises from a combination of pseudo-vertical transmission from mother to daughter, male-female transmission where males share a higher than random proportion of the same female mates, and the continuing use by an infected possum sub-population of den sites in an environmentally unfavourable area. Thus spatial clusters will tend to persist in the same approximate locations for far longer than if transmission were simply a function of contact rates as in simple models of infectious diseases.

6. The creation of new foci of infection both within endemically infected areas and (more visibly) in previously uninfected areas, arises from dispersal of pseudo-vertically infected juvenile males plus a small proportion of infected mature males which relocate to new home ranges. This process of dispersal of infection will create new foci if the male effectively establishes himself in a home range to the point where he begins to leave progeny and contemperaneously infects a number of females. Such foci are more likely to become apparent if the females with which he mates occupy a sub-optimal habitat. These new foci are important in maintaining stable prevalence in an area when other foci are dying out due to the death rate within certain groups exceeding the incidence for long enough that infection is extinguished.

7. Transmission from possums to domestic livestock results from a relatively small proportion of infected possums, because otherwise infection prevalence in domestic stock would be much higher than recorded figures, given that annual incidence in possums is apparently over 20%. It would appear from the study data so far collected that some terminally ill tuberculous possums changed their den sites and behaviour in the final weeks of life, and as a result were more likely to come in contact with domestic stock. A proportion of these animals would act as sources of infection for animals with which they interacted, both domestic livestock and wild animals. It is remarkable that of 7 previously unrecorded possums found dead on pasture in the study area, 5 had tuberculosis (when point prevalence was typically under 10%. It seems that tuberculous possums were much more likely to die on open pasture than were other possums, presumably because of behaviour changes. The evidence for the period reported does not make it possible to draw conclusions about whether cattle in the area became

infected by direct contact with live possums, or through contact with a possum carcass or organisms surviving the decomposition of a carcass (which would presumably raise soil nitrogen and produce a flush of growth at the spot, hence attracting grazing cattle). The apparent cessation of transmission to cattle after completion of the fence which excluded cattle from the main denning areas raises intriguing possibilities.

8. With the possible exception of possum carcasses as described in point 7, transmission among possums and from possums to cattle due to contamination of pasture is of negligible importance.

So if this theory of transmission proves to be correct, the two principal methods of transmission of infection within a local area are between the two sexes of breeding adults in association with courting and mating, and from mothers to joeys by pseudo-vertical transmission. Transmission between adult males causes some local dissemination of infection, and dispersal of the annual crop of juvenile males and occasional mature males creates new foci of infection both in infected areas and at the periphery of the infected areas. Transmission to domestic livestock and wild animals occurs largely from terminally ill or dead tuberculous possums, and the details of this process remain to be resolved. Other mechanisms, especially transmission through contamination of pasture or through simultaneous or sequential sharing of dens, are not important factors in the epidemiology of the disease.

The significance of this information for control policies will be taken up in the final chapter of the thesis.

CHAPTER 6

A COMPUTER SIMULATION MODEL OF THE DYNAMICS OF TUBERCULOSIS INFECTION IN A WILD POSSUM POPULATION

SIMULATION MODELLING

Shannon (1975) has probably given the most widely accepted definition for simulation. He writes: "Simulation is the process of designing a model of a real system and conducting experiments with this model with the purpose of either understanding the behavior of the system or of evaluating various strategies (within the limits imposed by a criterion or set of criteria) for the operation of the system." Zeigler (1991) gives a more narrow definition for simulation tools which are intended to facilitate a (hypothetical) description of the internal structure of a real system to the level of detail the modeler perceives as reality. Winston (1987) writes: "A simulation model takes the form of a set of assumptions about the operation of the system, expressed as mathematical or logical relations between the objects of interest in the system. In contrast to the exact mathematical solutions available with most analytical models, the simulation process involves executing or running the model through time, usually on a computer, to generate representative samples of measures of performance."

Modelling is one of the most important tools in systems analysis or operations research. Systems analysis needs models to predict the consequences that would follow were one of a set of alternatives to be chosen and implemented. Very often they are judgmental or mental models which are based on the assumptions and intuitions of an individual. Such models may have biases and gaps unknown to the person and undiscoverable by anyone else (Quade 1991). In this context computer models have a number of advantages over mental models (Meadows and Robinson 1984). They require assumptions to be specified explicitly, completely, and precisely. A computer model can manipulate more information than the human mind and can keep track of many more interrelationships at any one time. They can combine observations from many mental models into a more comprehensive picture than a human brain could ever handle. The human mind is likely to make errors in logic, especially if the logical chain is complex. Given that a computer model is programmed correctly, it can process very complex sets of assumptions to draw logical, error-free conclusions. Mental models are virtually unexaminable and uncriticizable, whereas a computer model has to be explicit, precise and unambiguous in order to communicate it to the computer. A computer model can test a wide variety of different conditions and policies, which is much less costly and time-consuming than tests within the real system.

Simulation modelling provides the balance between the two main types of scientific reasoning, induction and deduction. Inductive reasoning means inferring from the particular to the general. In this case empirical information is used to develop a hypothesis. Deductive reasoning begins with the development of a theory, which is then checked against the facts (Rountree 1977). When modelling a system, the researcher constantly alternates between both approaches.

EPIDEMIOLOGICAL SIMULATION MODELLING

The objective of epidemiological analysis is to describe and understand the interactions between factors in biological systems. Observational studies allow the researcher to develop a basic understanding of the system, to test and to generate hypotheses. Based on this information a model of biological reality can be developed to test these hypotheses and to identify areas of insufficient knowledge. In such a situation the model serves mainly as a research tool. A model which is capable of mimicking the operation of the real system closely enough can be used to conduct experiments on this system. It allows the evaluation and comparison of alternative ways of intervention with relationships in the system. In the field of animal health, models are now being used as decision support tools at levels ranging from an individual farm to a nationwide disease control program (Morris 1972). During the advent of the global AIDS epidemic public health authorities came to realize the importance of operational modelling in order to assist, improve and facilitate the decision-making process (Bailey 1991). Also in the context of the AIDS problem, Brandt (1989) emphasizes that modelling is our best approach at present to identify policy considerations for the future. But he warns that the expectations raised about the potential of these models should not be unrealistic.

Morris (1976) describes modelling as an essential part of an information system. Data gathered through a information collection system is used to produce parameter estimates for the model. Both the information system and the linked model are used repeatedly in order to progressively refine the model and improve its predictive ability.

Bradley (1982) describes the process of developing a mathematical model of an epidemiological process as follows. It starts from an empirical account of the process modelled, has the nature of a complex hypothesis and not of deductive logic. Progress is made by testing one hypothesis and then revising it prior to further testing. The proliferation of untested hypotheses is restricted by Occam's razor and scientific custom. Bradley quotes Sir Ronald Ross who defined *a priori* epidemiology as the synthesis of the known biology of transmission to build up a model and to compare it with observed data. In Bradley's words, first determine the biological processes and then put them together to produce a quantitative model and give it a sense of proportion. This hypothetico-deductive approach and improvement by falsification is consistent with the Popperian philosophy of scientific discovery.

In ecology, modelling has been used extensively to develop an understanding of the population dynamics of wild animals. Swartzman and Kaluzny (1987) write that simulation models are the only tool currently available for translating a collection of hypotheses for ecological processes into a representation of how a whole ecosystem functions. Models depict ecosystem function by changes over time and/or space in measurable quantities, which allows the user to test sets of hypotheses at the process level.

Bacon (1985) analysed the problem of rabies in wildlife using a systems analysis approach. He writes that any model attempting to represent the real world will incorporate differing degrees of the three fundamental aspects: generality, realism and precision. A high degree of any two of these three characteristics automatically excludes the possibility of a high degree of the third.

To understand the dynamics of *Mycobacterium bovis* infection in wild possum populations, simulation modelling is needed to test the provisional hypotheses which were generated from the results of observational field studies. Modelling can show which of the various hypotheses fit best to the data available from various field research studies.

SIMULATION MODELLING APPROACHES

A number of different classification schemes of simulation models have been used in the literature. Jørgenson (1986) gives a comprehensive list of pairs of model types. He includes research/management, deterministic/stochastic, compartment/matrix, reductionistic/holistic, static/dynamic, distributed/lumped, linear/nonlinear, causal/black box and autonomous/non-autonomous models. Most simulation models are based on combinations of these characteristics.

There are conflicting views among researchers about which approach is most suitable for a particular epidemiological simulation problem. Most epidemiological models are of the deterministic type. Such models are using sets of differential equations to describe the dynamics of a system. These models tend to be general and theoretical rather than realistic. Anderson and May (1981) have described a number of simple models for the population dynamics of invertebrates under the influence of a microparasitic disease. They defend this approach to ecological and epidemiological modelling, emphasizing the importance of generality at the cost of realism. In contrast Onstad (1988) believes that ecological theory cannot be generally applicable without being realistic. He points out that the coefficients in simple analytical models are highly aggregated. They do not expose many of the underlying assumptions. The processes and interactions expressed in these models are difficult to conceptualize and empirically estimate with statistical confidence. Onstad argues in favor of the development of complex, realistic theoretical models. The Anderson/May approach to epidemiological modelling typically involves the analysis of conditions leading to stable population equilibria. This concept has been questioned by a number of scientists. Onstad advocates the analysis of quantitative nonequilibrium results.

One of the major points of criticism of this modelling approach revolves around its treatment of uncertainty. Anderson (1976) writes that purely deterministic models disregard intrinisic uncertainties in the relationships described in the model. This results in the model only working 'correctly' under restrictive assumptions. He argues that only decision-makers who are indifferent to risk can afford to rely on single-valued responses like the mean which usually comprise the output from deterministic models. Anderson concludes from this

discussion that a stochastic model representing uncertainty can reflect the degree of understanding of the modelled system, including average and most likely performance, and dealing with the riskiness of the operation. Whenever a system is modelled imperfectly the model should become probabilistic in order to accurately represent the precision of understanding (Anderson 1974).

In the present study the approach chosen has been to develop a stochastic computer simulation model of the dynamics of *Mycobacterium bovis* infection in a wild possum population, so that the desired degree of realism could be built into the model design. The structure of the model had to be such that the model contained all major conceptual features derived from the field research, yet could be explained and demonstrated to people who are not familiar with mathematics and computing. This was necessary in order to allow constructive discussions to take place about the degree to which model behaviour and parameters accurately represented the field situation (Morris 1976).

DEVELOPMENT OF A SIMULATION MODEL

The simulation process can be structured into three different groups of activities (see figure 49; Ravindran *et al* 1987). The first group consists of *presimulation tasks.* The first step is the recognition of the problem which in turn leads to the study and analysis of the system. This information can then be used to establish the objectives which are directed towards solving the problem. At this stage it is necessary to decide on the modelling approach which is to be used.

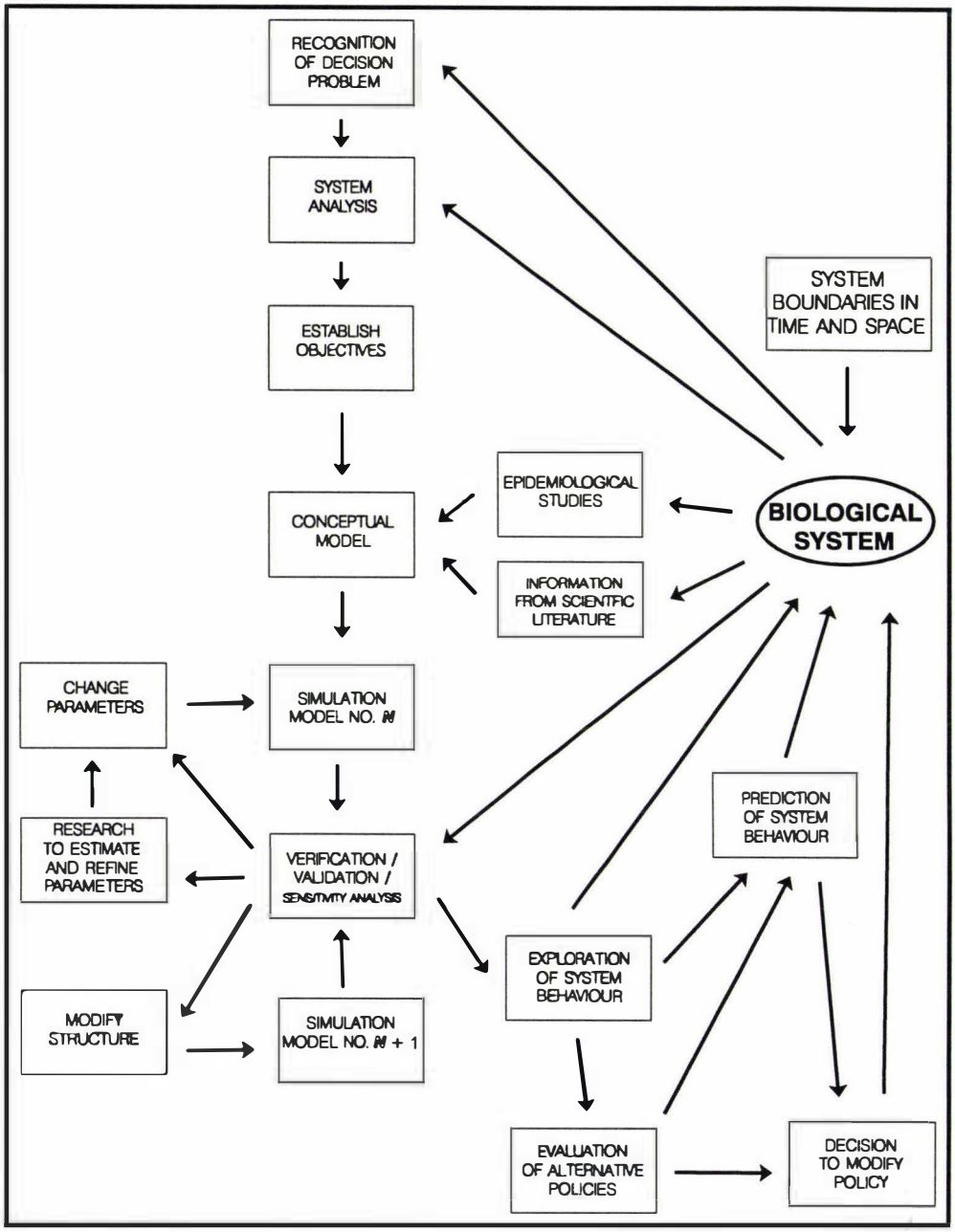
The next group of activities is concerned with *developmental activities.* The first step would be the *design and implementation* of the simulation model. It is followed by a *verification* of the model. Model verification is targeted at determining if the model is programmed properly and is operating in accordance with its design. A verified model then has to be validated. The objective of model *validation* is to ensure that the simulation program is a proper representation of the system being studied (sometimes called the simuland). It has to be recognized that a model is unlikely to ever be a completely comprehensive representation of the real system and that a real system is never completely understood (Payne 1982). In fact, the objective of modelling is to construct a system which is realistic enough to behave in a way comparable with the real system, but sufficiently simplified that its structure can be understood.

A verified and validated simulation model can be subjected to *sensitivity analysis.* This activity is concerned with learning about the soundness of the model by testing its sensitivity to changes in structural assumptions. It overlaps with the verification/validation stages in that it can lead to questioning the validity of the model and require the researcher to return to system analysis. This can be the case if the model is sensitive to changes in particular assumptions, about which there is considerable uncertainty. Anderson (1974) distinguishes between performance, decision and other variables in a simulation model. Performance

variables represent the behavioural features of the system the researcher is interested in. Decision variables are factors in the system under study which can be controlled by the researcher. Other variables in the model about which there exists uncertainty will have to be examined using sensitivity analysis. This can be done by changing one such parameter taking into account its dispersion, while leaving the others constant and measuring changes in performance variables (conditional sensitivity). The same or a limited number of known seeds should be used and the decision variables should have standard settings. In the case of several uncertain parameters sensitivity analysis becomes more complex. When using a statistical approach to sensitivity analysis the uncertain parameters are included into the set of decision variables and a formal experimental approach has to be used (Anderson 1974). If model behaviour changes relatively little in response to fairly wide-range changes in certain parameters, their accuracy does not seem to be important. When the model appears to be sensitive to a particular variable, it may be worthwhile to get better estimates of the factor, and that caution must be used in interpreting results unless the variable has been estimated precisely. Sensitivity analysis can be used to identify possible modifications which might usefully be made to the model. This could mean simplification through replacing stochastic variables by their mean value or dropping variables completely from the model. It is also possible that more complexity needs to be introduced into the model (Shannon 1975).

The next stage of the modelling process consists of *operational activities.* Simulation experiments have to be conducted to learn about the system under study. These can be based on a number of simulation runs where the model is run for a specific time, parameters are changed, and the model is run again. These loops are repeated until enough data is available to conduct a statistical analysis for interpretation of the results. This phase allows the researcher to analyze the behaviour of the system using different scenarios. Methods of experimental design as described by Hunter and Naylor (1970) are commonly used to provide a structure for the investigator's learning process. If the results of this analysis meet the objectives, the simulation study is complete at this point.

Figure 49: Structural steps of the simulation modelling process



Simulation, Model Verification / Validation and the Philosophy of Scientific Inquiry

Shannon (1975) emphasizes that the problem of validating a simulation model is no different from the question of validating or proving any hypothesis or theory in any field of science. He adds that unless our modelling efforts are to be pure exercises of science fiction, it will be one of the most crucial aspects of a simulation study to show that a model's output does bear some meaningful relationship to the behaviour of the real world. There are different theories of scientific inquiry which are likely to influence the approach a researcher chooses towards simulation modeling.

One of the major dilemmas of the scientific method is that it requires the scientist to be objective, but progress is made through following up subjective insights. Therefore,

development of a model can be based upon intuition, observation, opinion, insight etc. which are largely subjective processes. On the other hand verification should be almost exclusively objective (Blyth 1972).

The main conflict about the correct method to use for scientific inquiry is between rationalists and empiricists. Both groups begin with interpreting data from the real world. Rationalism is closely related to mathematics and logic. Models are considered to be a system of logical deductions from a series of synthetic premises which may or may not be open to empirical verification or appeal to objective experience. A purely rationalist perspective was represented by Immanuel Kant who described the premises of unquestionable truth (*synthetic a priori*) whose validity did not have to be established. Therefore validation by a rationalist requires a search for the basic assumptions underlying the behaviour of the system of interest (Naylor and Finger 1967). The empiricist is at the other end of the philosophical spectrum. He considers empirical science, and not mathematics, as the ideal form of knowledge. Empiricism refuses to admit any premises or assumptions that cannot be verified independently by experiment or analysis of empirical data. Hence, in its purest form empiricism requires that a model is based on proven or verifiable facts, not assumptions (Shannon 1975).

Shannon (1975) describes absolute pragmatism as the third major philosophy of scientific enquiry. If we consider a simulation model to be a black box transforming input variables into output variables, then the absolute pragmatist is not concerned with the validity of the model's internal structure, but with the input-output relationships. Both the pure rationalist and the pure empiricist, are primarily concerned with the internal structure of the model, while disagreeing over the nature of valid and admissible internal relationships.

Shannon (1975) concludes that most researchers during validation of a simulation model incorporate the viewpoints of the rationalist, the empiricist and the absolute pragmatist, which he calls a utilitarian approach. Naylor and Finger (1967) created the term multi-stage verification. They argue that each of the three methodological positions is a necessary procedure for validating simulation experiments but that none of them is a sufficient procedure for solving the problem of validation. In the first stage validity of the internal structure of the model is sought based upon *a priori* knowledge, past research and existing theory. This can be considered a modified rationalists approach which does not insist on Kant's *synthetic a priori* assumptions, but does require that the assumptions make sense. During the second stage the internal structure of the model is validated by empirically testing the hypotheses. Statistical methods can be used for this procedure which is based on the empiricist's viewpoint. In the third stage of validation the model's ability to predict the behaviour of the system under study is tested. Shannon (1975) emphasizes that this last stage is highly critical to gaining the user's acceptance and implementation. The three stages occur in an iterative manner throughout the modelling development process. The first two stages correspond to model verification and stage three to validation (Anderson 1974).

Swartzman and Kaluzny (1987) prefer to use the term *corroboration* instead of *validation*, because it essentially is a process of increasing confidence that the model meets its objectives. As has been pointed out by Popper (1959), hypotheses, including models, can never be proved right, they can only be proved wrong. Hence, the more difficult it is to invalidate a model, the more confidence we can have in it.

DEVELOPMENT OF A SIMULATION MODEL OF BOVINE TUBERCULOSIS IN A WILD POSSUM POPULATION

Objectives of the Modelling Undertaking

The work described in this thesis is the first part of an overall epidemiological study of tuberculosis in possums and domestic livestock which involves a number of investigators working in collaboration. The aim is to produce a valid understanding of the epidemiological processes which influence the behaviour of the disease in the field, and to contribute to the formulation of effective control policies. To do this it is necessary to synthesize the information arising from the various linked studies in a way which assists in their interpretation and helps to direct future research.

It was therefore decided that as part of the total research program a computer model of the disease would be developed, and that this work would proceed in stages with contributions from two or more investigators. This phase of the work is limited to formulating the structure of the model to the point where it can be considered to contain most of the structural features required to represent the understanding of the disease as presented in this thesis, and to examine the performance of the model when carrying out simulations, so that apparent limitations or deficiencies of this initial model formulation can be identified for later refinement.

It would be premature, given that only 22 of the planned 60 months of field studies are covered in this thesis, to take the model beyond this first stage of development. The task of continuing the development of the model and carrying out detailed verification and validation studies will be passed to another investigator, who will also extend the model to look at larger scale issues in both possums and domestic livestock, extending the model from its present form which simulates only possums, to a complete form which simulates both possums and domestic livestock on the same geographical area.

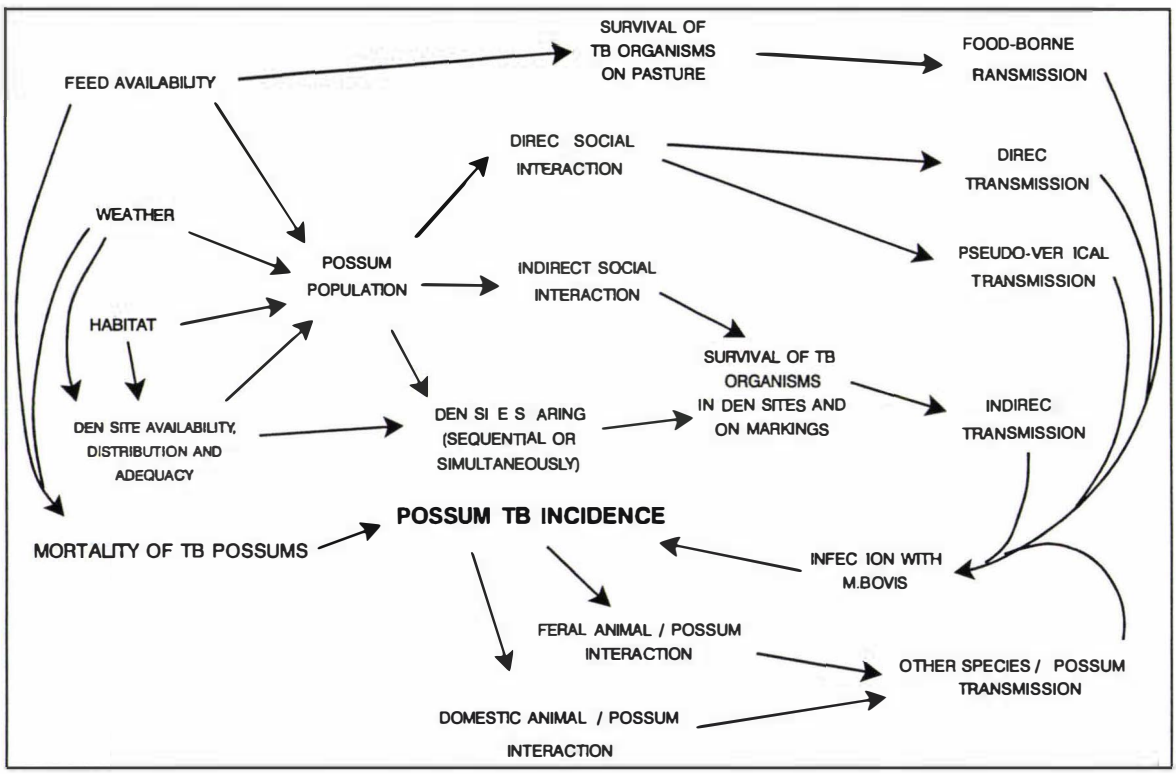
The work to be described below therefore deals primarily with the design of the model, not with its refinement and application.

General Model Characteristics

A stochastic modelling approach was selected to simulate the dynamics of bovine tuberculosis infection in a wild possum population. The model is capable of representing populations of any size, although clearly computation time will restrict the size of population simulated. It takes account of spatial heterogeneity by including geographical characteristics of a particular site, in the first instance the longitudinal study site. As shown at the end of the chapter, it can be extended to spatially heterogeneous sites, using information supplied about the spatial characteristics of the site being modelled. Within the model, each possum in the population is 'moved' through time on a day by day basis. Each day certain characteristics of a possum and its behaviour can change according to the probability of certain events taking place. The model can run simulations of any duration.

The development of a simulation model requires an understanding of the epidemiology of tuberculosis infection in a possum population. This was based on a review of the scientific literature and results from the cross-sectional and longitudinal studies described in the thesis. A diagram describing the major factors and processes in the epidemiology of the disease in possums was developed (see figure 50). This system of factors and relationships was used as the basis for the program development.

Figure 50: Important factors in the epidemiology of possum tuberculosis



Many of the processes in the model are subject to random effects. These were simulated by generating random variates from specific probability distributions. Numerical estimates describing the distributions were mainly obtained from the epidemiological studies described in this thesis and the scientific literature. In some cases it was necessary to use biologically reasonable guesstimates.

An object-oriented programming approach using Turbo-Pascal for Windows version 1.0 (Borland International, Inc., Scotts Valley, California, U.S.A.) was used to develop the model. It has been suggested that the object-oriented paradigm is particularly well suited to modelling animal-environment interaction (Coulson *et al* 1987). A listing of the programming code is provided in appendix VI.

The resolution of a system simulation model determines which entities, processes and activities are distinguishable and which remain hidden. If a model is too coarse, it will be inadequate. If it is too fine, it distracts by unimportant detail. In order to develop a properly

balanced model, the scientist has to decide which components of the system he wants to include and how much detail he wants to ascribe to them (Starfield and Bleloch 1986). Occam's razor should be kept in mind, when developing a simulation model. In modelling jargon less detailed models are also called 'lumped'. In this simulation model it was decided to model at the individual possum level. The two other dimensions which contribute to the resolution of the model are its temporal and spatial scale.

Temporal Scale

A model requires a sequencing variable which binds state transitions into processes. This sequencing dimension can be model time, using time slicing based on a monotonically increasing clock. Appropriate choice of the length of this time slice determines the model's balance between the two conflicting requirements of accuracy and computational efficiency (Kreutzer 1986). The clock mechanism provides for synchronization of the various elements and the occurrence of events in a simulation run. In the current model the clock is incremented in fixed-length intervals of one day. This modelling approach has been described as discrete-time modelling.

Spatial Scale

The effects of heterogeneous mixing are often not represented in epidemiological disease modelling because they have been technically difficult to include. Yet, they comprise a major component of the epidemiology of many diseases (Mollison 1986). Heterogeneous mixing can result from spatial aggregation and qualitative factors (Black *et al* 1987). Spatial heterogeneity may be one of the most important factors influencing population dynamics in diseases such as bovine tuberculosis. It can be represented in a model by the presence of a spatial coordinate system on which populations can interact and disperse. Some models also allow for environmental variability (Kareiva 1990).

Hanski and Gilpin (1991) make the distinction between three spatial scales. The *local* scale refers to the spatial scale at which individuals move and interact with each other in the course of their routine feeding and breeding activities. This is also called the local population and may represent a habitat patch. At the *metapopulation* scale individuals infrequently move from one place (population) to another, typically across boundaries between habitat types. The *geographical* scale includes a species' entire geographical range.

The habitat which is occupied by a possum population influences the population density and various aspects of the epidemiology of bovine tuberculosis infection in possums. Results from the longitudinal study suggest that the location of den sites is especially important. It is biologically reasonable to assume that the local density of possums and the extent to which activity areas are overlapping depends on geographical characteristics such as vegetation and topography. Local possum density would be limited by feed and more importantly by den site availability. After giving extended consideration to alternative ways of structuring the spatial aspects of model behaviour, it was decided to represent habitat by a map of potential den site

locations. This allows the use of a spatial coordinate system to model crucial aspects of the behaviour of possums. It indirectly accounts for environmental variability through varying the densities of den sites in different habitat types. The location of a den site may be optionally interpreted as the reference point for the possum's activity area during a particular night rather than necessarily as the physical den site itself. The model does not attempt to represent the actual movements of the possum during a night, but in keeping with the findings of the longitudinal study it sees the transmission of tuberculosis being determined by associations among sub-populations of possums which share use of a cluster of den sites to which they typically return each morning. Availability of den sites is considered a limiting resource for the purpose of this simulation study. The spatial scale of the model depends on the map of den sites which is used in the simulation. This influences the size of the population which is modelled. The simulation can currently be carried up to the metapopulation scale, and has been formulated to allow its extension to larger areas. The structure of the model does not require the metapopulation to be divided into local populations, which is frequently done when spatial heterogeneity is represented in deterministic models.

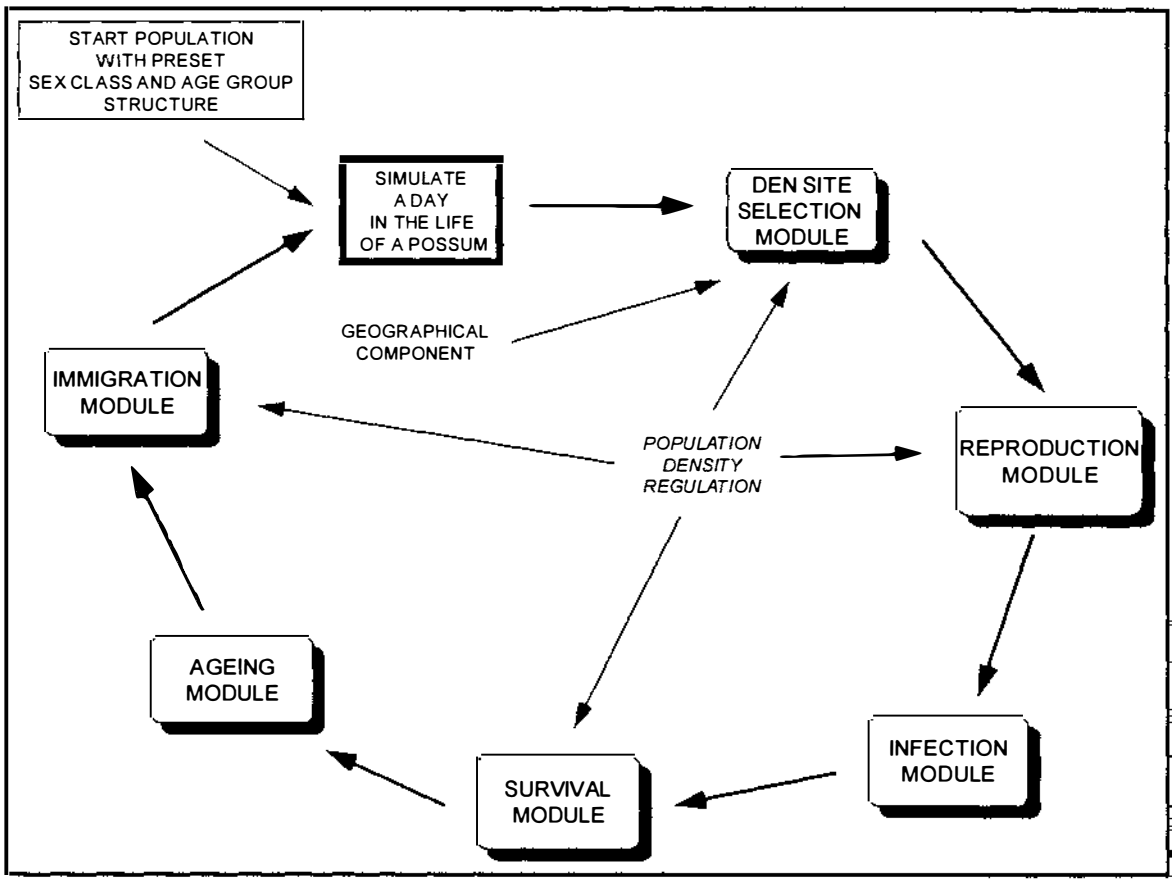
The den site map is currently based on radio-tracking information from the longitudinal study. Interaction between individual possums in the simulation model depends on proximity of den sites which are being used by the animals on a particular day. Environmental characteristics of a particular den site do not in this version of the model have an effect on interactions between possums or on transmission of tuberculosis infection.

The density of den sites in both the field and the model depends largely on the vegetation and topography of a particular site, which can be derived from available geographical data for New Zealand without necessarily making new field measurements. Using this approach, it will be possible to use the model at locations other than the longitudinal study area. A vegetation map of such areas allows random allocation of den site locations according to expected densities of den sites in particular vegetation types.

Description of Model Structure and Functionality

The model is divided up into operational modules, each of which represents a distinct biological process. The modules are processed in a logical sequence. Each of these modules represents an aspect of possum ecology which was considered important with regard to the dynamics of *Mycobacterium bovis* infection within the population. The first module covers the spatial component of the model which is based on the *den site selection* mechanism. The next module represents *reproduction* of possums. The *infection* module models the various modes of transmission of infection between possums. The *survival* module simulates the effects of environment, population density and tuberculosis on mortality and emigration. The *ageing* module controls the stages of physiological development in a possum life. Finally, there is an *immigration* module which controls the numbers of immigrating possums, and tuberculosis prevalence in them. Figure 51 shows a overview of the modular structure of the simulation model.

Figure 51: Overview of the model structure



At the beginning of a simulation run a map with available den site locations is read into computer memory. This can be derived from field data (for example, at the longitudinal study site) or can itself be created by simulation using random sampling to define a den map pattern which produces the density appropriate to the vegetation and topography of the area to be simulated (which can be obtained as digital map data for any part of New Zealand).

Then the program parameters and variables which can be edited are initialized. Before the simulation begins it is necessary to specify the length of the run, the display format and the summary statistics required by the user. It is also possible to specify a seed for the random number generator. A simulation run begins with a possum population of a given age and sex structure which is read from an ASCII formatted file into computer memory. Then the program randomly allocates a den site to each possum. If specified, clinical tuberculosis status is assigned randomly to a proportion of animals before the first day of the simulation. Each possum is represented for programming purposes as an "object" with the following attributes: birthdate, sex class, date of sexual maturity, pregnancy status, resident or immigrant status, a memory of den sites used, date of tuberculosis infection and date of onset of clinical tuberculosis. During the simulation possums are subjected to the main mechanisms which determine population dynamics - birth, ageing, reproduction and death. They are also exposed to the risk of infection with *Mycobacterium bovis* and infected possums eventually will

develop clinical tuberculosis. These processes are modelled for each possum, using probabilities of events occurring which are based on specific distributions chosen to fit the nature of the event. Each possum in the population is 'moved' daily through the sequence of program modules representing the above listed mechanisms, and this occurs for all possums which are currently "alive" in the modelled area until the end of the simulation run.

The settings for parameters in the model are read from ASCII files and each file stores the appropriate settings for the 12 months of a year. Cyclical changes as well as control measures can be introduced by reading in new parameter settings from ASCII files at the appropriate time during a simulation run.

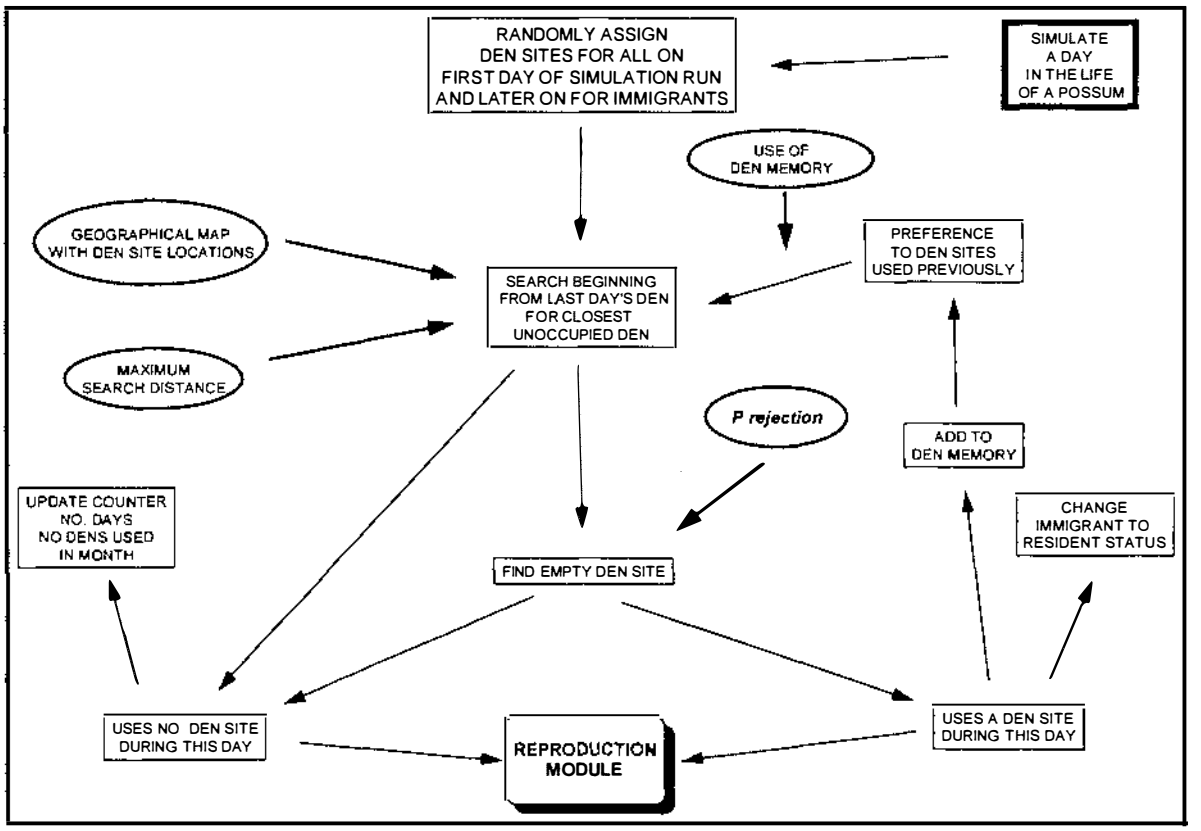
The functions and parameters of each logical program module are described in the following paragraphs. Variables which can be edited are specified with their acronyms and printed in italics. Program output relevant to specific modules is explained.

Den Site Selection

At the beginning of the simulation run available den sites are randomly allocated to each possum in the starting population. Only one possum can use a particular den at a time. Beginning with the previous day's den location a possum begins to search for a den site. Even if a den is not occupied, the animal may refuse to use it ($P_{rejection}$). In the case of refusal or if the den is occupied, the possum goes on to inspect the next den. This search continues until the possum has found an empty, acceptable den site or until it has travelled a *maximum den search distance* from last day's den. If it does not find a den site, the program allocates a 'dummy' den to the possum for this day. This is necessary to ensure that the animal is exposed to other mechanisms in the model which require a geographical location. It also updates a counter of the number of days on which the possum did not find a suitable den site. As soon as a possum with immigrant status has found a den site, it is changed to resident status. Each possum object also has a memory of den sites which it has used in the past. In its search for a den site the possum gives preference to locations which are recorded in its *'den memory'*. This produces the denning pattern seen in the longitudinal study where possums returned to the area where they denned the previous day, but not necessarily to the same den. If desired, this mechanism can be disabled so that possums den randomly. See figure 52 for a graphical representation of the den site selection module.

The program provides the option for output to a file of a monthly summary table with the categories representing the number of days without a den site during a period of the past 30 days and the counts standing for the number of possums in each category. A monthly summary of the number of possums counted by categories of number of different den sites used over the last 30 days can also be saved as a file on disk.

Figure 52: Structure of the den site selection module



Reproduction

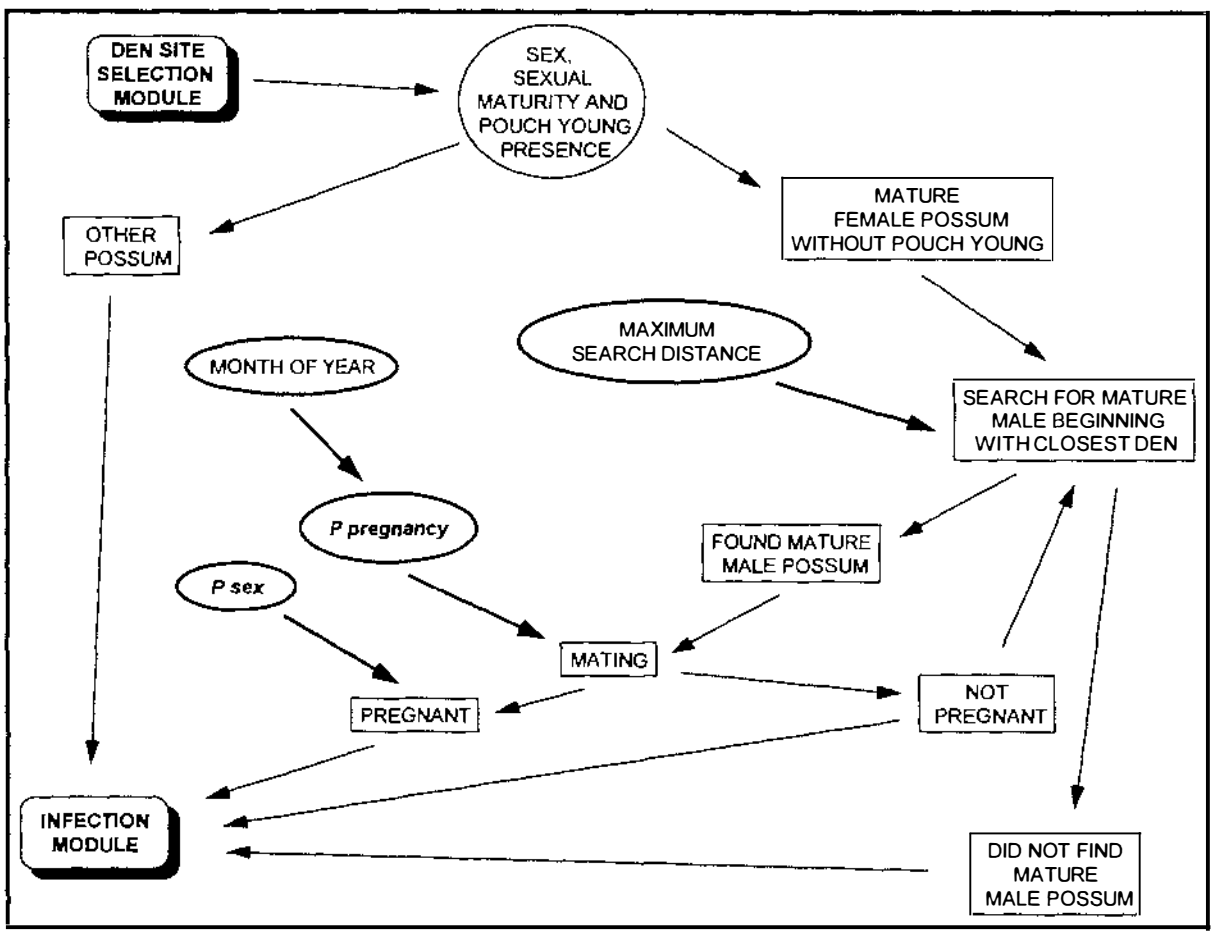
Sexually mature female possums which are not pregnant are processed by the reproduction module of the simulation model. Successful mating requires the presence of mature male possums denning in the proximity of the female. Based on her own current den location the female possum searches (beginning with the closest den site) for a mature male possum. For each encounter with a male during this search, there is a probability of a successful mating ($\boldsymbol{P}_{pregnancy}$). If mating was successful, she moves on to the infection module. The sex of the newborn is determined by a probability ($\boldsymbol{P}_{sex}$). If during a simulation day she does not become pregnant during an encounter with a particular male, the female tries to find another mature male, until mating is successful or she has reached the *maximum mating search distance* (see figure 53). This module is only processed if the $\boldsymbol{P}_{pregnancy}$ settings for a particular month are different from 0.

The structure of the reproduction module ensures that mating is based on direct contact between a mature male and female. If there is no mature male available within a given distance, no mating occurs for a particular female.

The proportion of mature females possums with a dependent young is part of the daily and monthly statistical summary produced on screen. From the age structure which can stored as a file on disk number of births can be derived from temporal changes in the total number of

dependent possums in the population. This figure is likely to be an underestimate, because it is influenced by mortality.

Figure 53: Structure of the reproduction module



Infection with Mycobacterium bovis

With regard to tuberculosis infection a possum can be in one of three possible states. Non-infected possums are *susceptible* to infection. Once a possum is *infected*, it goes through a period of *sub-clinical* disease (or incubation period) without being considered infectious. A transition from infected with *Mycobacterium bovis* back to susceptible is not possible. Infected possums cannot develop immunity. An infected possum which survives long enough eventually develops *clinical* disease and is then capable of exposing other animals in the population to the risk of infection. This transition from subclinical to clinical disease is based on a probability ($P_{clinical}$) which depends on environmental conditions varying between the months of the year. This mechanism is processed during a simulation run once at the beginning of each month. The date of onset is taken randomly from a uniform distribution of days within a month. Clinically diseased possums have four allowed mechanisms for exposing susceptible possums to the risk of infection. Firstly, they can use a den site in the proximity of a non-infected possum, and thus have the opportunity for social contact with

animals of the same and those of opposite sex. Secondly, they can leave their current den site contaminated with *Mycobacterium bovis* for a given period of time. They can also transmit the disease during courting and mating. Finally, a clinically diseased female will transmit the disease to its pouch young. Within the model "infectious" and "clinical" are treated as synonymous, and the model does not currently provide explicitly for intermittent infectious episodes, although because infection is stochastically determined intermittent infectiousness will be an emergent property of each infectious possum in the model.

The major mechanisms for transmission of *Mycobacterium bovis* infection within a possum population were listed in the discussion of the results of the longitudinal study, and each of them is implemented in the model so that their consequences can be explored. They include *direct* contact during social activities such as mating, fights, mutual grooming and simultaneous den sharing, *indirect* contact through marking activities and sequential den sharing and *pseudo-vertical* transmission. These mechanisms are implemented in the model as described in the following. Young possums living with their mother are at risk of infection, if the mother has clinical disease. The model assumes that in this case transmission is 100% effective. A susceptible independent possum has three possibilities of getting infected with *Mycobacterium bovis*. It is exposed to the risk of infection when using a den site which is bacterially contaminated after it had been used by a clinically diseased possum. Infection through bacterial contamination of den sites or shared activity in the vicinity of den sites is considered in the initial model formulation to have a high probability ($\boldsymbol{P}_{TB\ den}$). The second mechanism for transmission is represented by mating with a clinically diseased possum. Mating is used as a generic representation for male-female contacts which are concentrated within breeding seasons, i.e. depend on the level of breeding activity in the month. During the search process of each mature male, every mating contact provides a possibility for transmission for both sides if the male or the female has clinical disease. This mechanism is likely to have a high probability of infection ($\boldsymbol{P}_{TB\ mating}$). The presence of a clinically diseased possum within a given *TB buffer distance* from a particular day's den site is assumed to represent a lower risk of infection ($\boldsymbol{P}_{TB\ buffer}$). This last mechanism accounts for the possibility of transmission during social interaction such as fights and marking activities. See figure 54 for a graphical description of the structure of the tuberculosis infection module.

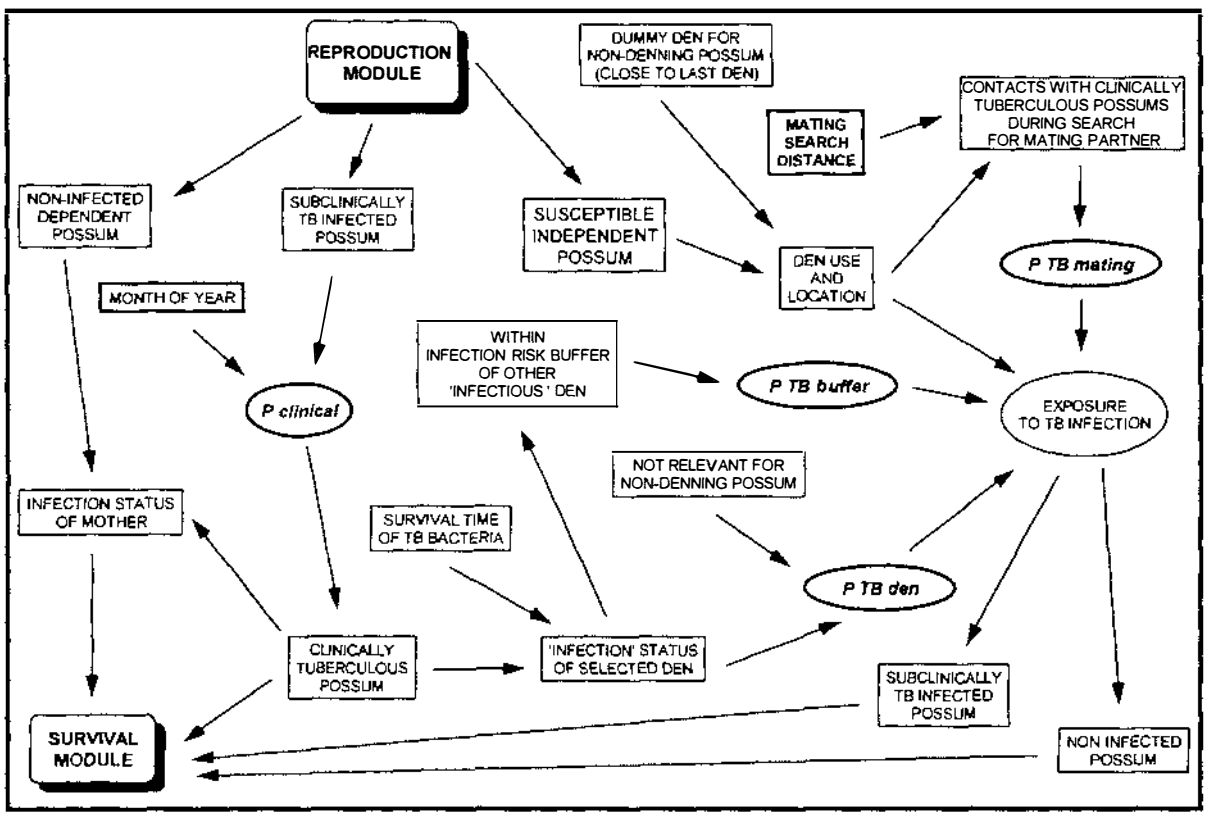
When requesting a map displaying the location of den sites on screen, different colors are used to mark 'infection status' of individual den sites. Dens which are bacterially contaminated are displayed in red. They turn green once the period of expected survival of the bacteria has expired. Dens which have never been used by tuberculous possums are displayed as white points on the screen. This map is updated daily. Current numbers of infected and of clinical possums, total number of 'infectious' dens and current tuberculosis prevalence are displayed on the top of the screen.

If the program is not running in graphics mode, three different types of tuberculosis infection summary statistics can be requested for display on screen. At daily, monthly or

yearly intervals a table of the total number of susceptible, sub-clinically infected and clinically diseased possums for each sex class is shown on screen. The total number of 'infectious' dens is included in the table.

The program provides the option for output of a monthly summary of numbers of possums, stratified by infection status, physiological development status and sex. It is also possible to write current or cumulative locations of 'infectious' dens to a file on a daily or monthly basis.

Figure 54: Structure of the tuberculosis infection module



Survival of Possums

It is difficult to distinguish mortality from emigration or dispersal when interpreting data from an ecological field study. When modelling a local population rather than a metapopulation, it is considered reasonable to represent both effects through the survival mechanism. For the purpose of modelling a metapopulation, emigration and dispersal should become emergent properties of the model. Therefore, the survival parameters in the model would have to be adjusted accordingly.

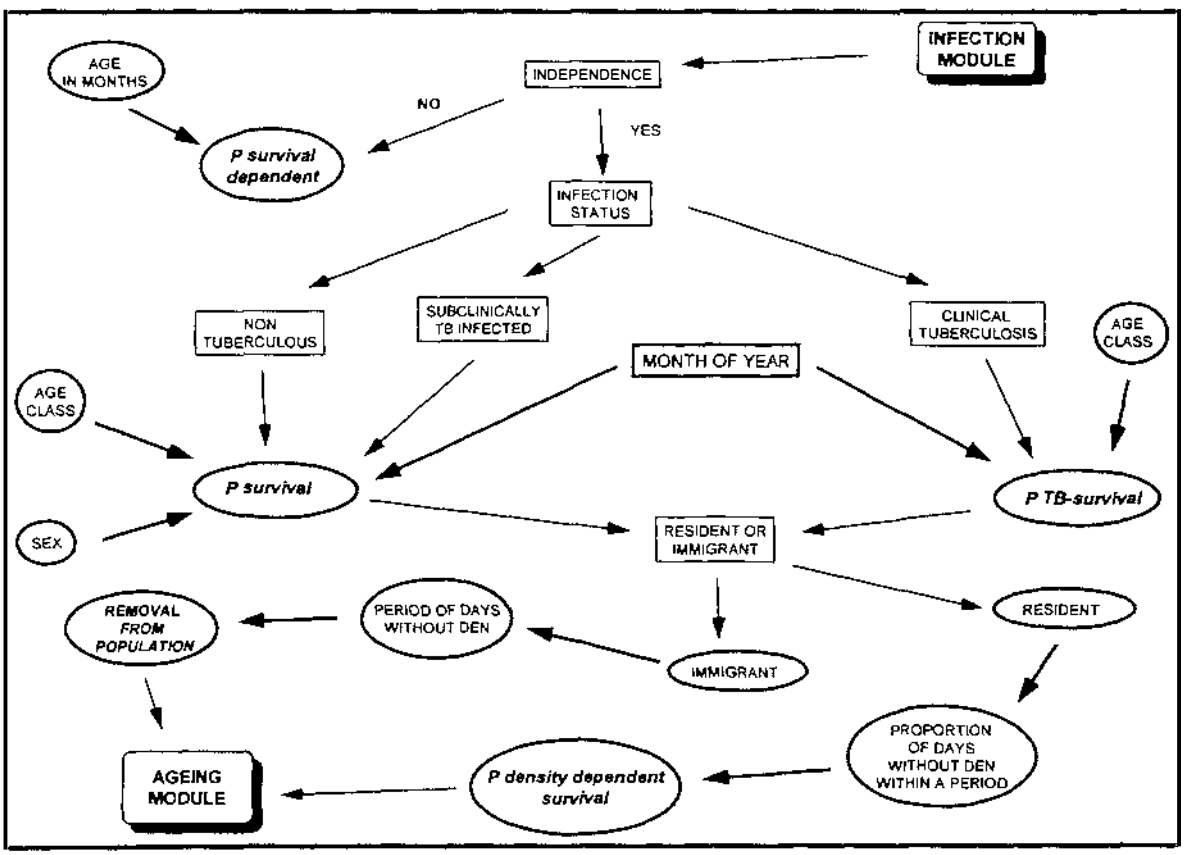
Survival of possums is represented in the model by four different mechanisms. Young possums dependent on their mothers are exposed to mortality risk with probabilities varying with age in months until independence ($P_{survival\ dependent}$). This effect is independent of

environmental factors. If the mother dies before independence of her joey, the young also dies.

For independent possums there are three mechanisms affecting survival. Non-infected and subclinically infected possums are subject to mortality ($P_{survival}$), which depends on age class, sex group and month of the year. Clinically diseased animals are exposed to a separate mortality mechanism ($P_{TB\ survival}$) which also varies between age classes and months of the year. All resident possums are affected by a third mechanism which represents density-dependent mortality ($P_{density\ dependent\ survival}$). It is controlled by the *proportion of days* within a given *period* a possum had been without a den, since this exposes them to more severe environmental stress and is likely to be a good reflection of density-dependent ecological pressures in the possum. Possums with immigrant status are removed from the population, if they have been unable to find a den for a certain *number of days*.

Figure 55 graphically describes the structure of the survival mechanisms in the model.

Figure 55: Structure of the survival module

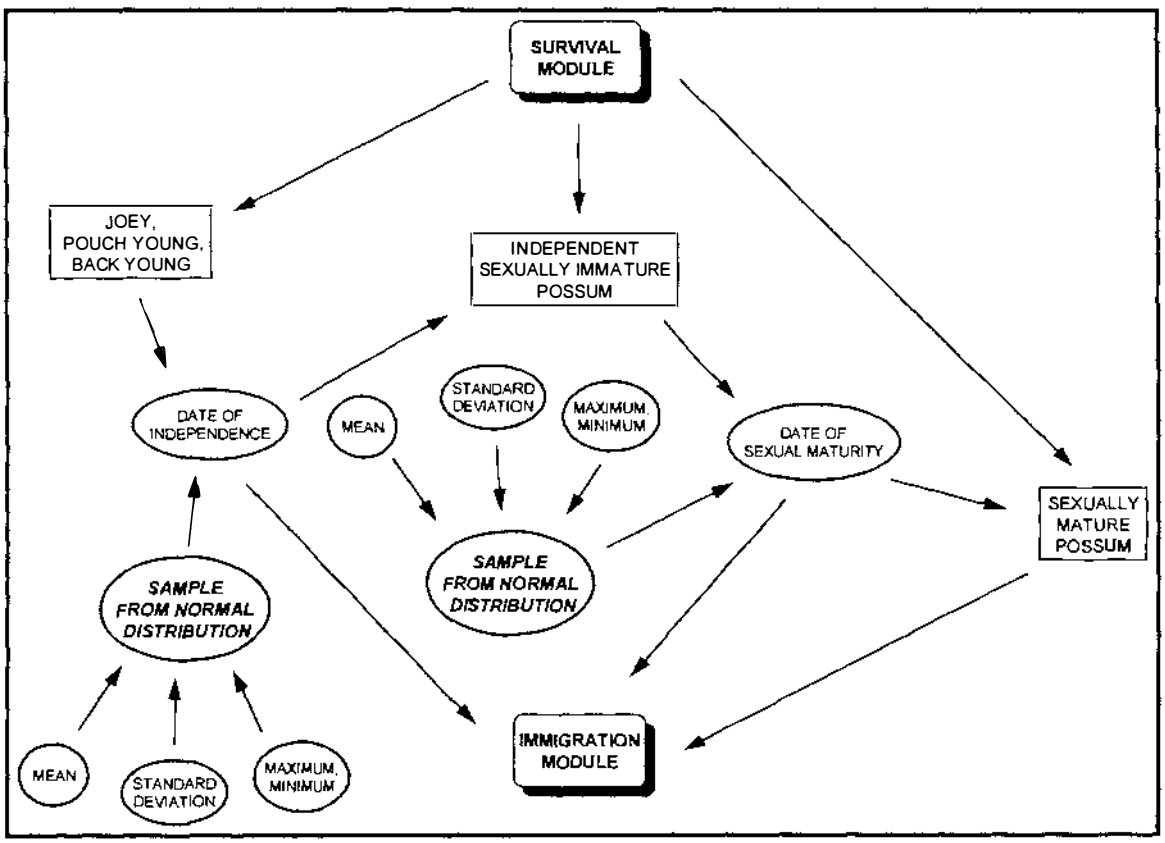


Ageing Mechanisms for Possums

Each possum has a birth date as one of its attributes. This means that it will automatically age during a simulation run. There are three major stages in the physiological development of a possum which are important for population dynamics and the epidemiology of tuberculosis

infection. The first stage is the time between birth and independence during which the young possum lives in close association with its mother. Then there is the time between independence and sexual maturity and finally the stage when the animal has reached sexual maturity. In the model, both the date of independence and the maturity date are randomly sampled from a normal distribution with a given mean, standard deviation, minimum and maximum value. Once the possum has reached the chosen age, it changes its status from dependence to independence or from sexually immature to sexually mature. At independence a female possum copies its mother's den memory. Figure 56 describes the ageing mechanisms in the model representing physiological development.

Figure 56: Structure of the ageing module



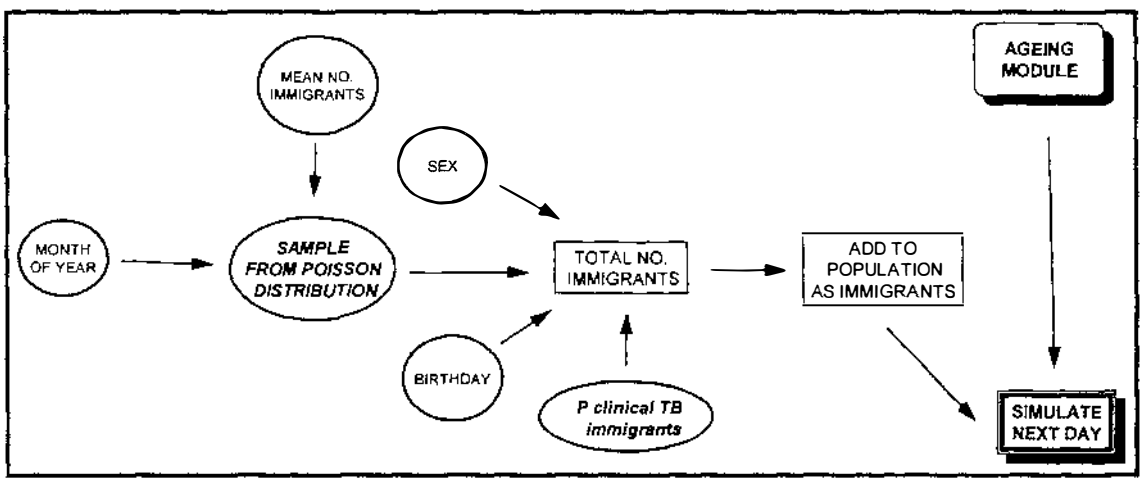
Immigration of Possums

Immigration is another major factor influencing the population dynamics of a possum population. It has to be taken into account when simulating a local population. In this simulation model a *mean number of immigrants* is given for each month of the year and each sex class. As immigration is processed daily, this average monthly figure is divided by the number of days to determine a daily number. The actual number of immigrants on a particular day is then sampled for each sex class separately based on a poisson distribution with the expected mean number of immigrants per day. A birth date in March of the previous year is assigned to each immigrant. Immigrating possums can have clinical tuberculosis

($P_{clinical\ TB\ immigrants}$). At the end of the simulation day the pool of immigrants is added to the population. Each immigrant has immigrant status until it finds its first den (see den site selection module). If it does not find an empty den for a given sequence of days, it is removed from the population (see survival module). When simulating a metapopulation, immigration should be an emergent property of the model.

Figure 57 graphically describes the structure of the immigration mechanism in the model.

Figure 57: Structure of the immigration module



Input Parameters for the Model

The model can be adapted to different simulation scenarios by adjusting specific parameters. Individual scenarios can be described by specific parameter sets which can be stored on disk. For this epidemiological study, the model is set up to represent a possum population with characteristics similar to the one which was studied during the longitudinal study. The *base* parameter settings used during the simulation experiments are discussed in the following paragraphs.

Start Population

The composition of the start population in terms of sex/age structure and size can be varied according to the objectives of the specific simulation exercise. For this study, a start population similar to the longitudinal study population with the age and sex structure given in table 16 was created and stored in an ASCII file on disk. The file was created using the random number generation tool of the computer spreadsheet software Microsoft EXCEL for Windows version 4.0. Based on a discrete distribution with given probabilities for 8 different age categories, a population of subjects with a given size was generated. Populations of different age and sex structures can be created using the worksheet. For the following simulations a population size of about 150 possums was used for the start population.

Table 16: Structure of the start population

	SEX		
AGE	F	M	Total
1	11	25	36
2	18	29	47
3	19	17	36
4	19	4	23
5	5	1	6
6	0	2	2
Total	72	78	150

Den Site Parameters

At the beginning of each simulation a list of available den sites is read into memory from a file which is stored on disk. The euclidean distances in two-dimensional space from each den to all other dens are calculated and stored on disk as separate files for each den site. If the same list of den sites is used for further simulation runs, this calculation does not have to be repeated. The den locations are stored in a space-delimited ASCII formatted file with three columns representing den site identification number, X- and Y-coordinate. This file can be produced by a geographic information system. During the longitudinal study den sites which had been located using radio telemetry were digitized from aerial photographs of the study area and stored as digital map coverages using the GIS-software PC-Arc/Info. This software allows the generation of ASCII files in the above format (using the command 'UNGENERATE'). After deletion of the 'END' statement at the end of the file, they can be used directly as input for the simulation model.

The distances for each den site are stored on disk as space-delimited ASCII files with file names 'CDidno.DAT' ('idno' standing for the individual den identification number). They contain one column of values standing for the identification number of all other den sites on the map and a second column with the distance to the particular den site. The data is stored in the order of increasing distance.

It is impossible to obtain a complete map of all potential den site locations for a possum population. The den locations which are used for this simulation are an underestimate of the true number of dens. This situation is dealt with by allowing possums to not occupy a den site if none is available, which is equivalent to occupying a low-grade den site.

For the longitudinal study the mode of the maximum distance between all known den sites for individual possums was 125m for males and 76m for females. In the simulation model a maximum den search distance of 100m was considered appropriate. Once possums have found an empty den, there is a probability of 0.15 that they reject it and try to find another den. A den memory for individual possums was included in the model to simulate preferential or territorial behaviour. The den rejection probability and the option of use of a

den memory influence the size of the activity area of a simulated possum. The total number of days on which a possum did not find an empty den is recorded and used to influence survival.

Reproductive Parameters

There are five parameters affecting reproduction which can be varied between simulation runs. The first variable is the age of maturity for individual possums which will be discussed under ageing parameters. The second variable is the probability of a successful mating for mature female possums. Data from the longitudinal study and reports from the literature indicate that possums are very successful breeders. The data also suggests that there is a principal mating season in March/April and a subsidiary one in September/October. About 90% of available females were found to have become pregnant during the late summer/autumn mating season, but in spring the proportion varied between years from 20 to 50%. It is also known that given the right environmental conditions female possums are biologically capable of breeding all year round. In the model the probability of successful mating is simulated by taking a random number from a Bernoulli distribution. In order to achieve the observed birth pattern the probability of a successful mating for a mature female possum without dependent young is varied over the course of a year. The mating mechanism also has implications for the dynamics of tuberculosis infection within the model population. The more mating contacts a female has until she is mated successfully the more likely it is that she eventually meets a tuberculous male. Table 17 lists the sequence of probabilities describing the pattern of mating probabilities. The monthly probabilities for a succesful mating were chosen relatively low in order to achieve multiple mating contacts. During the months with a probability of zero no mating contacts were simulated.

Table 17: Monthly probabilities of a successful mating

MONTH	1	2	3	4	5	6	7	8	9	10	11	12
P pregnancy	0.00	0.05	0.20	0.20	0.05	0.00	0.00	0.00	0.10	0.10	0.05	0.00

The fourth parameter influencing reproduction in the model is the maximum distance a female possum travels in order to find a mature male for mating. This parameter was introduced for studies of meta-populations to mimic the effects of locally low population numbers. In reality evidence suggests that the males will travel further than the females to find a mating partner. During the present simulation a maximum search distance of 100m was used. This figure was based on estimates of average activity radii of possums, which were calculated from the longitudinal study data. The latter were about 60m and 70m for females and males respectively. A maximum travel distance of 100m was considered a reasonable estimate for the simulation to allow for temporally increased home ranges during mating season. As a fifth parameter the sex of a newborn possum is determined by taking a random number from a Bernoulli distribution. A probability of 0.50 was assumed for the sex of the newborn being female. The model does not allow mating of mature females if a dependent young is present.

Infection Parameters

The transmission mechanisms implemented in the model are designed to respresent most aspects of the epidemiology of *Mycobacterium bovis* infection in possums. There is currently no direct quantitative information about the different modes of disease transmission available. Results from the analysis of the longitudinal study data allow the formulation of some hypotheses. These are mainly based on data on occurrence of cases with clinical disease and the survival of these cases from detection until death. The greatest uncertainty is associated with the nature of events during the period between infection and onset of clinical disease, because no test is available to detect infection of an animal. This affects repesentation of temporal changes in the risk of infection for the different modes of transmission.

Studies of the epidemiology of HIV infection in humans had to deal with a similar problem. Fusaro *et al* (1989) have reviewed the subject and provided an annotated bibliography. They emphasize that the distribution of the incubation period is particularly hard to estimate, in part due to its the length, but also due to the heterogeneous structure of the infected population and the fact that the data is often based on prevalent cohorts rather than incident cohorts. Jewell (1990) provides a review of methods used for estimating the incubation period distribution. He discusses issues regarding data based on prevalent cohorts and interval- censored information about the occurrence of infection. In the situation with HIV infection the presence of antibodies in the blood can be used as an indicator for time of infection. At present there is no satisfactory serological test for the detection of *Mycobacterium bovis* infection in possums. Disease information from the first months of the longitudinal study can be considered as coming from a prevalent cohort, because the time of infection or onset of clinical disease was not known. New cases with tuberculous lesions in later months of the study are based on interval-censored data. The interval was defined by the time period between the date of previous physical examination and date of lesion detection. For the survival analysis the date of lesion detection was used as the date of onset of clinical disease. It is therefore likely that the period of clinical disease is underestimated.

The transition of infected possums from the subclinical to the clinical stage is likely to vary over the course of a year because of environmental and possibly behavioural factors. The data on the occurrence of new clinical cases in the longitudinal study population allows estimation of the risk of developing clinical disease for infected possums, assuming for the purposes of the analysis that they had been infected since entry into the study and hence that date of infection was not confounding the analysis. While clearly inaccurate, this may not be a very distorting assumption. Results from proportional hazards regression analysis using monthly data for animals which eventually developed clinical disease suggests that during spring (September-November) the risk of developing tuberculous lesions was 4 times as high as the baseline risk. Given the relatively small sample size there were no other factors which appeared to be of importance. In 1990 the risk of developing clinical tuberculosis averaged 0.02 per month. The population which was used as the denominator for this cumulative

incidence estimation included non-infected possums as well as (unidentified) subclinical cases of tuberculosis infection. Therefore this figure must be an underestimate of the true probability for transition from subclinical to clinical disease status. The cumulative risk (cumulative incidence) of developing tuberculosis lesions (including only possums into the denominator which eventually showed lesions on examination) was 0.90 over 12 months. As this figure is likely to be an overestimate in this simulation exercise 0.70 was used as the cumulative risk for developing clinical tuberculosis in infected animals. Analysis of the longitudinal study data suggests that the risk increases during spring season as reflected in a hazard ratio of 4.0 and it is reduced in adult possums as shown by a hazard ratio of 0.25. These figures are also likely to be overestimates of the true effects. For the development of the monthly probabilities of transition from subclinical to clinical disease status it was assumed that the hazard ratio was 2 during spring and 0.5 for adult possums. A computer worksheet using the 'goal seeking' function in the spreadsheet software Microsoft EXCEL for Windows 4.0 was used to model the monthly parameters using the above discussed the parameters (see table 18). Once an animal has developed clinical tuberculosis it is assumed to be infectious until its death.

Table 18: Worksheet for modelling of parameters for probability of transition from subclinical to clinical tuberculosis

MONTH	1	2	3	4	5	6	7	8	9	10	11	12
Adult	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.1303	0.1303	0.1303	0.0652
Immature	0.1303	0.1303	0.1303	0.1303	0.1303	0.1303	0.1303	0.1303	0.2607	0.2607	0.2607	0.1303

BASELINE RISK	0.130345857
ANNUAL Survival	0.30

	Spring	Adult Age
Hazard Rate Ratio	3.88	0.25
95% CI	1.47-10.2	0.06-1.02
Figure used	2	0.5
ln HRR	0.69314718	-0.693147

Proportion in Population	
Adults	0.76
Immature	0.24

Transmission of infection can occur through *direct* or *indirect contact*. In the first case the source of infection is the clinically diseased possum, in the second instance a clinically diseased individual bacterially contaminates a site, for example when using a den or when marking locations. Direct contact is likely to have the highest probability of successful transmission as it only depends on the quantity of organisms shed and the duration of exposure. Infection through indirect contact depends on the same factors plus dilution effects and survival of the organism in the environment. Two modes of *direct contact* are implemented in the model. One is *pseudovertical infection* from mother to dependent young. The young possum spends on average about 5 to 6 months in a close physical relationship with its mother. It seems unlikely that it would escape infection during this time, given that the mother has clinical tuberculosis. Therefore a 100% probability of successful pseudovertical transmission was assumed in the simulation model. The second mechanism

with direct contact is related to *mating*. Given that one of the mating partners has clinical tuberculosis there is a probability that the other one gets infected. In the simulation model it is assumed that possums do not form continuing consort relationships during the mating season. This means that transmission can only occur in association with courting and the act of mating. Transmission via *indirect contact* can occur during use of a bacterially contaminated den site and possibly during marking activities. The first is implemented in the model based on the assumption that a clinically diseased possum potentially contaminates a den site. If a susceptible possum subsequently uses the contaminated den site within a given period of time, it can potentially become infected (*temporal proximity*). This is the only transmission mechanism in the model where a clinically diseased possum produces a temporally persistent source of infection independent of the possum's presence. The time of persistence is limited by the survival of the bacteria within a contaminated den site. Mitscherlich and Marth (1984) reviewed survival of *Mycobacterium bovis* under a number of environmental conditions. Bacterial survival in a typical den site mainly depends on protection from direct sunlight. Taking into account the survival estimates reported in Mitscherlich and Marth and the circumstances which can be expected in a den site it seems reasonable to assume that on average a large enough quantity of bacteria for infection of a susceptible possum would be present for up to 10 days. This figure is likely to be subject to substantial variation as it depends on the quantity of bacteria excreted and daily climatic factors. The second transmission mechanism for indirect contact depends on the presence of a clinically diseased possum within a certain distance from a susceptible individual (*spatial proximity*). This mechanism may as represent direct as well as indirect contact. Possums denning within a certain distance from each other are more likely to have close physical encounters during social interactions other than mating. They are also more likely to come in contact with bacterially contaminated sites such as territorial markings or on runs. The radius of this area associated with increased risk of transmission around a clinically diseased possum is assumed to be about 50% of the average activity radius of mature possums (=30m). This estimate appears to be reasonable taking into account the distribution of distances between activity centres of individual tuberculous possums and a variogram of differences between prevalence estimates for different lags (see figure 58 a,b and c).

Figure 58a: Distribution of distances to the tuberculous possum with nearest arithmetic center of activity

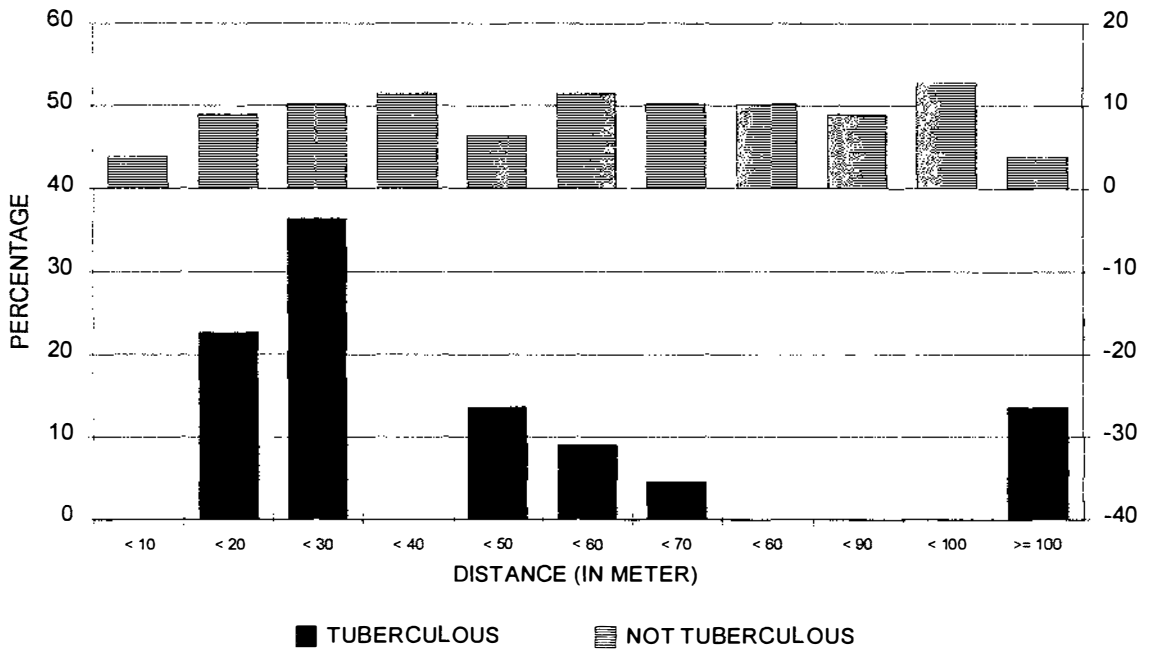


Figure 58b: Variogram of difference in prevalence between trap locations on longitudinal study site

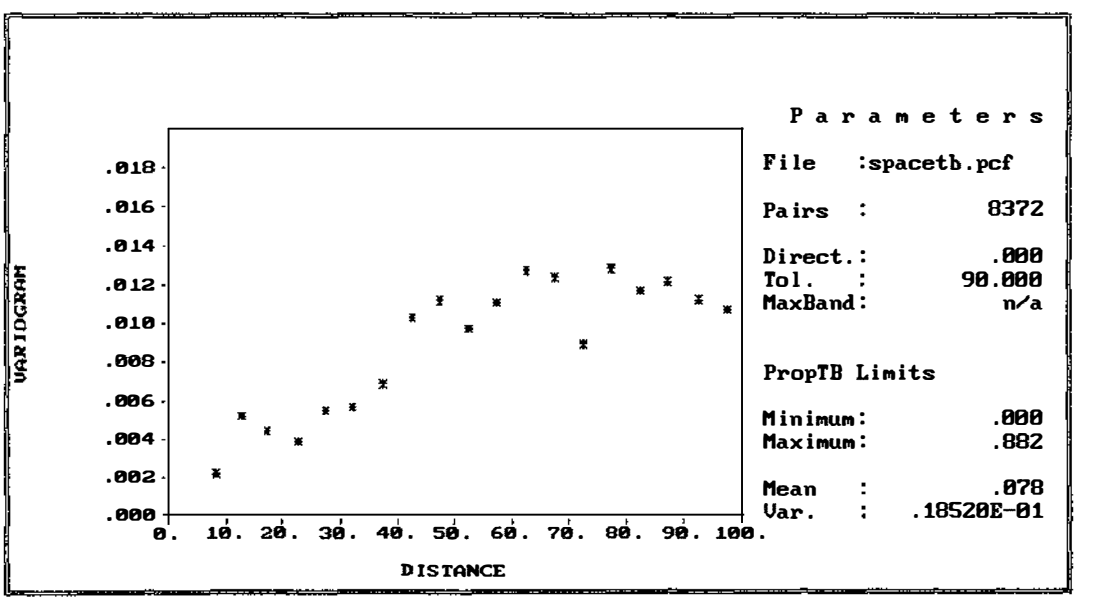
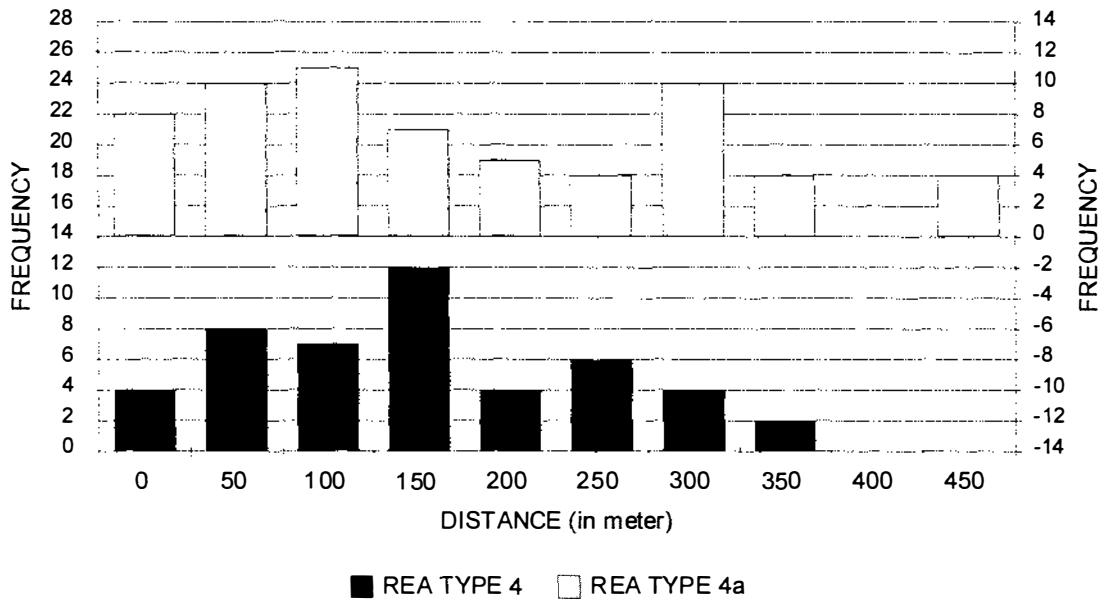


Figure 58c: Frequency histogram of geographical distances between centres of activity for tuberculous possums detected within 3 month intervals from each other stratified by REA type



A worksheet model was developed in the computer spreadsheet software MS Excel for Windows version 4.0 to estimate infection probabilities for the different transmission mechanisms implement in the model (see table 18). The estimates for the individual transmission mechanisms (excluding pseudo-vertical transmission) are linked with a baseline daily risk of successful transmission through a weighting scheme representing their relative importance. The probability of successful transmission during *mating* is assumed to be five times as high as through *spatial* or *temporal proximity*. For this simulation exercise a monthly average of ten contacts during *mating* and through *spatial proximity* and three contacts through *temporal proximity* has been assumed. The *spatial* and *temporal proximity* infection mechanisms are both active during the whole year, whereas the *mating* mechanism is active only during six months of the year. The individual probabilities are derived from a baseline risk of successful transmission within a local group of possums using this weighting scheme. This baseline risk of infection was estimated taking into consideration the spatio-temporal pattern of tuberculosis infection during the longitudinal study. A map representing the spatial pattern of period prevalence over the 22 month study period was produced by first summarizing disease and locational information on a grid system and then interpolating grid cell values (see figure 59a). For estimation of the baseline risk only areas with an above average risk of infection were considered. In these high risk areas period prevalence during the 22 months study period ranges from 0.20 to 0.50. The median prevalence was 0.034 over the whole study area (see figure 59b). A variogram of the original point data was generated. This technique is used to describe spatial continuity in the data. The variogram is generated by plotting half the squared difference between all paired data values at a particular distance from

each other on the y-axis against the distance on the x-axis. The variogram therefore provides a measure of the variability between data values relative to their distance from each other. In this analysis the variogram was required to decide on the appropriate cell size for generating a histogram of values for period prevalence in the study area in order to identify high infection risk clusters. Inspection of the variogram for period prevalence shows that variation between local estimates increased significantly above lags of 20m (see figure 59b). Therefore a grid cell size of $20m^2$ was considered appropriate to describe the variation of average area prevalence using a frequency histogram (see figure 59c). Areas with a prevalence of at least 10% were considered clusters of high infection risk. The median prevalence in cells with more than 10% prevalence was 0.22. This value relates to a period of 22 months. It only includes tuberculous possums with lesions which are detectable by clinical and by post mortem examination. There is evidence from this and other epidemiological studies that about 50% of possums develop lesions which are detectable using clinical examination. This suggests that the true clinical prevalence in local clusters with infection could be up to 0.44 over 22 months. The model requires parameters for each month during a twelve month period. Therefore an average cumulative infection risk of 0.40 over a 12 month period in population subgroups with prevalent infection is used for estimation of the parameters for the three variable transmission mechanisms.

It is possible to model a disease-free population by setting all transmission mechanisms and the TB prevalence in immigrants to zero.

Figure 59a: Contour map of the spatial distribution of proportion tuberculous possums in total catch

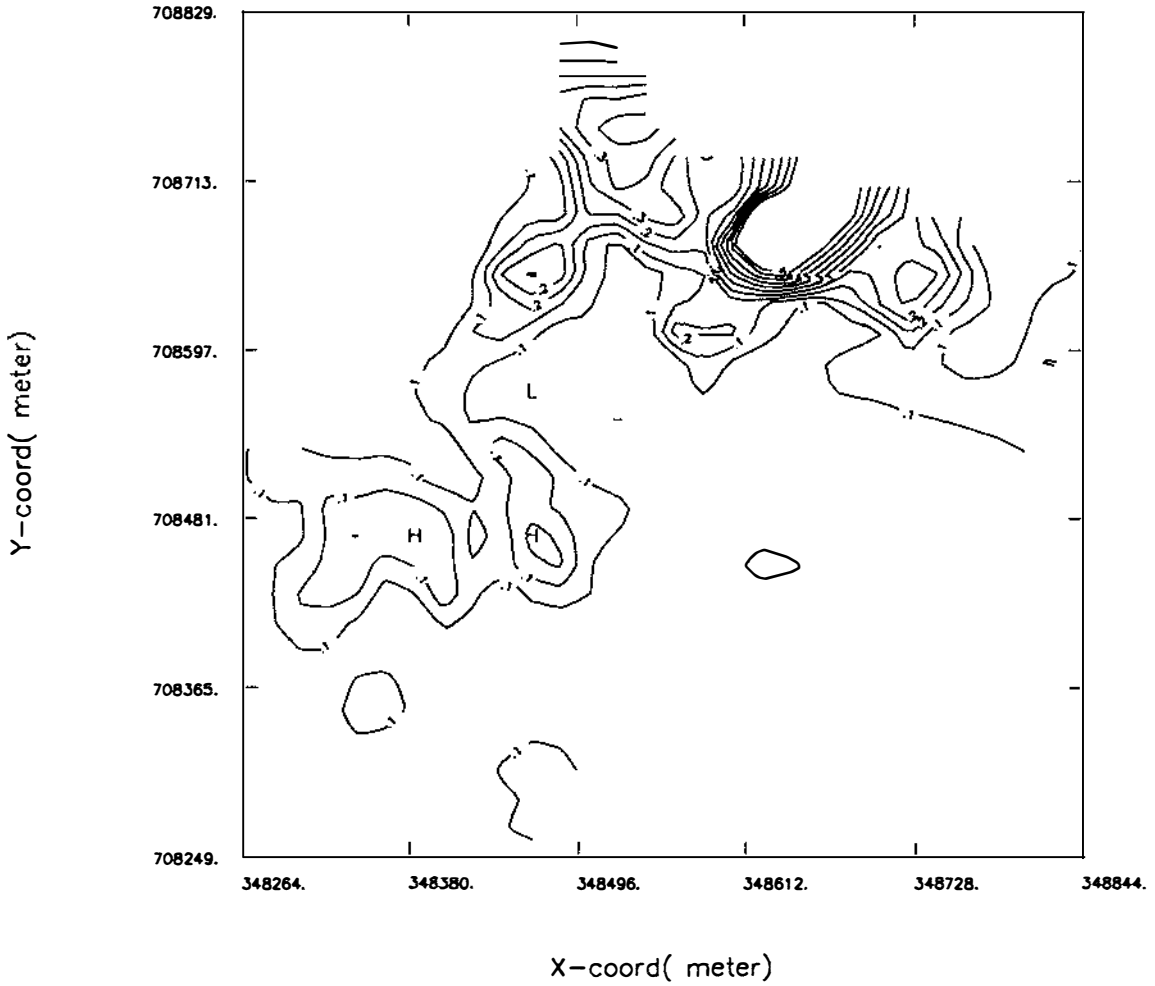


Figure 59b: Histogram of period prevalence per trap location

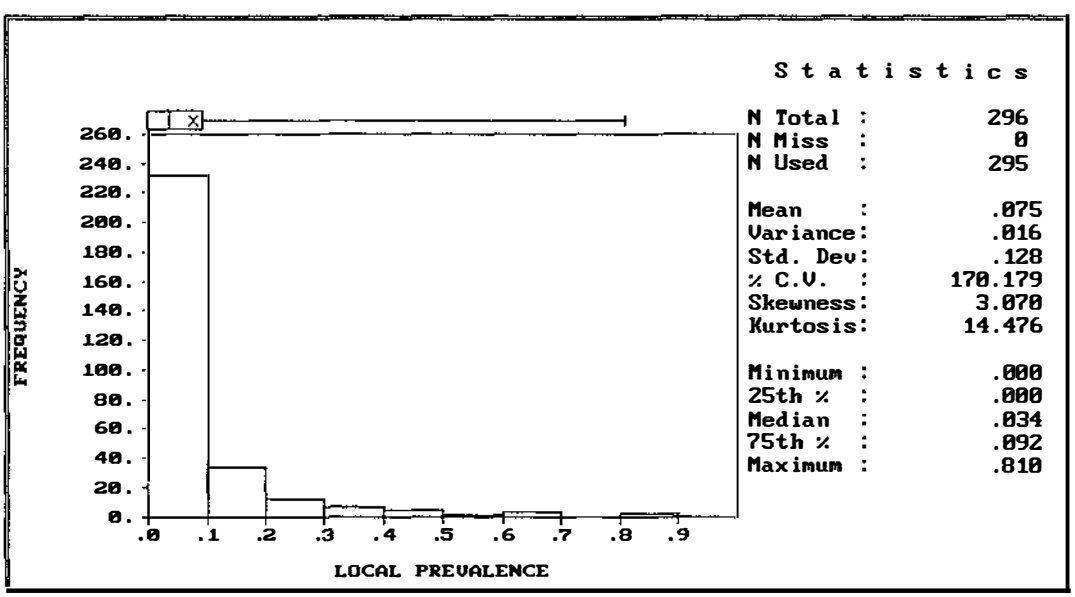


Figure 59c: Histogram of period prevalence for locations with tuberculosis based on a 20m grid cell size

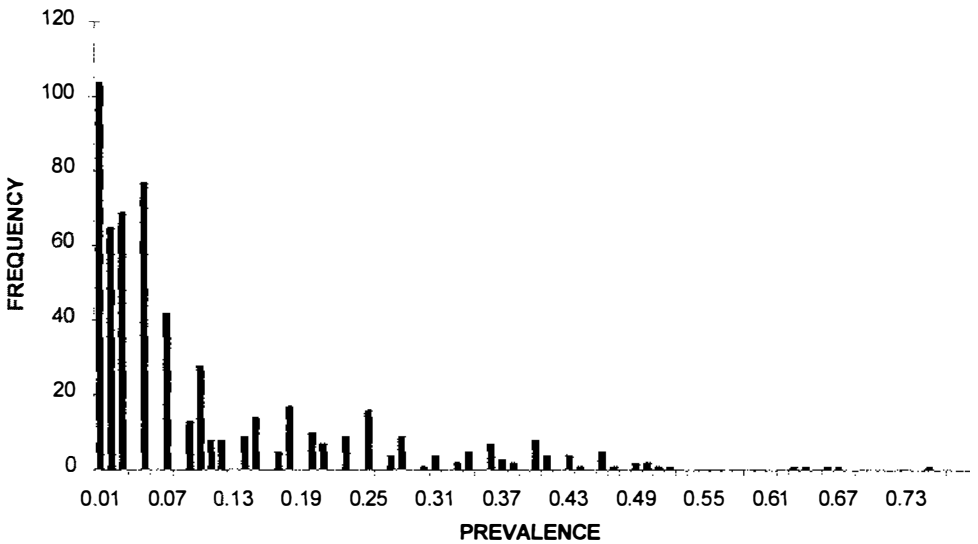


Table 18: Worksheet model for estimation of infection probabilities for the three variable transmission mechanisms in the model

Transmission Path	Mating	Spatial Proximity	Temporal Proximity
Relative Risk	5	1	1
Risk	0.0056	0.0011	0.0011
Contacts per Month	10	10	3
Months active	6	12	12
Daily Baseline Incidence	0.0011		
Yearly Survival	0.60		

Longitudinal Study Annual TB Incidence	0.22
95% CI	
Figure used	0.40
Annual Survival	0.60

Survival Parameters

Different survival mechanisms are operating in the model for dependent and independent possums. The survival module is processed once every month. Dependent possums are subject to mortality mechanisms which vary between months of age and are assumed to be independent of environmental factors. Data from the longitudinal study suggests that of 87 females with a pouch young, 30 (34.5%) successfully reared it until independence. This figure was used to develop a worksheet to calculate monthly mortality based on a weighting scheme

(see table 19). It was assumed that the risk of death decreased with age as reflected in the changing risk rating for different months of age.

Table 19: Worksheet for calculation of monthly survival in dependent possums

Age (months)	1	2	3	4	5	6
Cumulative Survival	0.81	0.65	0.53	0.45	0.39	0.35
Monthly Survival	0.81	0.81	0.81	0.86	0.86	0.90
Risk of Death	0.19	0.19	0.19	0.14	0.14	0.10
Risk Rating	2	2	2	1.5	1.5	1
Baseline Monthly Risk	0.10					
Total Survival	0.35					

The survival mechanisms for independent possums implemented in the model combine the effects of true mortality and emigration. The term *removal* is used in the following for both effects. Susceptible possums and possums with subclinical tuberculosis are exposed to removal varying between age groups, sex classes and the months of the year. A baseline risk is estimated and adjusted using a risk rating scheme. The results of proportional hazards regression analysis based on longitudinal study data are used to determine the risk weightings for the three risk factors age, sex and season (see table 20). An annual failure probability of 0.58 was estimated for the whole study population using the life-table method available in SAS procedure LIFETEST. For this simulation exercise a removal risk of 0.50 was used. This figure forms the basis for the worksheet model shown in table 20 which models the monthly mortality risk for possums in the different age, sex and disease strata. The individual probability estimates take into account the relative contribution of the different cohorts to the total population.

Table 20: Worksheet for estimation of monthly survival probabilities which are not density-dependent

MONTH	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
P death mature male	0.0158	0.0158	0.0229	0.0229	0.0229	0.0321	0.0321	0.0321	0.0229	0.0229	0.0229	0.0158
P death mature female	0.0158	0.0158	0.0229	0.0229	0.0229	0.0321	0.0321	0.0321	0.0229	0.0229	0.0229	0.0158
P death immature male	0.0989	0.0989	0.1433	0.1433	0.1433	0.2006	0.2006	0.2006	0.1433	0.1433	0.1433	0.0989
P death immature female	0.0989	0.0989	0.1433	0.1433	0.1433	0.2006	0.2006	0.2006	0.1433	0.1433	0.1433	0.0989
P TB death mature	0.0949	0.0949	0.1376	0.1376	0.1376	0.1926	0.1926	0.1926	0.1376	0.1376	0.1376	0.0949
P TB death immature	0.5932	0.5932	0.8598	0.8598	0.8598	1.2037	1.2037	1.2037	0.8598	0.8598	0.8598	0.5932

MONTHLY BASELINE RISK	0.1433
ANNUAL SURVIVAL	0.4991

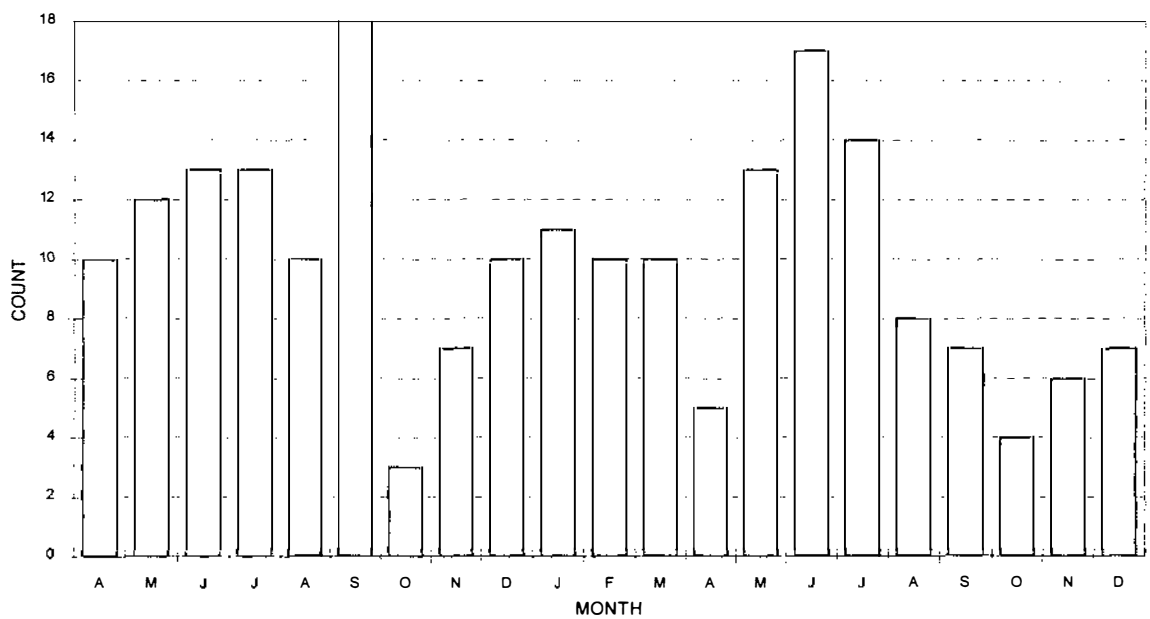
	Winter	*Summer*	*Adult Age*	*Clinical TB*
Odds Ratios	1.41	0.69	0.16	7.62
95% CI	0.94-2.12	0.43-1.12	0.10-0.26	4.31-13.5
Figure used	**1.4**	**0.69**	**0.16**	**6**
ln OR	0.33647	-0.37106	-1.83258	1.791759

Population Structure	Orginal
MATURE MALES	0.33	0.33
MATURE FEMALES	0.26	0.26
IMMATURE MALES	0.12	0.12
IMMATURE FEMALES	0.07	0.07
TB MATURE	0.17	0.17
TB IMMATURE	0.04	0.04

The population density dependent survival mechanism affects all independent possums. It handles possums with immigrant and resident status differently. The individual probabilities are estimated separately from the probabilities for other survival mechanisms in the model.

The mechanism can be used in order to stabilize variation in population numbers during the simulation. The data from the longitudinal study does not provide information to estimate these parameters. During the preliminary simulation runs, the parameter settings are adjusted according to the population dynamics. Figure 60 was used to provide a description of the observed temporal pattern of possum disappearance during the longitudinal study. Any adjustments based on the population density-dependent survival mechanism should not change this particular pattern. It was decided to use the same monthly probabilities for this mechanism throughout the year. If a possum had not used a den during 15 days within a period of 30 days, it was subjected to this mechanism. It was decided to use a probability of death or disappearance of 0.05 for this mechanism except during the winter and spring months which had monthly probabilities of 0.30 and 0.15 respectively, because during the last two periods density-dependent mortality is most likely to have an effect. If necessary, these probabilities could be varied in order to achieve the total population size (including sexually immature and mature animals, excluding dependent young) with a mean as observed in the longitudinal study (about 150 possums).

Figure 60: Temporal pattern of possum disappearance in the longitudinal study



Ageing Parameters

The parameters describing physiological development of individual possums are based on age of transition from dependent to independent and from sexually immature to sexually mature status. The same values are used for both sex classes. The actual age for individual possums is taken as a random observation from a normal distribution with a given mean, standard deviation, maximum and minimum value. The distribution of age of independence uses a

mean of 5 months, a standard deviation of 10 days, a maximum of 6 and a minimum of 4 months (see figure 61a). The age of sexual maturity for both sexes is sampled from a normal distribution with a mean of 1.5 years, a standard deviation of 1.5 months, a minimum of 1 year and a maximum of 2 years (see figure 61b). Possums which have survived other removal causes are removed from the simulations once they have reached an age of 7 years.

Figure 61a: Distribution for sampling age of independence in possums

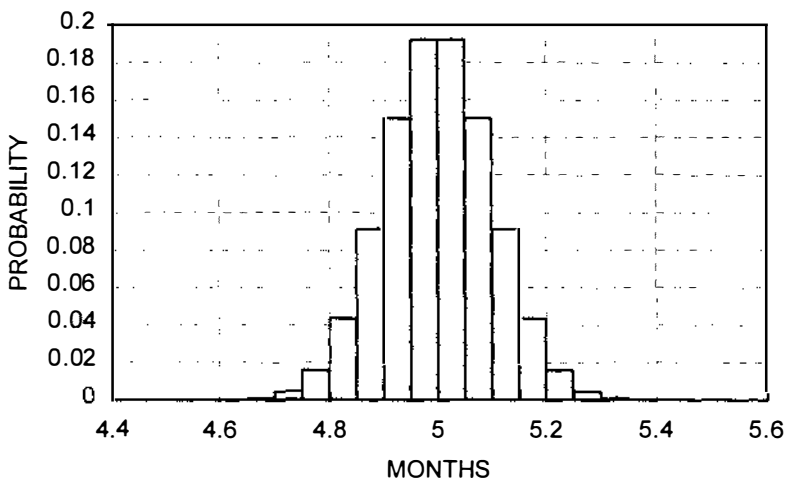
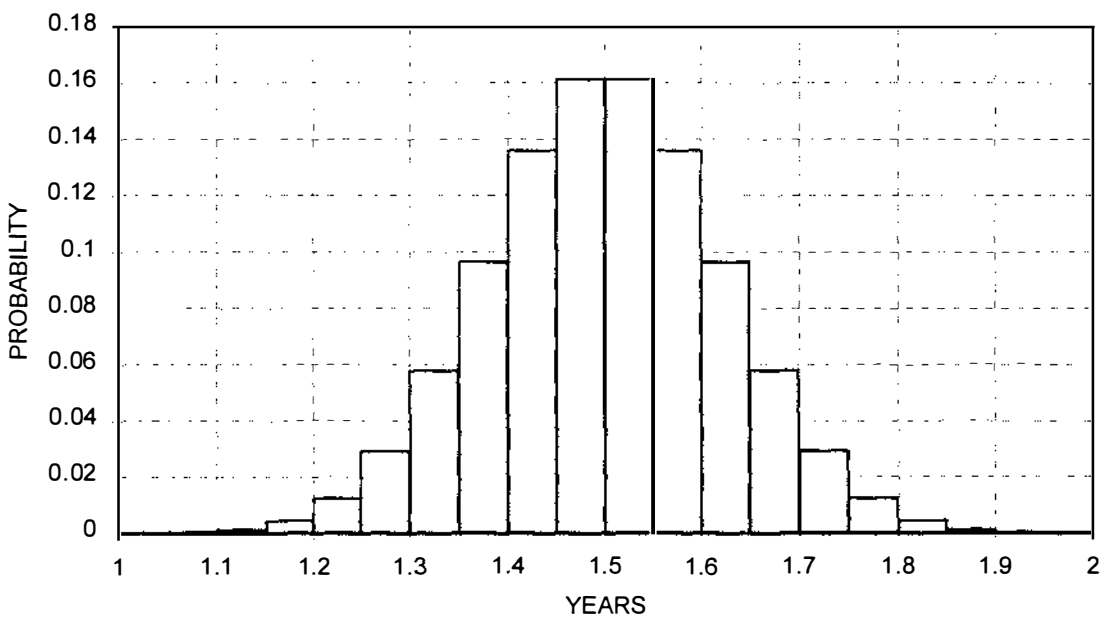


Figure 61b: Distribution used for sampling age of sexual maturity



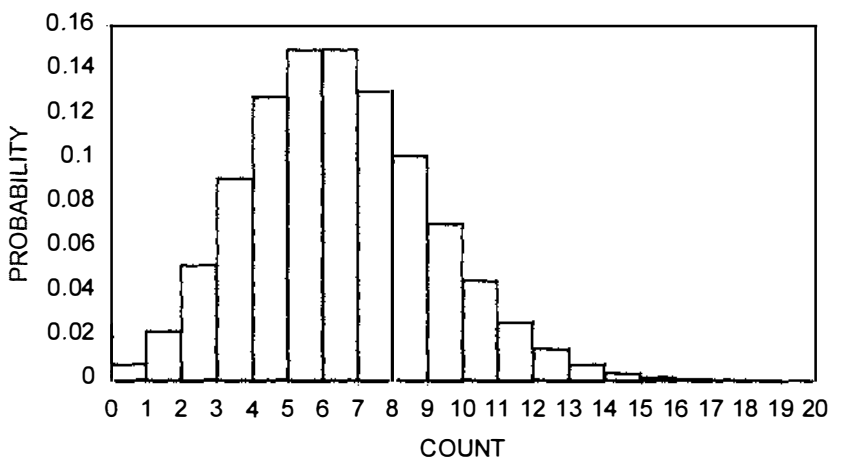
Immigration Parameters

Immigration of possums is implemented in the model by sampling the total number of immigrants from a poisson distribution with a given mean. The expected average number of immigrants per month is based on the monthly number of new captures during 1990 in the longitudinal study (see table 21). The expected number of immigrants per day for each month is derived from this figure by dividing it by the number of days in the particular month (see example in figure 62).

Table 21: Monthly distribution of average number of immigrants per year

MONTH	1	2	3	4	5	6	7	8	9	10	11	12
MALES	11	5	5	2	7	0	0	1	4	8	1	6
FEMALES	4	3	3	1	5	2	1	1	1	1	1	2

Figure 62: Example of a poisson probability distribution with a mean of 7 expected immigrants per month



Cyclical Effects

To introduce cyclical effects into the model, parameter settings representing three different "types" of years (*bad*, *average* and *good*) were used in the base simulation runs. Previously discussed parameters developed from longitudinal study field data were used as the basis for the *bad* year modelling scenario. Parameter settings for *average* and *good* years had to be adjusted using field experience. Cluster analysis was used to identify *good*, *average* and *bad* years from a sequence of weather data for Castlepoint weather station from 1972 to 1990. For each year the average of monthly rainfall, average monthly temperature and average monthly temperature fluctuation during the winter months was used for the analysis. Only the winter months were used because environmental conditions during these months appeared to have a more significant effect than the other months of the year. The *k*-means procedure (McLachlan 1992) was used to assign each year to the cluster whose sample mean was closest to its feature

vector defined by rainfall, average temperature and temperature fluctuation. Table 22 shows the characteristics for each of the three clusters. A year was classified as a *bad* year if winter rainfall and temperatures had been low. The sequence of *good*, *average* and *bad* years identified in this analysis was used during the subsequent simulation runs.

Table 22: Characteristics of the three types of years (*good*, *average*, *bad*) based on results of *k*-means cluster analysis

	GOOD		*AVERAGE*		*BAD*	
Variable	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**
average ratio of minimum and maximum daily temperature	0.641	0.039	0.638	0.011	0.646	0.009
average temperature (°C)	14.824	1.396	14.369	0.565	14.099	0.472
average monthly rainfall (mm)	59.392	5.829	76.617	5.411	99.597	6.972
N	6		8		6	

The parameter settings for the three different types of years are shown in figures 63a, b and c. The settings for the *good* and *average* years were estimated using probability arrays which favoured survival and reduced the probability of transition from subclinical to the clinical disease stage. The estimates were derived by adjusting relevant parameters in the spreadsheets which were used to develop the parameters for the *bad* year modelling scenario. During model experimentation these parameter settings will be referred to as *base* parameter settings. The combination of the *start* population, den site locations and *base* parameter settings will named *standard* conditions. Table 23 lists the parameters which were changed to develop the settings for the three different types of years.

Figure 63a: Parameter settings for the *bad* year simulation scenario

SURVIVAL PROB.		JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC.
ADULT	MALE	0.9842	0.9842	0.9771	0.9771	0.9771	0.9679	0.9679	0.9679	0.9771	0.9771	0.9771	0.9842
	FEMALE	0.9842	0.9842	0.9771	0.9771	0.9771	0.9679	0.9679	0.9679	0.9771	0.9771	0.9771	0.9842
IMMATURE	MALE	0.9011	0.9011	0.8567	0.8567	0.8567	0.7994	0.7994	0.7994	0.8567	0.8567	0.8567	0.9011
	FEMALE	0.9011	0.9011	0.8567	0.8567	0.8567	0.7994	0.7994	0.7994	0.8567	0.8567	0.8567	0.9011
IMMIGRATION	FEMALES	4	3	3	1	5	2	1	1	1	1	1	2
	MALES	11	5	5	2	7	0	0	1	4	8	1	6
CONCEPTION PROB.		0.0000	0.0500	0.2000	0.2000	0.0500	0.0000	0.0000	0.0000	0.1000	0.1000	0.0500	0.0000
	MONTHS	0	1	2	3	4	5	6	7	8			
JOEY SURVIVAL		1.0000	0.8100	0.8100	0.8100	0.8600	0.8600	0.9000	0.9500	0.9500			
DISAPPEARANCE		0.0500	0.0500	0.0500	0.0500	0.0500	0.3000	0.3000	0.3000	0.1500	0.1500	0.1500	0.0500
		15	DAYS WITHOUT DEN DURING					30	DAYS				
TRANSIENCE OF IMMIGRANTS				IF	15	DAYS WITHOUT DEN							
TB SURVIVAL	ADULT	0.9051	0.9051	0.8624	0.8624	0.8624	0.8074	0.8074	0.8074	0.8624	0.8624	0.8624	0.9051
	IMMATURE	0.4068	0.4066	0.1402	0.1402	0.1402	0.0000	0.0000	0.0000	0.1402	0.1402	0.1402	0.4066
TB PROB.	BACKGROUND	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	IMMIGRATION	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
DEVELOPMENT	Mature	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.1304	0.1304	0.1304	0.0652
OF CLINICAL TB	Immature	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.2607	0.2607	0.2607	0.1304

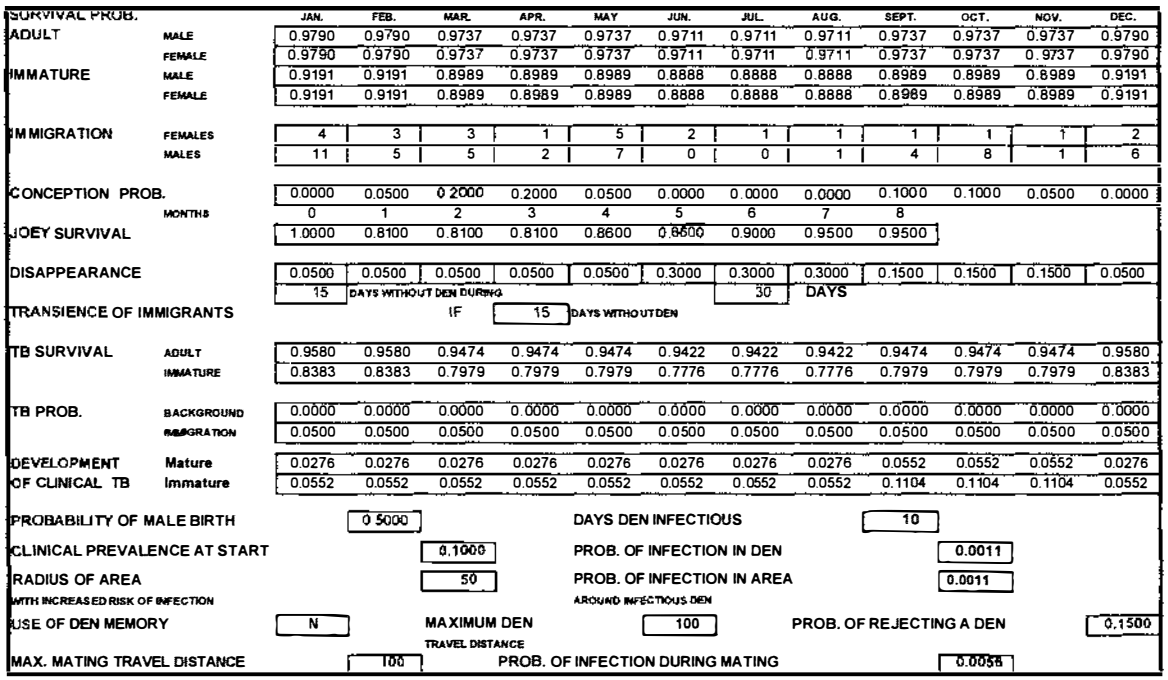
PROBABILITY OF MALE BIRTH	0.5000	DAYS DEN INFECTIOUS	10		
CLINICAL PREVALENCE AT START	0.1000	PROB. OF INFECTION IN DEN	0.0011		
RADIUS OF AREA WITH INCREASED RISK OF INFECTION	50	PROB. OF INFECTION IN AREA AROUND INFECTIOUS DEN	0.0011		
USE OF DEN MEMORY	N	MAXIMUM DEN TRAVEL DISTANCE	100	PROB. OF REJECTING A DEN	0.1500
MAX. MATING TRAVEL DISTANCE	100	PROB. OF INFECTION DURING MATING	0.0055		

Figure 63b: Parameter settings for the *average* year simulation scenario

SURVIVAL PROB.		JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC.
ADULT	MALE	0.9877	0.9877	0.9821	0.9821	0.9821	0.9750	0.9750	0.9750	0.9821	0.9821	0.9821	0.9877
	FEMALE	0.9877	0.9877	0.9821	0.9821	0.9821	0.9750	0.9750	0.9750	0.9821	0.9821	0.9821	0.9877
IMMATURE	MALE	0.9229	0.9229	0.8882	0.8882	0.8882	0.8435	0.8435	0.8435	0.8882	0.8882	0.8882	0.9229
	FEMALE	0.9229	0.9229	0.8882	0.8882	0.8882	0.8435	0.8435	0.8435	0.8882	0.8882	0.8882	0.9229
IMMIGRATION	FEMALES	4	3	3	1	5	2	1	1	1	1	1	2
	MALES	11	5	5	2	7	0	0	1	4	8	1	6
CONCEPTION PROB.		0.0000	0.0500	0.2000	0.2000	0.0500	0.0000	0.0000	0.0000	0.1000	0.1000	0.0500	0.0000
	MONTHS	0	1	2	3	4	5	6	7	8			
JOEY SURVIVAL		1.0000	0.8100	0.8100	0.8100	0.8600	0.8600	0.9000	0.9500	0.9500			
DISAPPEARANCE		0.0500	0.0500	0.0500	0.0500	0.0500	0.3000	0.3000	0.3000	0.1500	0.1500	0.1500	0.0500
		15	DAYS WITHOUT DEN DURING					30	DAYS				
TRANSIENCE OF IMMIGRANTS				IF	15	DAYS WITHOUT DEN							
TB SURVIVAL	ADULT	0.9488	0.9488	0.9229	0.9229	0.9229	0.8921	0.8921	0.8921	0.9229	0.9229	0.9229	0.9468
	IMMATURE	0.6675	0.6675	0.5181	0.5181	0.5181	0.3254	0.3254	0.3254	0.5181	0.5181	0.5181	0.6675
TB PROB.	BACKGROUND	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	IMMIGRATION	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
DEVELOPMENT	Mature	0.0374	0.0374	0.0374	0.0374	0.0374	0.0374	0.0374	0.0374	0.0749	0.0749	0.0749	0.0374
OF CLINICAL TB	Immature	0.0749	0.0749	0.0749	0.0749	0.0749	0.0749	0.0749	0.0749	0.1498	0.1498	0.1498	0.0749

PROBABILITY OF MALE BIRTH	0.5000	DAYS DEN INFECTIOUS	10		
CLINICAL PREVALENCE AT START	0.1000	PROB. OF INFECTION IN DEN	0.0011		
RADIUS OF AREA WITH INCREASED RISK OF INFECTION	50	PROB. OF INFECTION IN AREA AROUND INFECTIOUS DEN	0.0011		
USE OF DEN MEMORY	N	MAXIMUM DEN TRAVEL DISTANCE	100	PROB. OF REJECTING A DEN	0.1500
MAX. MATING TRAVEL DISTANCE	100	PROB. OF INFECTION DURING MATING	0.0055		

Figure 63c: Parameter settings for the *good* year simulation scenario



SURVIVAL PROB.		JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC.
ADULT	MALE	0.9790	0.9790	0.9737	0.9737	0.9737	0.9711	0.9711	0.9711	0.9737	0.9737	0.9737	0.9790
	FEMALE	0.9790	0.9790	0.9737	0.9737	0.9737	0.9711	0.9711	0.9711	0.9737	0.9737	0.9737	0.9790
IMMATURE	MALE	0.9191	0.9191	0.8989	0.8989	0.8989	0.8888	0.8888	0.8888	0.8989	0.8989	0.8989	0.9191
	FEMALE	0.9191	0.9191	0.8989	0.8989	0.8989	0.8888	0.8888	0.8888	0.8989	0.8989	0.8989	0.9191
IMMIGRATION	FEMALES	4	3	3	1	5	2	1	1	1	1	1	2
	MALES	11	5	5	2	7	0	0	1	4	8	1	6
CONCEPTION PROB.		0.0000	0.0500	0.2000	0.2000	0.0500	0.0000	0.0000	0.0000	0.1000	0.1000	0.0500	0.0000
	MONTHS	0	1	2	3	4	5	6	7	8			
JOEY SURVIVAL		1.0000	0.8100	0.8100	0.8100	0.8600	0.8600	0.9000	0.9500	0.9500			
DISAPPEARANCE		0.0500	0.0500	0.0500	0.0500	0.0500	0.3000	0.3000	0.3000	0.1500	0.1500	0.1500	0.0500
		15	DAYS WITHOUT DEN DURING					30	DAYS				
TRANSIENCE OF IMMIGRANTS				IF	15	DAYS WITHOUT DEN							
TB SURVIVAL	ADULT	0.9580	0.9580	0.9474	0.9474	0.9474	0.9422	0.9422	0.9422	0.9474	0.9474	0.9474	0.9580
	IMMATURE	0.8383	0.8383	0.7979	0.7979	0.7979	0.7776	0.7776	0.7776	0.7979	0.7979	0.7979	0.8383
TB PROB.	BACKGROUND	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	IMMIGRATION	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
DEVELOPMENT OF CLINICAL TB	Mature	0.0276	0.0276	0.0276	0.0276	0.0276	0.0276	0.0276	0.0276	0.0552	0.0552	0.0552	0.0276
	Immature	0.0552	0.0552	0.0552	0.0552	0.0552	0.0552	0.0552	0.0552	0.1104	0.1104	0.1104	0.0552

PROBABILITY OF MALE BIRTH	0.5000	DAYS DEN INFECTIOUS	10
CLINICAL PREVALENCE AT START	0.1000	PROB. OF INFECTION IN DEN	0.0011
RADIUS OF AREA WITH INCREASED RISK OF INFECTION	50	PROB. OF INFECTION IN AREA AROUND INFECTIOUS DEN	0.0011
USE OF DEN MEMORY	N	MAXIMUM DEN TRAVEL DISTANCE	100
PROB. OF REJECTING A DEN	0.1500		
MAX. MATING TRAVEL DISTANCE	100	PROB. OF INFECTION DURING MATING	0.0058

Table 23: Parameters used to estimate different probability arrays for the three types of years (*good*, *average*, *bad*)

	Parameter	*GOOD*	*AVERAGE*	*BAD*
Mortality	**OR Winter**	1.1	1.4	1.4
	OR Summer	0.8	0.69	0.69
	OR Adult Age	0.26	0.16	0.16
	OR Clinical TB	2	4.31	6
	Cumulative Annual Risk	0.42	0.42	0.50
Transition from subclinical to clinical TB	**Cumulative Annual Survival**	0.60	0.50	0.30

(OR = odds ratio)

Simulation Program Operation

After execution of the program file a startup screen appears. The menu option 'Run Model' has to be selected which opens another window on the computer screen. The program then asks for input of the name of the following three files: start population file, den map file and parameter file. It also asks if the user wants to change the parameter settings during the simulation run, at what intervals and for how long until the original parameter settings are restored. The above parameters can be entered interactively or as command line parameters. The program responds by first reporting the amount of available computer memory. Then the program initializes by reading in the respective parameters. After completion of initialization it prompts the user with the options: begin simulation run, calculate distances between den sites or quit the program (see figures 64a and b).

Figure 64a: Startup screen after program execution

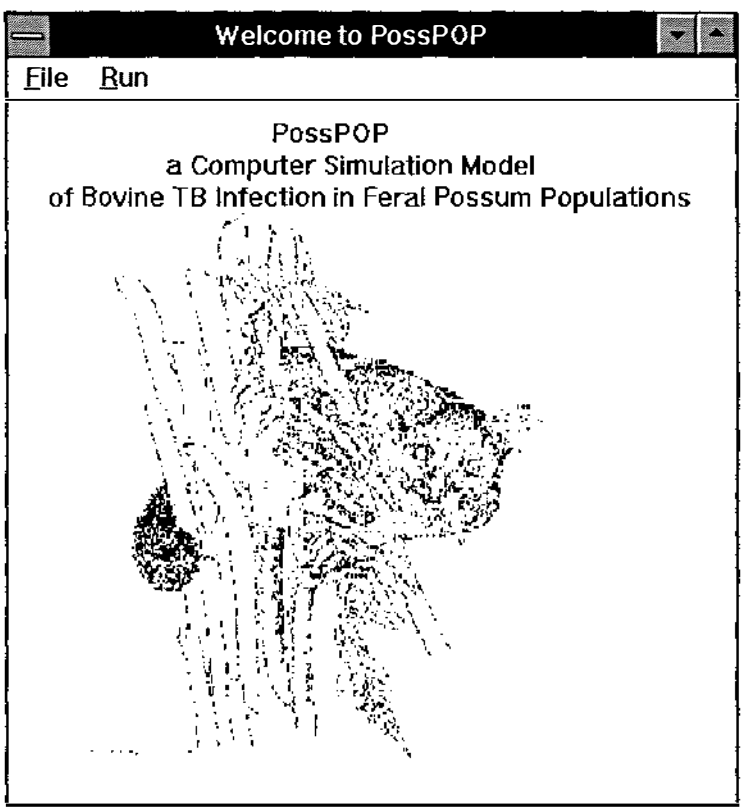
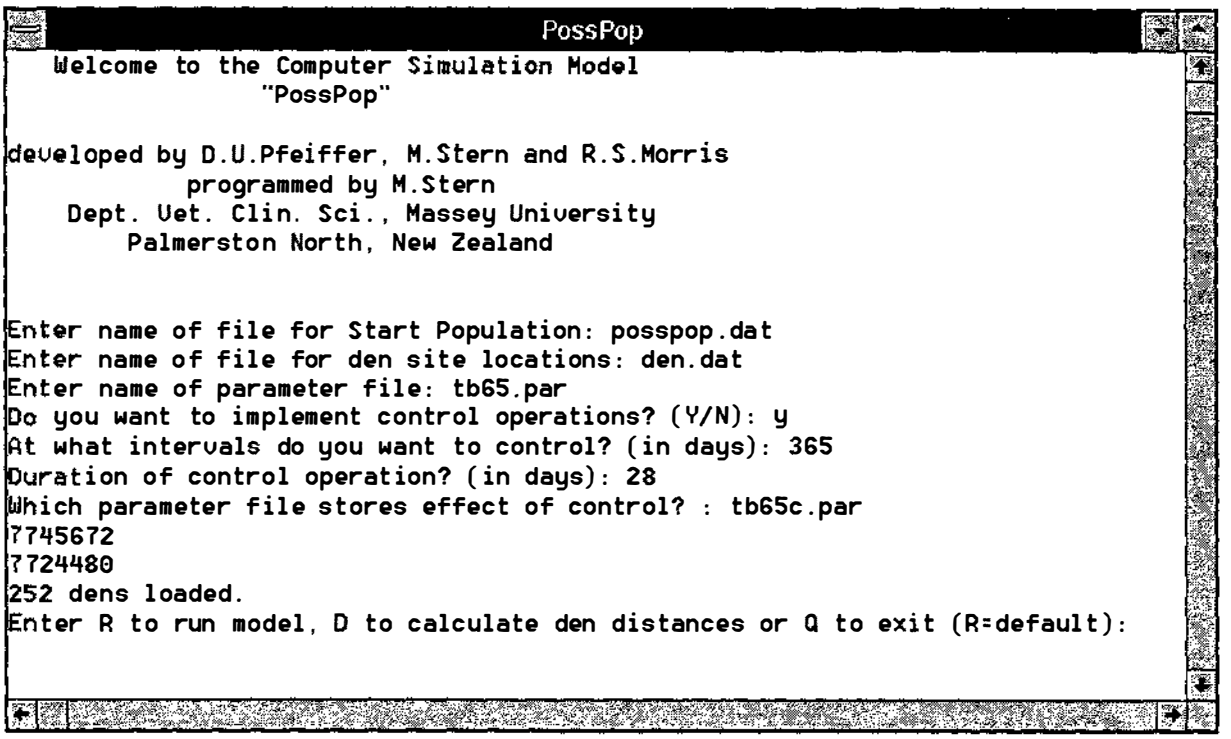
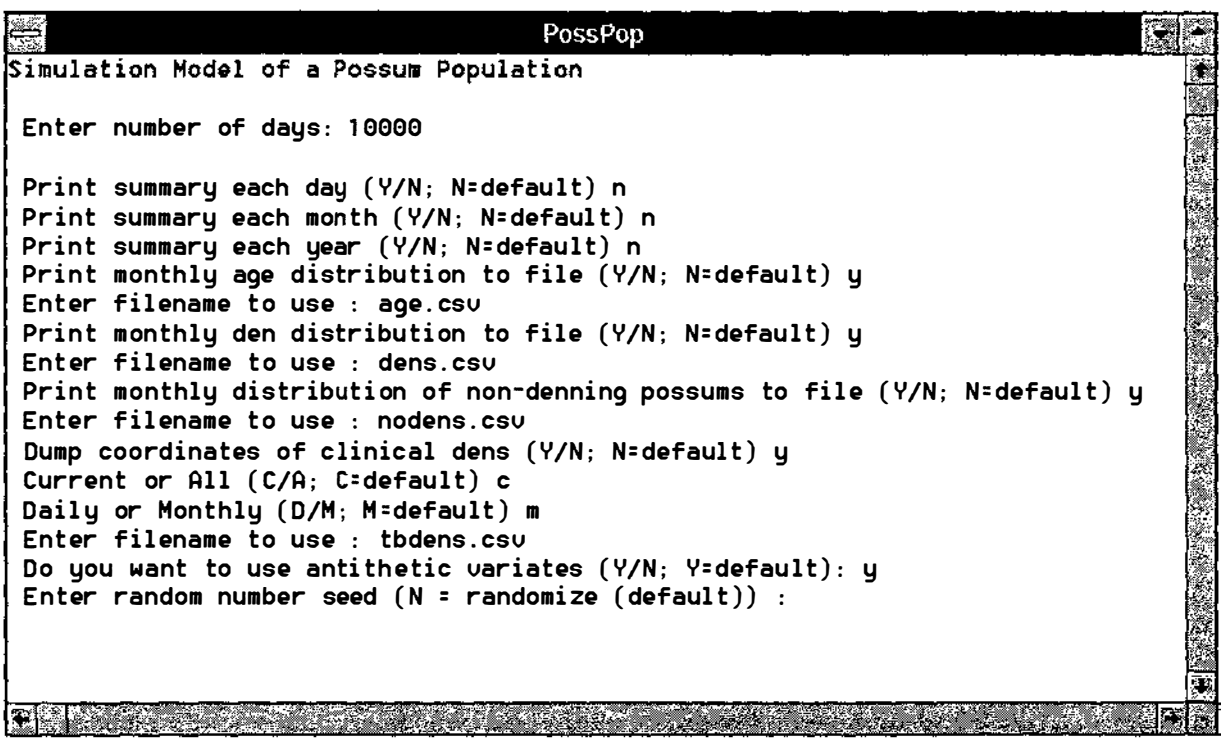


Figure 64b: First screen for defining simulation run parameters



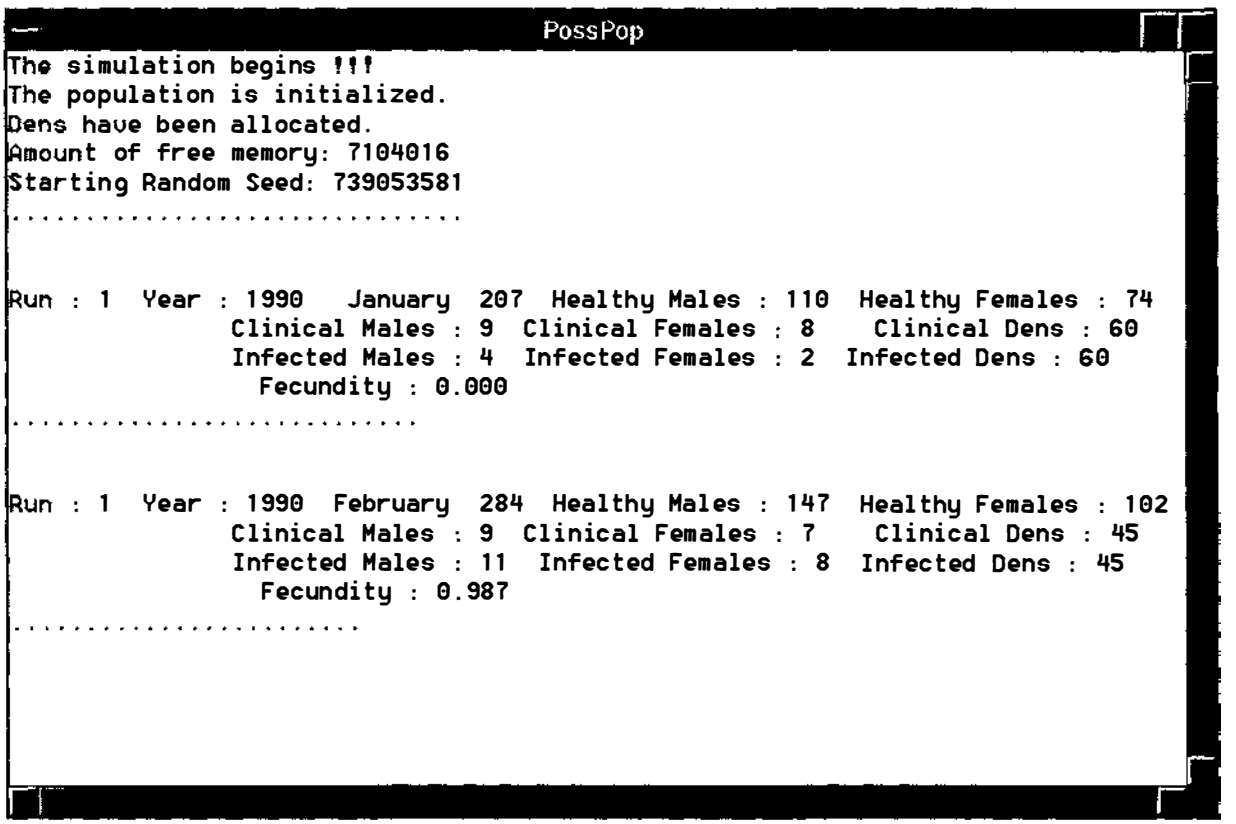
On selection of the option 'run simulation' the screen clears and the user has to enter information regarding the length of the simulation run in days, the simulation output required, about the use of antithetic variates and it is possible to enter a random seed or have the computer generate it (see figure 65).

Figure 65: Second entry screen for defining simulation run parameters



At this stage the simulation run begins. The screen displays summary information reporting the status of the simulated population which has been requested by the user (see figure 66).

Figure 66: Screen display during computer simulation run



Parameter Settings for Population and Disease Mechanisms used in Simulation Run

The parameter settings which are used to control the mechanisms in the simulation model are stored in ASCII-formatted files which have to be specified at the beginning of a simulation run. A spreadsheet model has been developed which allows editing of the parameters (see figure 67).

Figure 67: Worksheet model for creation and editing of parameter files

SURVIVAL PROB.		JAN.	FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC.
ADULT	MALE	0.9842	0.9842	0.9771	0.9771	0.9771	0.9679	0.9679	0.9679	0.9771	0.9771	0.9771	0.9842
	FEMALE	0.9842	0.9842	0.9771	0.9771	0.9771	0.9679	0.9679	0.9679	0.9771	0.9771	0.9771	0.9842
IMMATURE	MALE	0.9011	0.9011	0.8567	0.8567	0.8567	0.7994	0.7994	0.7994	0.8567	0.8567	0.8567	0.9011
	FEMALE	0.9011	0.9011	0.8567	0.8567	0.8567	0.7994	0.7994	0.7994	0.8567	0.8567	0.8567	0.9011
IMMIGRATION	FEMALES	4	3	3	1	5	2	1	1	1	1	1	2
	MALES	11	5	5	2	7	0	0	1	4	8	1	6
CONCEPTION PROB.		0.0000	0.0500	0.2000	0.2000	0.0500	0.0000	0.0000	0.0000	0.1000	0.1000	0.0500	0.0000
	AGE (months)	0	1	2	3	4	5	6	7	8			
JOEY SURVIVAL		1.0000	0.8100	0.8100	0.8100	0.8600	0.8600	0.9000	0.9500	0.9500			
DISAPPEARANCE		0.0500	0.0500	0.0500	0.0500	0.0500	0.3000	0.3000	0.3000	0.1500	0.1500	0.1500	0.0500
TRANSIENCE OF IMMIGRANTS		15	DAYS WITHOUT DEN DURING PERIOD OF				30	DAYS					
				IF	30	DAYS WITHOUT DEN							
TB SURVIVAL	ADULT	0.9051	0.9051	0.8624	0.8624	0.8624	0.8074	0.8074	0.8074	0.8624	0.8624	0.8624	0.9051
	IMMATURE	0.4068	0.4068	0.1402	0.1402	0.1402	0.0000	0.0000	0.0000	0.1402	0.1402	0.1402	0.4068
TB PROB.	BACKGROUND	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	IMMIGRATION	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500	0.0500
DEVELOPMENT OF CLINICAL TB	ADULT	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.0652	0.1304	0.1304	0.1304	0.0652
	IMMATURE	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.1304	0.2607	0.2607	0.2607	0.1304
PROBABILITY OF MALE BIRTH			0.5000			DAYS DEN INFECTIOUS				10			
CLINICAL PREVALENCE AT START				0.1000		PROB. OF INFECTION IN DEN					0.0011		
RADIUS OF AREA WITH INCREASED RISK OF INFECTION				50		PROB. OF INFECTION IN AREA AROUND INFECTIOUS DEN					0.0011		
USE OF DEN MEMORY		N		MAXIMUM DEN TRAVEL DISTANCE			100		PROB. OF REJECTING A DEN				0.1500
MAX. MATING TRAVEL DISTANCE			100			PROB. OF INFECTION DURING MATING					0.0056		

Random Number Generation

The generation of random numbers is an essential element in the Monte Carlo simulation of stochastic models. The algorithms used in stochastic models derive their randomness from a supply of random numbers, which is usually based on an independent sequence of random variables uniformly distributed between 0 and 1. Ripley (1987) points out that most users of simulation are content to remain ignorant of how such numbers are produced and rely on standard functions. He writes that such attitudes are dangerous, because the random numbers form the foundation of any such simulation exercise and problems at higher level can frequently be traced back to faulty foundations. However these theoretical concerns are difficult to support when reputable and reliably designed random number generators are used. Simulations which are conducted on a digital computer typically use *pseudo-random* numbers, which are based on a deterministic sequence of numbers having the same relevant statistical properties as a sequence of *random* numbers, but have the advantage that if desired the same sequence can be repeated on future occasions. Those in use in major computer software packages are derived from well-tested and extensively investigated generators which meet statistical requirements for samples drawn from a rectangular R(0,1) distribution.

Knuth (1981) writes that the most popular random number generators in use today are based on the linear congruential method. This technique was introduced by Lehmer in 1949. It uses four 'magic' numbers: m (modulus; $m > 0$), a (multiplier; $0 \leq a < m$), c (increment; $0 \leq c < m$) and X_0 ($0 \leq X_0 < m$). The sequence of random numbers X_n is obtained from the following equation:

$$X_{n+1} = (aX_n + c) \bmod m$$

The congruential generator will always generate a cyclic pattern consisting of a long stream of numbers, which it eventually repeats endlessly. This cycle is called the *period*. The terms *multiplicative congruential method* and *mixed congruential method* are commonly used to describe congruential generators with $c = 0$ and $c \neq 0$ respectively. The *period* of the generator cannot have more elements than the modulus m. The choice of m also determines the speed of generation. It is convenient to choose numbers for m using a base of 2 on binary computers and an exponent determined by the computer's word size (e.g. 2^{31}). An extensive discussion explaining the effects of the choice of m, a and c can be found in Knuth (1981). For the current simulation model a multiplicative congruential random number generator with the following settings was used: $a = 742938285$, $c = 0$ and $m = 2147483647$. With these settings it will not repeat itself before 2,147,483,646 random numbers have been generated (Fishman and Moore 1986). Different streams can be created by selecting different seed numbers to start the process. The Turbo Pascal source code for this generator was taken from Hultquist (Hultquist 1991).

Each of the biological processes within the model used a separate independent stream of random numbers, created using different seeds and then drawing the next group of digits from the stream each time a probability value was required. At the beginning of each run, a different random number generator was used to generate random seeds for each random number stream (positive integers between 0 and 2^{31}). The principal component of this random number generator is the Subtract-With-Borrow (SWB) generator which was described by Marsaglia and Zaman (Marsaglia *et al* 1991). This generator was implemented in PC-assembler programming code by Zaman and Marsaglia (FSU - ULTRA version 1.05). The generator has an extremely long period (10^{356}) and it is very fast by taking advantage of the 32bit architecture of 80386/80486 microprocessors. This random number generator normally mixes a SWB sequence with a random number stream from a congruential generator ($a=69069$, $c=0$, $m=2^{32}$). In order to increase speed this feature was disabled in its implementation in this simulation model.

Generation of Random Variates from Non-Uniform Probability Distributions

Techniques for generation of random variates from non-uniform probability distributions typically draw on a sequence of random numbers drawn from a uniform or rectangular distribution as a starting point. There is a vast literature on algorithms with differing characteristics for converting these into samples from univariate distributions with a specific probability density function (Dagpunar 1988, Devroye 1986, Knuth 1981, Press *et al* 1986). The following algorithms were selected in order to minimise execution time without sacrificing validity of the derived sample. They use sequences of uniform pseudo-random numbers generated by the above described multiplicative congruential random number generator.

Random deviates with a normal probability distribution were generated using the polar method by Marsaglia and Bray which is a modification of the Box-Muller method. This method was recommended by Ripley (1983). The polar method uses two uniform variates which preferably should be independent. This can be achieved by using two independent random number streams. The Pascal program code for the implementation of the polar method was adapted from Cooke *et al* (1985).

The algorithm which was used for the generation of samples from a poisson distribution in the simulation model was recommended by Ripley (1987) for distributions with a mean of $\mu \leq 5$ to 20. The Pascal program code was taken from Cooke *et al* (1985).

Samples can also be drawn from purely empirical distributions derived from field data which does not fit any particular probability density function, by defining the nature of the cumulative distribution function, and using that to allocate observations randomly to specific values or to ranges according to the nature of the empirical cumulative distribution function.

Variance Reduction Techniques in Simulation Modelling

Stochastic simulation is a sampling experiment where the experimentation consists of running the computer program. This sampling process can be manipulated without introducing bias using variance reduction techniques. Kleijnen (1974) writes that these techniques replace 'straight on' or 'crude' sampling by more sophisticated sampling. A variance reduction technique should reduce the variance of the estimator by replacing the original sampling procedure by a new procedure that yields the same expected value but with smaller variance. Kleijnen describes a number of techniques for variance reduction including stratified sampling, selective sampling, use of control variates, importance sampling, use of antithetic variates and use of common random numbers. Ripley (1987) points out that variance reduction techniques are often known as *swindles.* They often do not work, especially if more than one technique is used. He adds that variance reduction may obscure the essential simplicity of simulation.

The method of antithetic variates is considered one of the most important variance reduction techniques and was employed for simulation runs of the current model. This technique tries to create a negative correlation between observations by generating one random observation from the random number r and the other observation from its *antithetic* partner $(1 - r)$. Negative correlation between the two responses decreases the variance of the estimated response. The mean response of the system is estimated by calculating the average of the two responses (original and antithetic). It is possible that the *antithetic* run for a particular variable may need less random numbers than the original run. It has been suggested that this does not have a serious effect but tends to weaken the correlation. In a simulation model with multiple stochastic effects synchronization is necessary in order to ensure that the i'th random number does not for example generate a survival event in the original run and a

den site selection event in the *antithetic* run. Synchronization is simplified if each stochastic variable in the model has its own random number generator (Kleijnen 1974).

In the current model the variance reduction technique of *antithetic variates* was implemented by creating independent random number seeds for each stochastic variable using the SWB random number generator. Each random number seed was stored and it was subtracted from m = 2147483647 to calculate the seed for the antithetic run. Each stochastic effect had its own random number generator. The proof for the validity of this approach is provided in Kleijnen (1974).

The method of antithetic variates can reduce the variance in outcome variables and hence can be useful to make predictions of expected values from a smaller number of runs, without the fear that in a very small series of runs one will produce extreme predictions which will unduly bias the estimate of expected value. However in doing so it will by definition underestimate the true variability in model behaviour, which in some cases is of particular interest since it shows the likelihood of an unusual but important outcome (such as the probability of failure of a control or eradication program which is expected to succeed "on average"). Hence in this study, which is concerned with developing a model rather than applying it, the method of using repeated runs to estimate mean and standard deviation of outcome variables will be first compared with the method of antithetic variates, and then the method of antithetic variates will be used to explore in a preliminary way the representation of different control strategies in the model, since at this stage of development it is the expected outcome in such exploratory exercises which is of interest rather than the variance.

Verification and Validation of the Simulation Model

The conceptual model, the logic by which it was implemented, and the computer model structure were verified as outlined by Morris (1976) by reviewing the program code and examining the functioning of the program. Each component of the model structure was carefully checked in terms of its logic and behaviour during preliminary simulation runs. Verification is the first major step in model validation (Sargent 1984), and is an essential prerequisite to further testing of its behaviour.

After the simulation model is verified, a structured testing program should be carried out to determine if its output is an accurate, and therefore valid, representation of the real system. A key concept is validation by module or building block showing each section produces valid small scale results, and demonstrating emergence of biologically important properties not designed in. In just the same sense that it is impossible to determine whether the results of a specific field experiment truly represent the “state of nature”, it is not feasible to prove that a model is completely valid. Normal practice is to progressively build confidence in the biological accuracy of the model, and eventually to test its predictive accuracy. To achieve this, there are a number of commonly used components of model validation (Gass 1983). These include for example comparison of the output of the model to

the real system, the Delphi method, the Turing test and validation by analysis of behaviour at extremes (Hoover and Perry 1989). The first method has been used in this simulation study. This technique requires comparison of performance indicators calculated by the simulation model with the equivalent indicators taken from the real system.

In a non-terminating simulation the researcher is generally interested in the steady-state performance of a system (Kleijnen 1987). Analysis of output from nonterminating simulations has to deal with specific problems. The behaviour of the system during the early part of the simulation when it goes through a transient phase may be misleading or irrelevant with respect to the objectives of the simulation run. It is therefore necessary to choose a run length which will allow any initial transient results to be discarded, but does not require excessively long run times.

Hoover and Perry (1989) discuss the advantages and disadvantages of four commonly used methods for analyzing output from non-terminating simulations. These include the method of replication, batching, autocorrelation methods and the regenerative method. For the analysis of output from this simulation study the method of batch means was used. This approach is based on one long simulation run, which is then divided into batches of equal size. Sample means are computed for each batch, which are then used to compute an estimate of the variance of the grand sample mean over all batches, assuming that all batch means are independent (Fishman 1978). The justification for using this approach is that if the number of observations per batch is sufficiently large, the statistics accumulated during each interval may be considered for practical purposes to be independent, even though strictly speaking this is not true because the ending state of one interval is the start state of the next interval. The statistics during the transient phase will normally be discarded. If the duration of the transient phase is underestimated, its effect will be diminished as the number of batches increases. The main difficulty with the method of batch means is determining the the length or size of each batch (Hoover and Perry 1989). Kleijnen (1987) writes that most simulation practitioners intuitively pick a fixed length for each batch. He recommends to use at least 100 batches in order to test the independence of batch responses. If dependence is detected the batch length has to be made longer. For calculation of confidence intervals of the mean response 10-20 batches are needed. Hoover and Perry (1978) recommend use of the Runs test as a statistical test of independence.

Model validation requires that the output from the model is compared with data from the real system. This model was validated against data from the longitudinal study. It is not recommended to use the same data for model development and validation, but at the time of this analysis no other suitable data set was available (McCarl 1984). The actual comparison between the model and the real system is a statistical comparison and the differences in performance measures have to be tested for statistical significance. This results in a number of problems. The performance measures taken from the real system generally are based on shorter time frames than the ones generated by the simulation. Often there are effects in the

real system, which intentionally or unintentionally have not been implemented in the model. It may be difficult to identify and adjust the performance measures for the presence of such effects. Hoover and Perry (1978) emphasize that if the performance measures of the real system and the simulation model are not statistically similar, one should not conclude that the model is invalid. It may indicate that the model needs further refinement in its structure or parameter estimates. Balci and Sargent (1981) point out that the concept of validation should not be considered a binary decision variable where a model is absolutely valid or absolutely invalid.

Voigt *et al* (1985) developed a simulation model of the epidemiology of rabies infection in fox populations using a similar modelling approach. Given a one-sample estimate of reality they write that it is impossible to estimate the variance to be used to test whether model output is a member of the real world of populations. Therefore the reverse approach is to test if the real world sample can be considered to be a member of the population of distributions produced by the model. Voigt *et al* concentrated on validating model output characteristics which could be directly related to field evidence. They tested whether the model produced a 'steady state' fox population with a realistic juvenile:adult ratio, in the absence of rabies. They also tested if the model was able to reproduce temporal and spatial patterns of rabies epidemics in fox populations.

Due to the availability of only 22 months data, validation of this simulation model was possible only to a limited extent. It was decided that a more extensive model validation will have to await the availability of a longer run of data from the longitudinal study. At this stage of the research program, the objectives have been limited to exploratory investigations of the behaviour of the model. This includes asessing the most appropriate way in which to represent the field data as parameter estimates in the model (since the validity of the model depends in part on the methods chosen to estimate influential parameters for the simulation), and to test how well the model handles a range of control policy evaluations which require parameters to be varied outside the range for which field data from the study was available.

First, a *preliminary model experimentation phase* is conducted in order to understand the general behaviour of the model given the base parameter settings. For these simulation runs it is assumed that no tuberculosis infection is present in the population or in immigrating possums. Population size, proportion of sexually mature animals and of female possums are used as the main indicator variables describing general population dynamics. The method of batch means is used to calculate estimates of these variables for individual parameter settings by estimating averages for each month of the year over the whole simulation period. An additional simulation run using antithetic random variates (*antithetic run*) was conducted after each *original run.* The preliminary model experimentation phase results in a set of model parameters which should allow adequate simulation of the longitudinal study population (for the purposes of this simulation exercise).

The dynamics of tuberculosis infection in the population are analyzed in the next step of model validation/experimentation. In this case it is assumed that tuberculosis infection is present in the start population and in immigrants. The variables which are comparable with data from the longitudinal study include prevalence and incidence of possums with clinical tuberculosis and the spatio-temporal distribution of den sites used by tuberculous possums. This data cannot be analyzed using batch means, because for a given random number stream it is possible that tuberculosis infection completely disappears from the population. Therefore, for each set of parameters the model is run multiple times using different random number streams. Each of these runs is repeated with an antithetic random number stream. This step completes the validation/experimentation stage. A set of parameters will be available which should produce results similar to the data from the longitudinal study.

Using this adjusted parameter set more detailed analyses of model output are conducted in order to understand the temporal and spatial dynamics of the disease and specifically the interaction between different parameters.

The final stage of model output analysis consists of a sensitivity analysis and another experimentation phase.

Methods of Analysis

The analysis of model input and output was done using a set of worksheets which were created in computer spreadsheet software Microsoft EXCEL for Windows version 4.0. One of these worksheets (*parameter editor*) is designed for retrieval, editing and creation of files with specific model parameter settings. Another worksheet (*analysis worksheet*) is used for retrieval and analysis of the ASCII files with model output from individual simulation runs. Time series charts are linked to this information. The individual worksheets are organized in a work group file. Retrieval of ASCII files is performed using the software Q+E for Microsoft Excel. For further statistical analyses in PC-SAS 6.04 and SOLO 4.0 data can be exported from EXCEL into dBase file format .

Each model run produces an ASCII file with monthly summary information of the population structure stratified by sex group (male,female), age class (dependent, immature, mature) and disease status (non-infected, subclinical, clinical TB) distinguishing between incident and prevalent cases (subclinical and clinical). This information is used to calculate a number of population parameters using the *analysis worksheet*. These variables include population size, sex and age structure, incidence and prevalence of clinical and subclinical tuberculosis cases stratified by age group and sex class.

Preliminary Simulation Experiment

As a first step in model experimentation a preliminary simulation experiment is conducted which examines the behaviour of the model with regard to its representation of possum population dynamics.

The length of this preliminary simulation run was 10000 days. Model behaviour was controlled by the *base* parameter settings, except for initial tuberculosis prevalence and tuberculosis prevalence in immigrants which were both set to 0 (see tables 24a). The random number seed was selected by calling the Borland Turbo Pascal function RANDOMIZE in the program. Table 24b shows a summary of a descriptive analysis of the simulation output. A time series plot of population size, proportion of sexually mature and of female possums reveals that the model produces results which are comparable to what has been found during the longitudinal field study (see figures 68a, b and c). Error bar charts summarizing the data for the 12 months of each year were prepared for average age structure of the population, population size, proportion of females in the population, proportion of immatures in the population (see figures 68d, e, f and g). There is only limited variation between years for the variables proportion of males and immatures in the total population. The error bars for the results for the original and the antithetic run overlap extensively for all variables.

Table 24a: General characteristics of preliminary simulation experiment

PARAMETER FILES	BADYR0.PAR, MEDYR0.PAR, GOODYR0.PAR
PARAMETER FILE SEQUENCE	YEAR.SEQ
DATE	10/11//93
TIME BEGIN	9:37
TIME END	9:59
RANDOM SEED	773196069
ANTITHETIC RUN	YES
LENGTH in days	10000
AGE DISTRIBUTION	AGED0.TXT
AGE STRUCTURE	AGES0.TXT
DEN USAGE	DENS0.TXT
NO DEN USAGE	NODENS0.TXT
LOCATION TB DENS	TBDENS0.TXT
SIMULATION TIME	0:22
SIMULTANEOUS RUNS	1

Table 24b: Results of descriptive analysis of output from preliminary simulation experiment

	Mean	95%C.L.	Median	Mode	S.D.	Variance	Kurtosis	Skewness	Min	Max	N
ORIGINAL RUN											
POPULATION SIZE	164.8	2.61	165	179	24.09	580.4	-0.02	0.15	104	232	328
FEMALES/TOTAL POP.	0.49	0.00	0.49	0.50	0.02	0.00	0.37	-0.05	0.43	0.57	328
IMMATURE/TOTAL	0.21	0.01	0.21	0.18	0.05	0.00	-0.29	-0.23	0.06	0.34	328
ANTITHETIC RUN											
POPULATION SIZE	158.8	2.42	156	157	22.33	498.6	0.30	0.62	107	233	328
FEMALES/TOTAL POP.	0.49	0.00	0.48	0.47	0.04	0.00	-0.89	0.15	0.41	0.58	328
IMMATURE/TOTAL	0.20	0.01	0.20	0.20	0.05	0.00	-0.28	-0.01	0.08	0.34	328

Figure 68a: Time series plot of monthly data for population size from the original and antithetic run of the preliminary simulation experiment

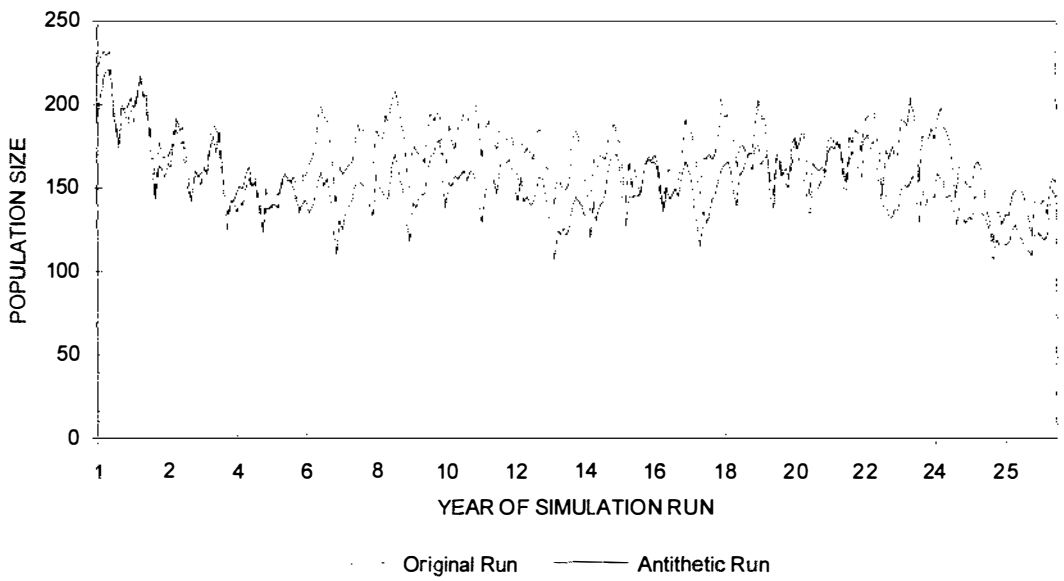


Figure 68b: Time series plot of monthly data of the proportion of sexually mature animals in the population based on data from the original and antithetic run of the preliminary simulation experiment

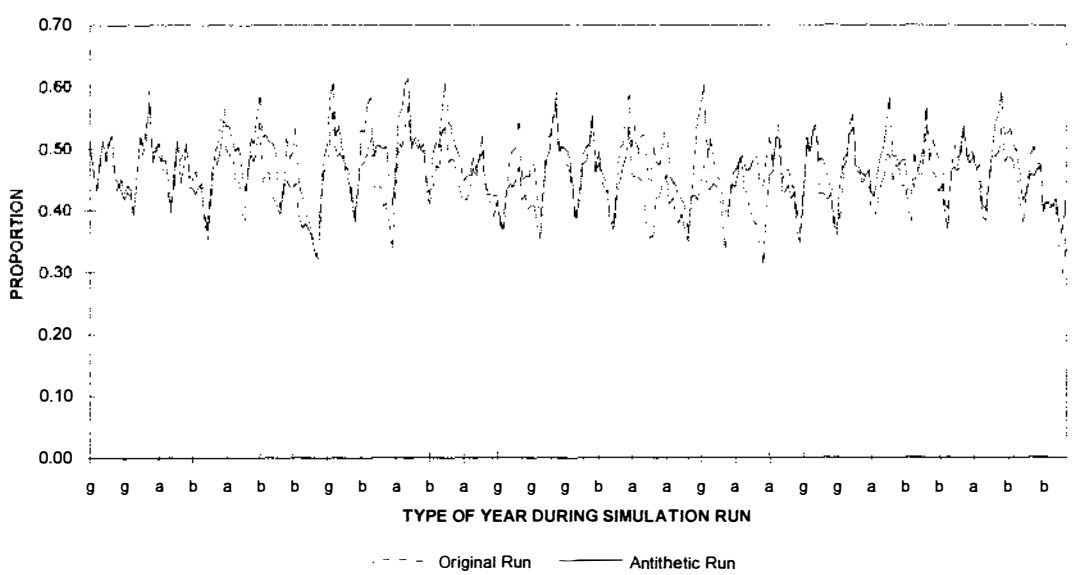


Figure 68b: Time series plot of monthly data of the proportion of female animals in the population based on data from the original and antithetic run of the preliminary simulation experiment

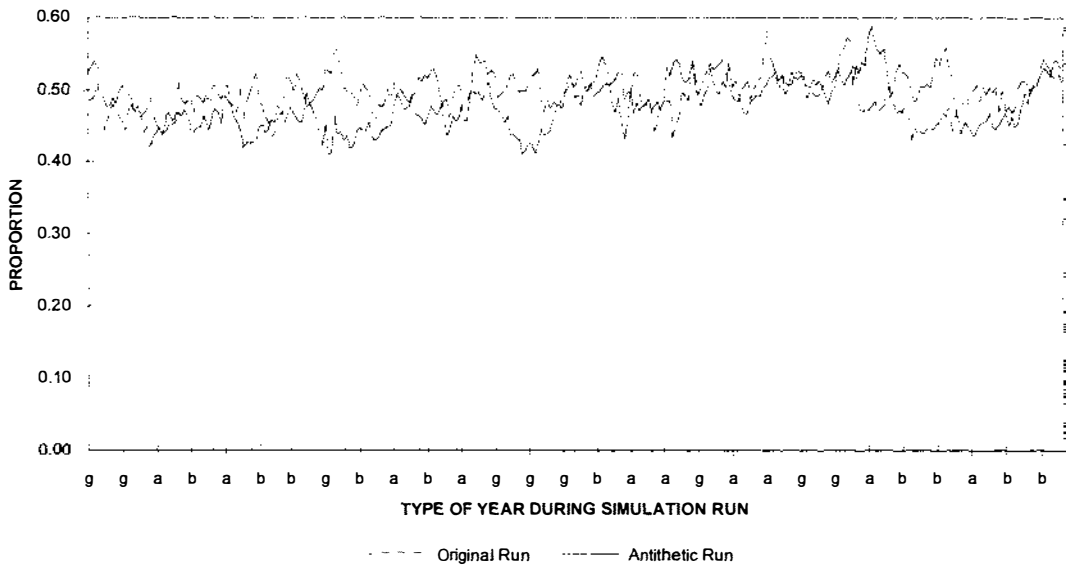


Figure 68d: Average age structure in simulated population of preliminary simulation experiment

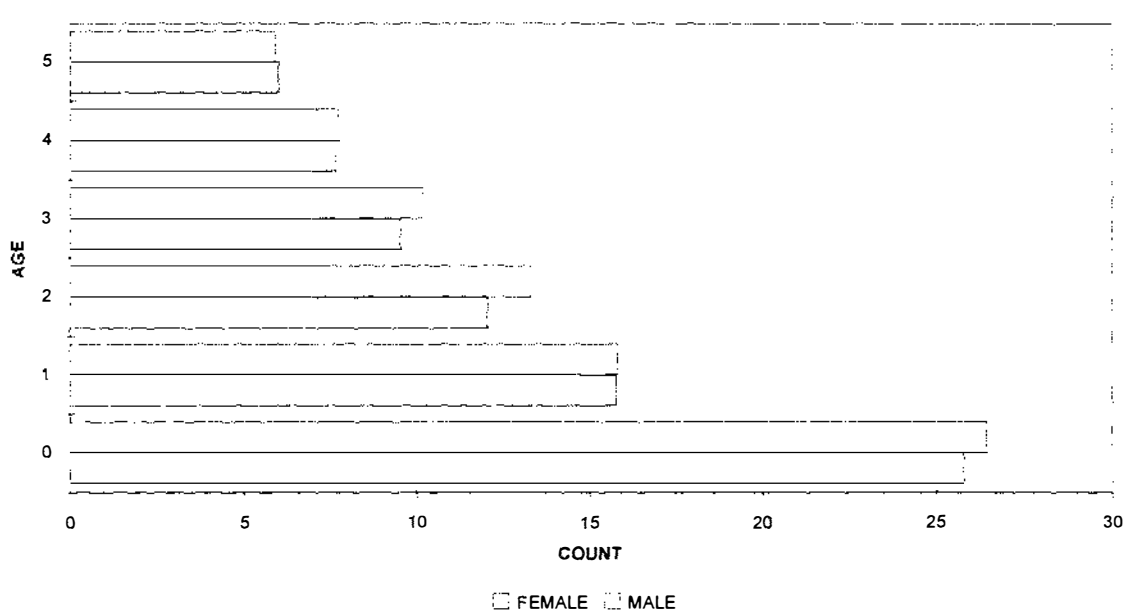


Figure 68e: Error bar chart for population size for each month of the year based on data from the original and antithetic run of the preliminary simulation experiment

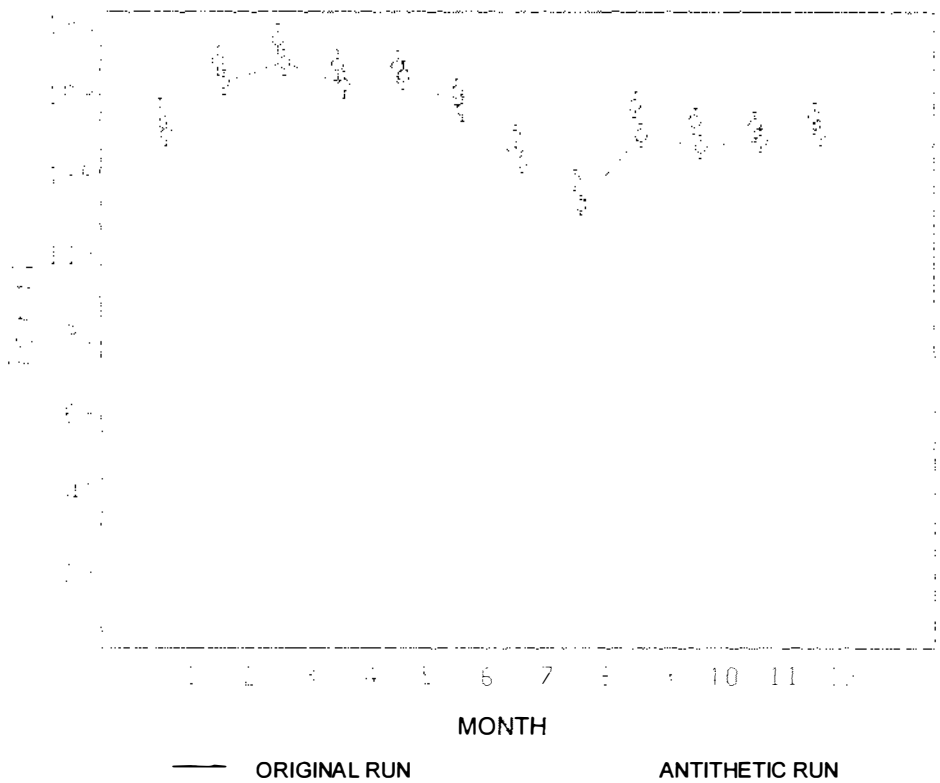


Figure 68f: Error bar chart for proportion of female possums in total population for each month of the year based on data from the original and antithetic run of the preliminary simulation experiment

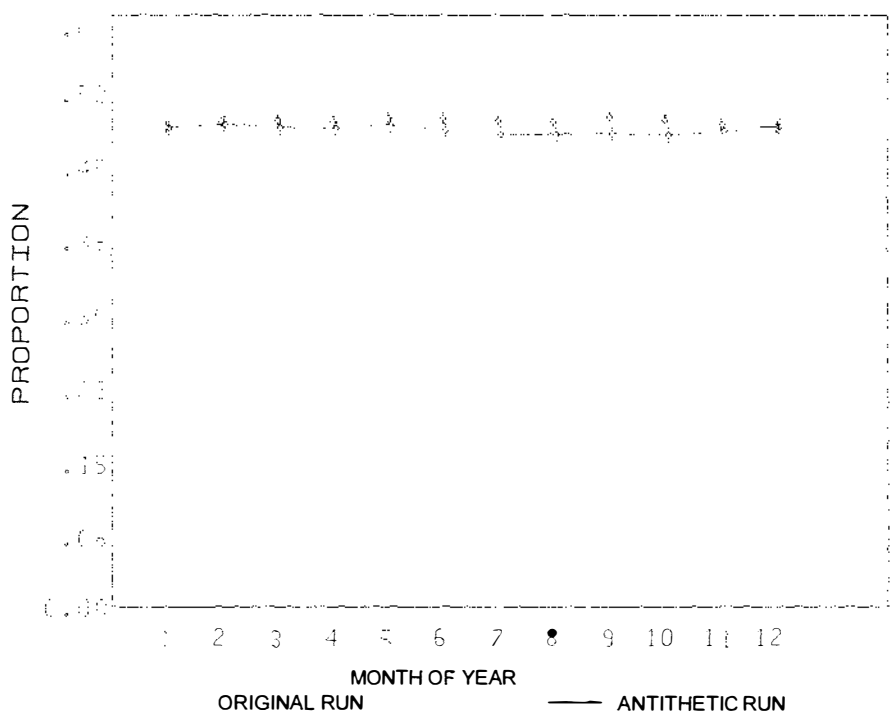
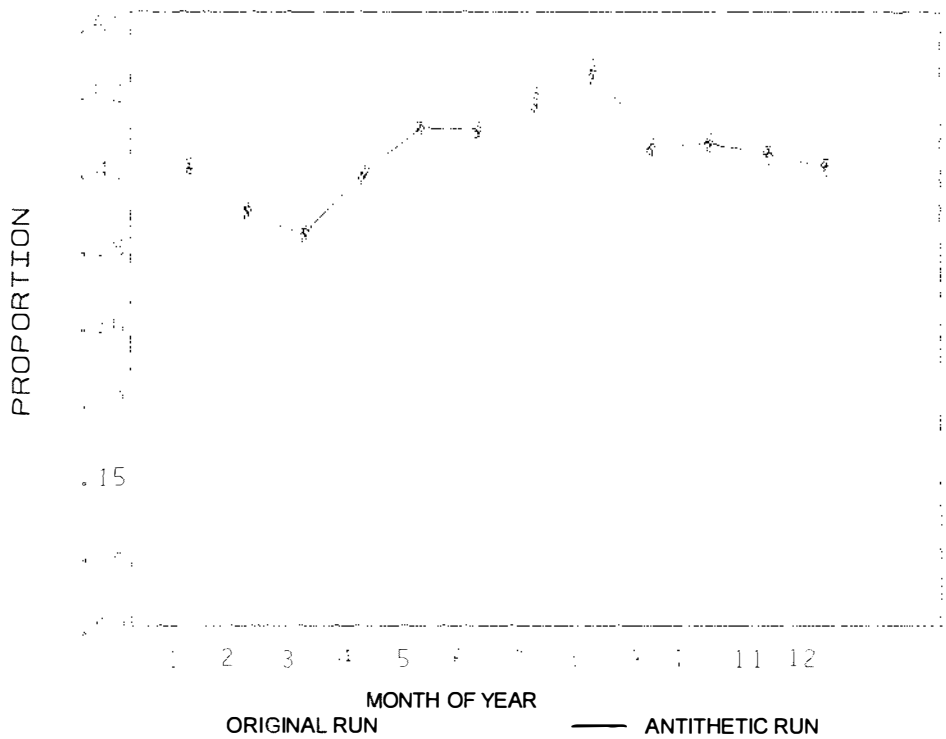


Figure 68g: Error bar chart for proportion of immature possums in the total population for each month of the year based on data from the original and antithetic run of the preliminary simulation experiment



Simulation of a Population with Tuberculosis Infection

A simulation was conducted using the same *base* parameter settings, but this time assuming an initial prevalence of clinical tuberculosis of 0.10 and a monthly prevalence in immigrants of 0.05. Given the currently available data it is hypothesized that these simulation conditions represent the epidemiology of *Mycobacterium bovis* infection in the longitudinal study possum population. These simulation conditions will be described as *standard* simulation conditions. The following simulation runs are conducted in order to investigate if the simulation model using *standard* conditions provides an adequate representation of the tuberculosis infection dynamics in a possum population. Table 25 shows the general characteristics of this simulation.

Table 25: General characteristics of the simulation experiment

PARAMETER FILES	BADYR.PAR, MEDYR.PAR, GOODYR.PAR
PARAMETER FILE SEQUENCE	YEAR.SEQ
DATE	10/11//93
TIME BEGIN	8:45
TIME END	8:58
RANDOM SEED	269551662
ANTITHETIC RUN	YES
LENGTH in days	10000
AGE DISTRIBUTION	AGED1.TXT
AGE STRUCTURE	AGES1.TXT
DEN USAGE	DENS1.TXT
NO DEN USAGE	NODENS1.TXT
LOCATION TB DENS	TBDENS1.TXT
SIMULATION TIME	0:13
SIMULTANEOUS RUNS	1

General Population Dynamics

The summary statistics for the main population parameters are presented in table 26a. Over the 2 simulation runs population size averaged 123 for the *original* and 132 for the *antithetic* run. On average the proportion of females and the proportion of immatures in the total population was 50% and 47% respectively for both runs. The influence of cyclical effects is shown in table 26b which presents the summary statistics for these variables by type of year. Population size, proportion of female and immature possums in the population was highest during *good* and *average* years and lowest during *bad* years. There was considerable variation between simulation runs when comparing the same types of years. A graphical comparison of the temporal pattern of population size, proportion of female and immature animals over the course of the year between data from the simulation run and data obtained from the longitudinal study is presented in the following series of figures. Visual inspection of figures 69a and b reveals a strongly seasonal pattern for the main population characteristics - population size, proportion of males and proportion of immatures in the population. The

period of initial stabilization for these variables during this simulation run appears to be about 2 to 3 years, so results should only be evaluated after this stage of the run.

The average seasonal pattern of the same population parameters was examined in figures 69c to e. The data from the longitudinal study for both the Jolly-Seber and the Jackknife estimator shows a peak for population size during the late autumn months and a minimum in late winter. A similar effect was produced by the simulation model, although with slightly lower mean population size and marginally different temporal pattern than were seen in the field study. It was decided that this small difference was within the operational tolerance of the model, and moreover would be consistent with the differences expected due to tuberculosis infection in the population. It also has to be taken into account that estimates based on field data will be subject to an "edge effect". This means that they are likely to include animals which mainly live outside the study area but were occasionally captured in the area and therefore were included in the population size estimation. The proportion of females in the total population produced by the simulation model was similar to what was suggested by the field data. Most of the time the actual field values were within one standard deviation of the average value for the simulation output. Capture-mark recapture studies suffer from the problem of unequal trappability, and it appeared that male possums were easier to capture than females, especially while females were carrying pouch young. Overall, this variable was considered to give close agreement with field data. The proportion of immature possums in the study population was comparable to data produced by the simulation model following the same seasonal pattern. The field methods for classification according to sexual maturity status are not precise, especially in the case of male possums.

Figure 69f shows the temporal pattern for proportion of adult females with dependent young, for both the model and the longitudinal study. This shows the greatest discrepancy of all the variables, but because of the nature of the field measurement process, they are not measuring the same items. In the model a joey is classified as a dependent young from the time that the model records a conception through birth approximately 17 days later, until the date when the joey is reclassified as independent (determined by sampling from a normal distribution). Throughout the growth process there is a monthly probability of death of the joey, which also modifies the numbers. In contrast, the field data records a joey from when it is first seen in its mother's pouch after birth until it is no longer recorded with the mother, and head length is used to calculate back to an estimated birth date, which is used to define commencement of the rearing period. In comparing model predictions with field data, it has to be taken into consideration that the method of collecting data about the reproductive state of breeding females in the field study was subject to inherent bias. Female possums with pouch young appeared to change their trap response, so that they became more difficult to catch while they had a pouch young. This compounded the second problem, that because possums were never examined at intervals less than two months, information about the reproductive state of individuals was unavoidably patchy. Moreover, many dependent young possums

approaching independence were not necessarily captured with their mothers in the same trap, and hence their date of independence and fate were uncertain. Thirdly, the model has been enhanced beyond a simple representation of conception as a monthly probability to make mating success depend on social interaction, with each breeding female having a probability of 0.2 or less of conceiving at each mating. If conception does not occur, then the female continues her search for males and hence her likelihood of achieving an infectious contact with a tuberculous male. This method of representation increases the realism of the model in representing tuberculosis transmission, but since no direct measurements have been made of mating success the parameters used in this part of the model must be regarded as very provisional.

It is clear that the reproductive aspects of the model are probably the most difficult to build correctly so that they represent the tuberculosis transmission possibilities of male-female interactions as well as the breeding process itself. This module will need considerable critical evaluation and further refinement before the full model is ready for use in policy assessment. However development has been completed to the point where the social interaction process has been incorporated in a biologically rational way to represent both breeding success and tuberculosis transmission success. Further development of this part of the model will require additional field data and a careful assessment of the implications of different parameter settings for model behaviour. Given the evidence accumulating from the study that breeding behaviour is the fundamental driving force in tuberculosis transmission, such an investment of further development effort would be very worthwhile.

It is important that denning behaviour be realistically represented in the model, because of the direct or more likely indirect influence which it has on tuberculosis transmission. On average about 30% of possums used at least 14 different den sites per month (see figure 69g). The number of possums using at least 15 den sites per month peaked at the time of maximum possum population, demonstrating that the modelled search process for a den was accurately reflecting the degree of competition at different times of the year (see figure 69h). For the population density-dependent mortality/survival mechanism the number of days spent by possums without finding a suitable den site was an important factor. On average 80% of possums found a suitable den site every night during a 30 day period (see figure 69i). There was a seasonal pattern for the number of nights without suitable den site, which basically followed the pattern of population size (see figure 69j).

Table 26a: Summary statistics of population parameters for simulation run using *base* parameter files

	Mean	95%C.L.	Median	Mode	S.D.	Variance	Kurtosis	Skewness	Minimum	Maximum	Count
ORIGINAL RUN											
POPULATION SIZE	123.5	3.47	117	95	32.08	1029.1	-0.06	0.77	69	220	328
FEMALES/TOTAL POP	0.50	0.01	0.50	0.50	0.05	0.00	-0.59	-0.17	0.36	0.60	328
IMMATURE/TOTAL	0.22	0.01	0.22	0.19	0.06	0.00	0.17	0.20	0.05	0.41	328
ANTITHETIC RUN											
POPULATION SIZE	131.9	2.82	130	122	26.06	679.2	-0.15	0.42	78	217	328
FEMALES/TOTAL POP	0.47	0.00	0.47	0.48	0.04	0.00	0.67	0.34	0.35	0.60	328
IMMATURE/TOTAL	0.20	0.01	0.20	0.21	0.05	0.00	0.07	-0.16	0.02	0.35	328

Table 26b: Summary statistics of population parameters by simulation run and type of year using *base* parameter files

Run	Type of Year	Population Size			Proportion Females in Population			Proportion Immatures in Population		
		Mean	N	S.D.	Mean	N	S.D.	Mean	N	S.D.
original	g	135.6	96	32.5	0.511	96	0.046	0.233	96	0.079
original	a	128.1	120	32.8	0.500	120	0.0459	0.213	120	0.051
original	b	108.1	112	24.1	0.477	112	0.0482	0.210	112	0.052
antithetic	g	130.6	96	25.7	0.471	96	0.038	0.210	96	0.067
antithetic	a	136.9	120	24.4	0.471	120	0.033	0.208	120	0.046
antithetic	b	127.6	112	27.2	0.465	112	0.042	0.186	112	0.048
All		127.7	656	29.5	0.482	656	0.045	0.209	656	0.059

(g= good, a=average, b=bad)

Figure 69a: Time series plot of monthly data for population size, proportion of females and immature animals in the population for the *original* simulation run using *base* parameter files

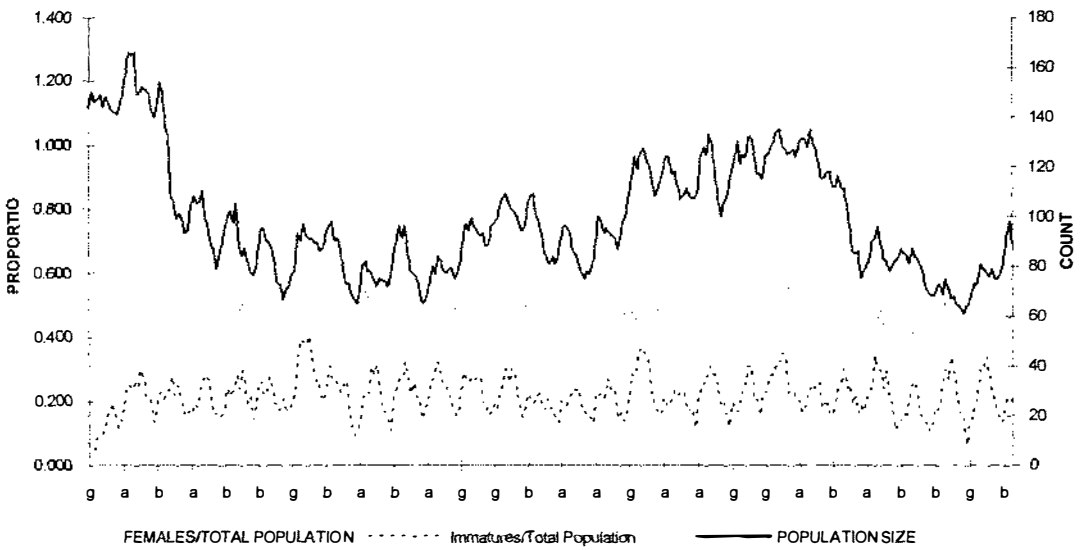


Figure 69b: Time series plot of monthly data for population size, proportion of females and immature animals in the population for the *antithetic* simulation run using *base* parameter files

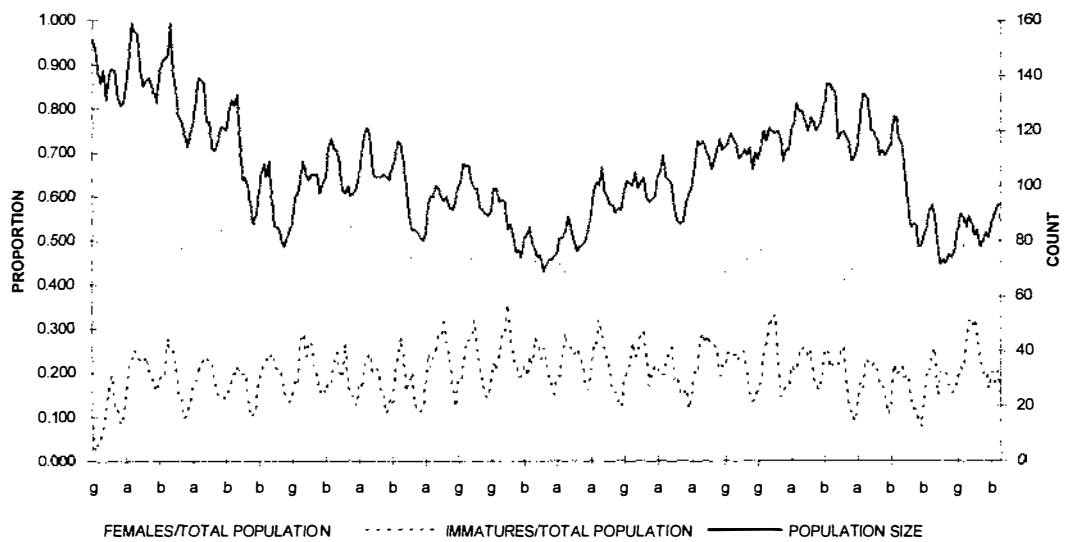
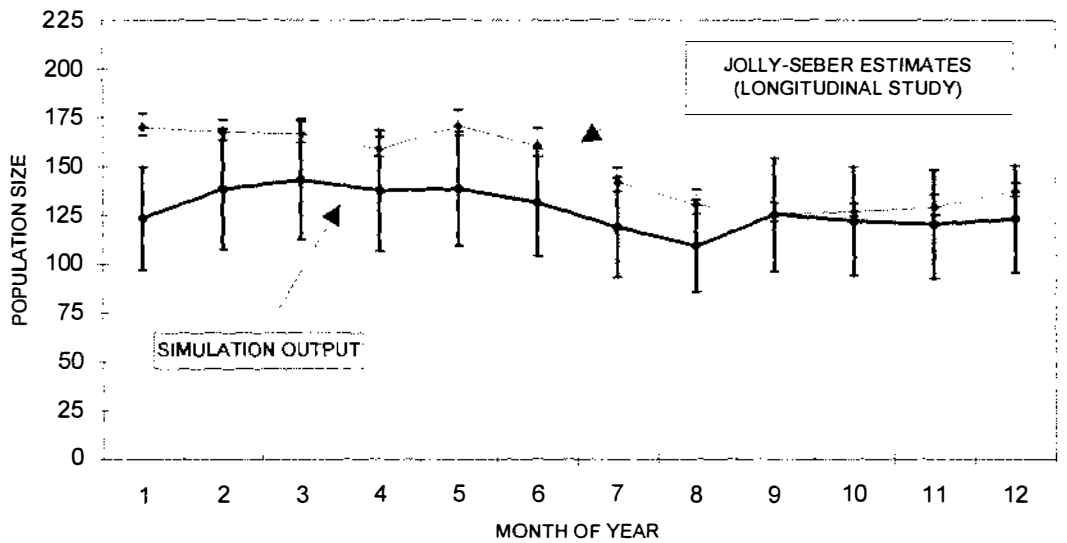
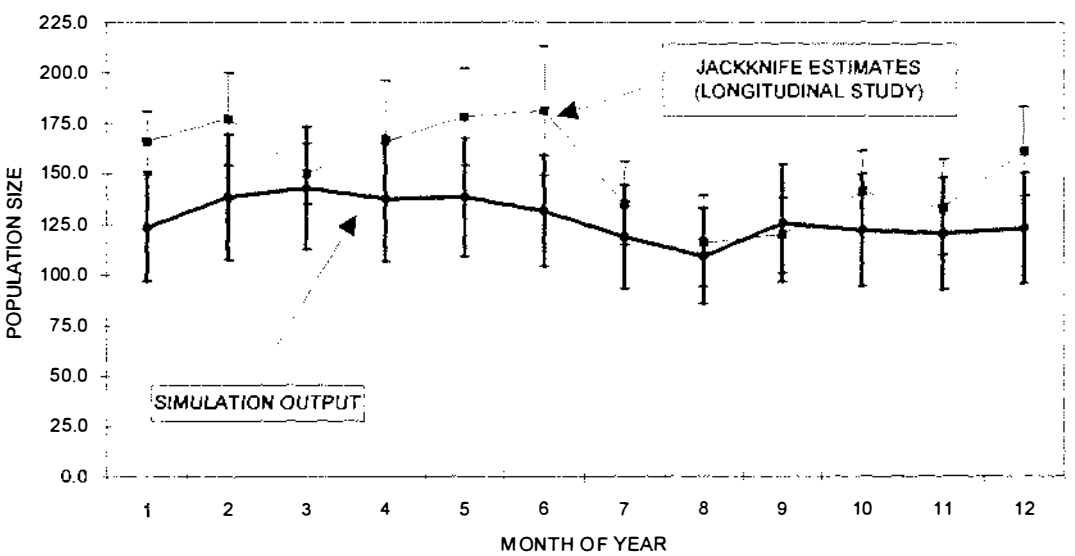


Figure 69c: Graphical comparison of average population size (incl. standard deviation bars) during the course of a year between output from the simulation model for *base* parameter files and data obtained from the longitudinal study



Population size based on Jolly-Seber estimates



Population size based on Jackknife estimates

Figure 69d: Graphical comparison of average proportion females in total population (incl. standard deviation bars) during the course of a year between output from the simulation model using *base* parameter files and data obtained from the longitudinal study

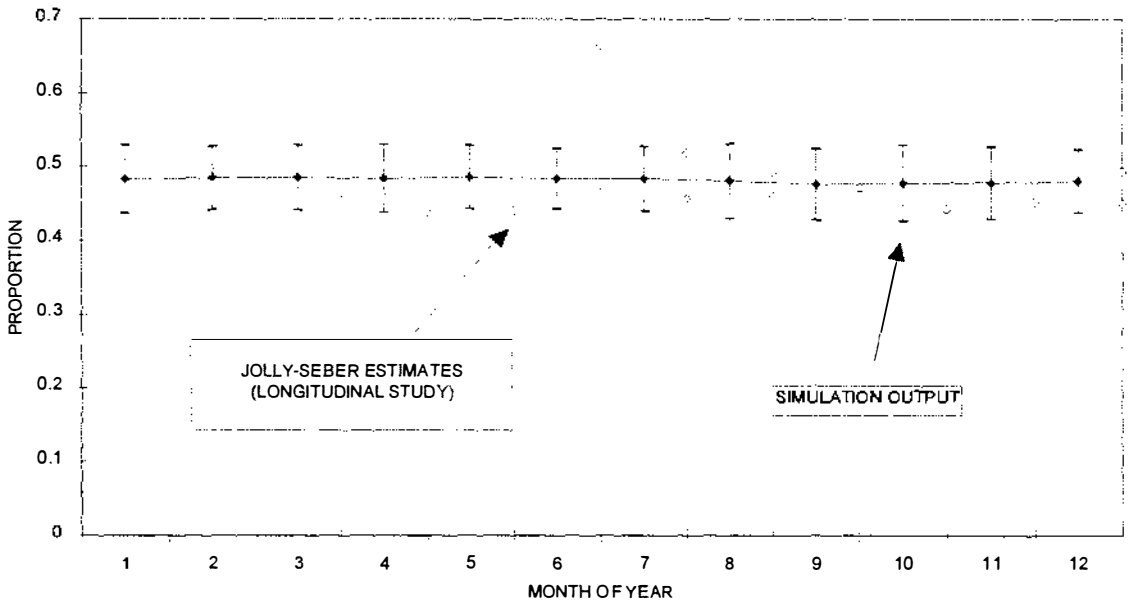


Figure 69e: Graphical comparison of average proportion immatures in total population (incl. standard deviation bars) during the course of a year between output from the simulation model using *base* parameter files and data obtained from the longitudinal study

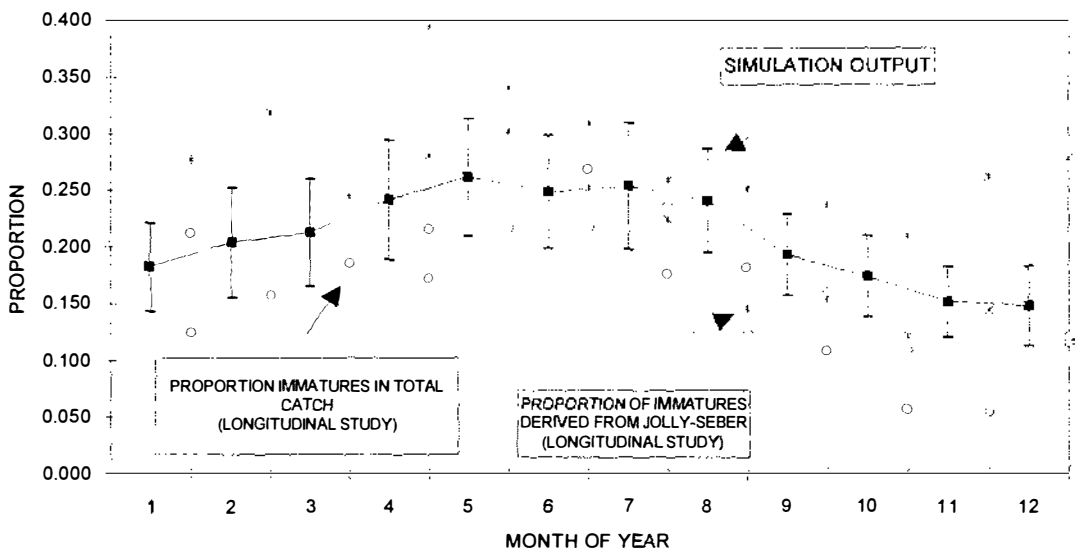


Figure 69f: Proportion of adult female possums with a dependent young present for each month of the calendar year based on summarized simulation output (*base* parameter files; incl. standard deviation bars) and data from the longitudinal study

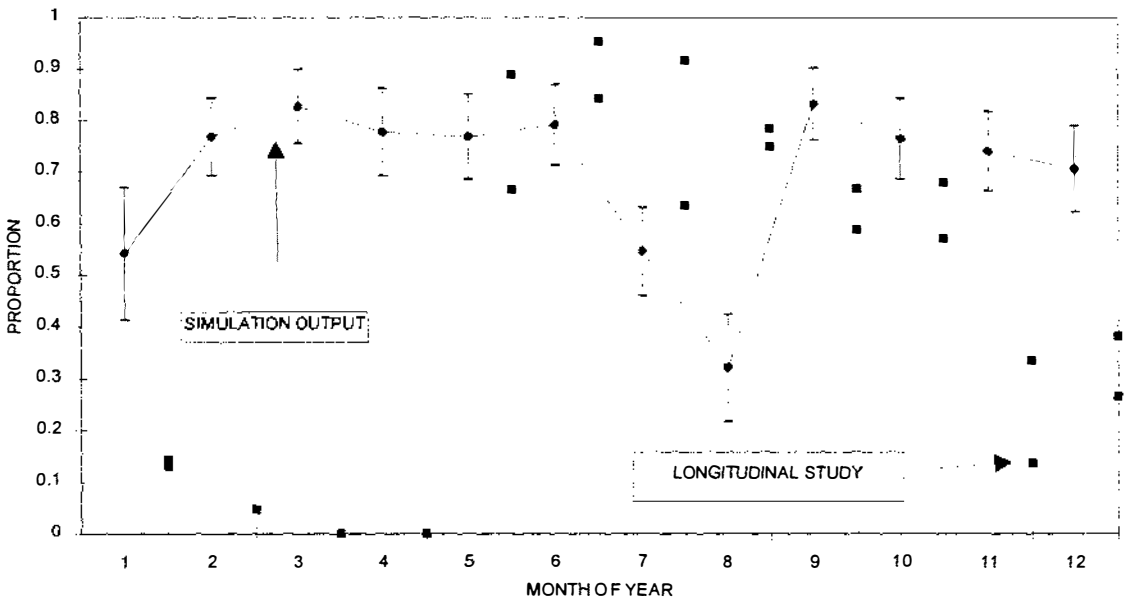


Figure 69g: Average and cumulative number of possums represented as bars using different number of den sites during the period of a month summarized over the whole simulation period (*base* parameter files)

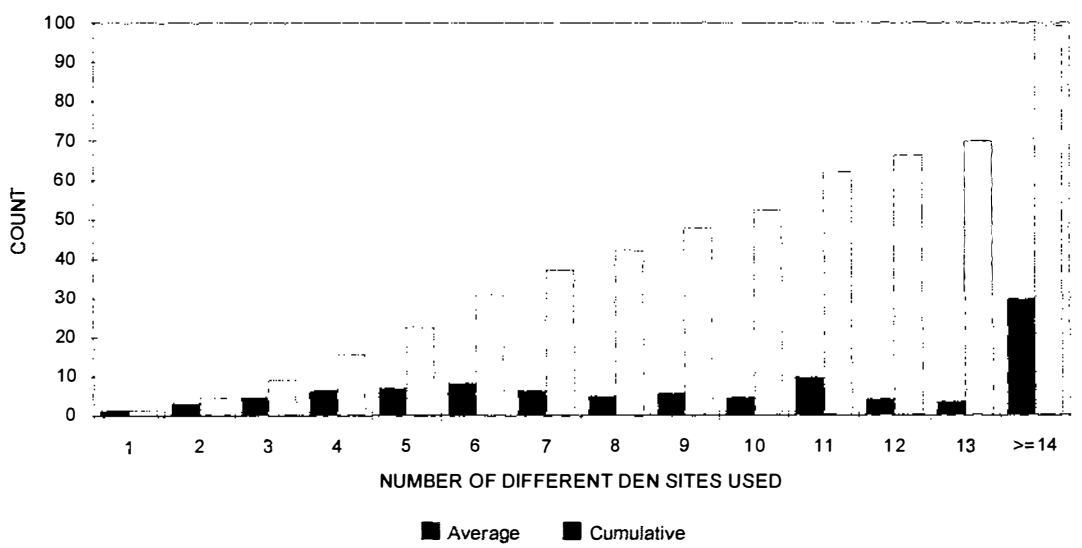


Figure 69h: Average number of possums (y-axis) using different numbers of den sites categorized into three groups (shaded areas) during the months of a year summarized over the whole simulation period (*base* parameter files)

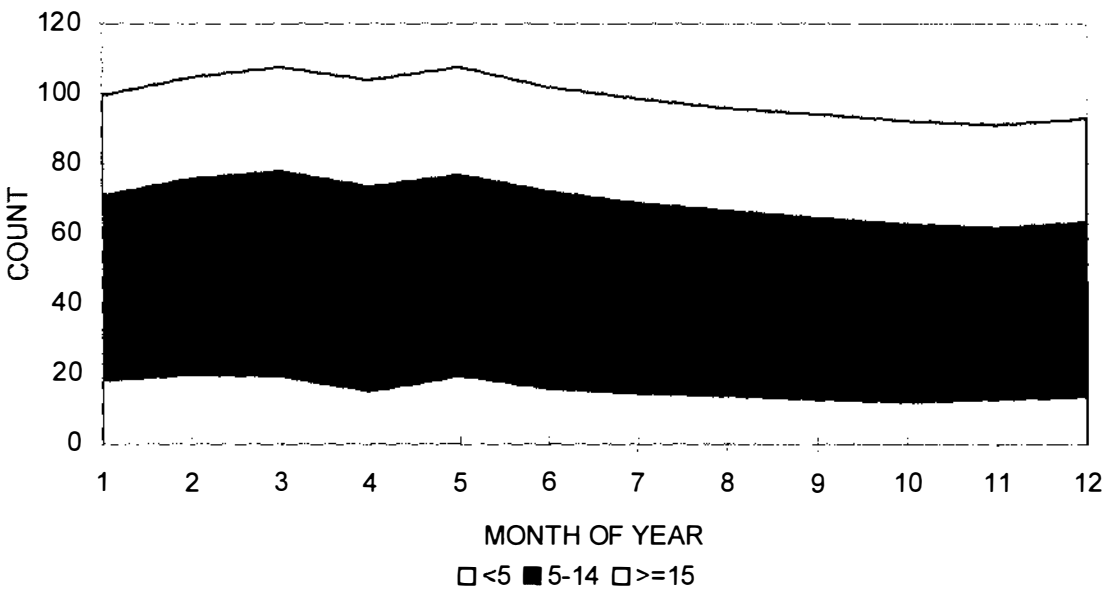


Figure 69i: Average number of possums (as vertical bars) spending a given number of days per month without finding a suitable den site summarized over the whole simulation period (*base* parameter files)

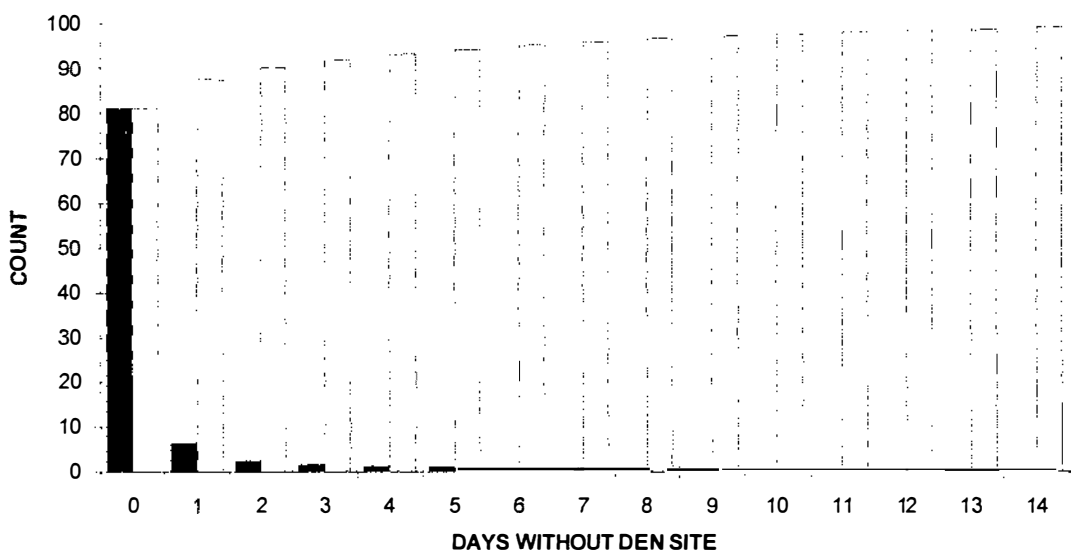
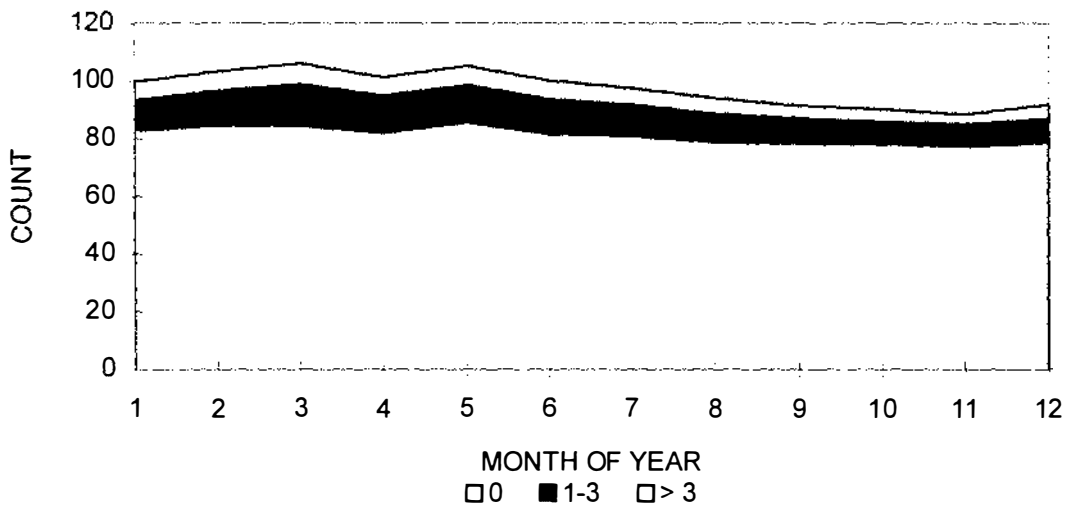


Figure 69j: Average number of days (y-axis) spent by possums without finding a suitable den site over the course of a year summarized over the whole simulation period (*base* parameter files)



Tuberculosis Infection Dynamics

A graphical comparison between computer simulation output using base parameter settings and data obtained from the longitudinal study was conducted for monthly incidence of clinical tuberculosis and monthly prevalence of clinical tuberculosis for the whole population and for immature, mature, male and female possums separately. Figures 70a and b present the temporal pattern of population size, prevalence and incidence of clinical tuberculosis during the *original* and *antithetic* simulation run. During the *original* simulation run clinical tuberculosis is prevalent at varying levels during the whole simulation period. Cases with clinical tuberculosis are incident every year, but not during every month. The output from the *antithetic* run is quite different as clinical tuberculosis prevalence, averaging around 1%, is quite low during the first 20 months of the simulation, but increases significantly up to about 11% during the last 8 months of the simulation.

Figure 70a: Time series plot of monthly data for population size, prevalence and incidence of clinical tuberculosis in the population for a simulation run using base parameter files based on simulation output from *original* run

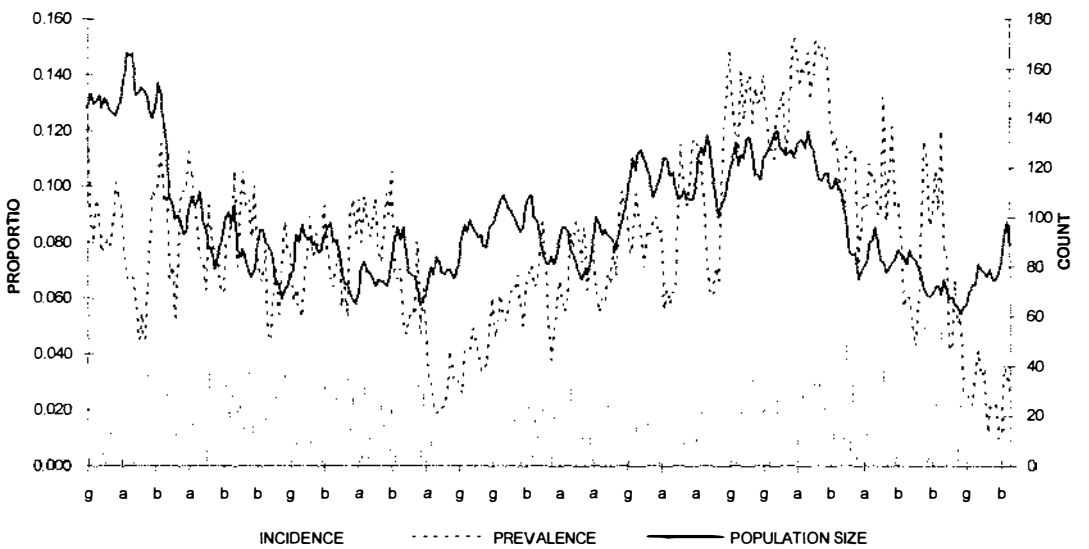
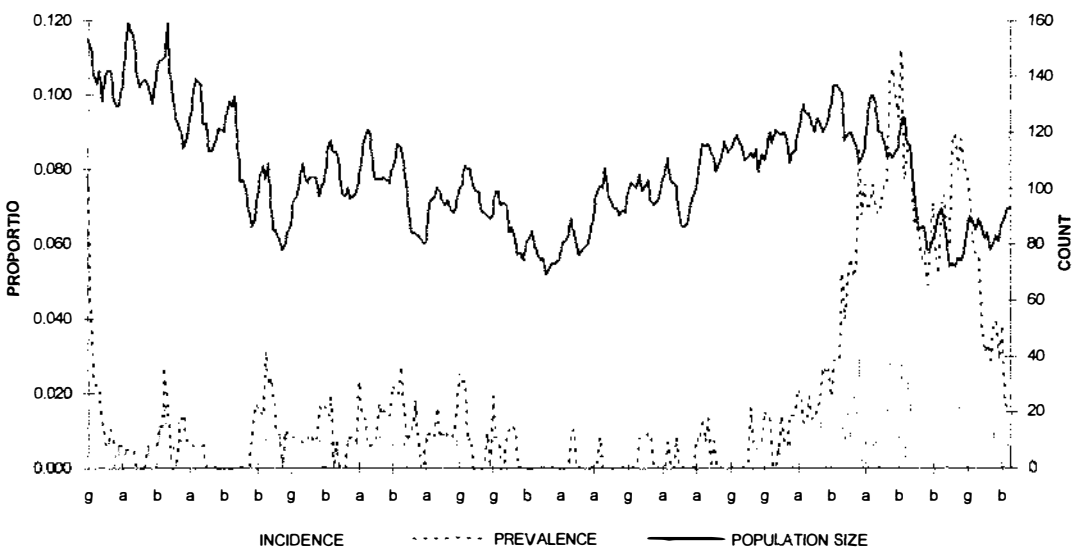


Figure 70b: Time series plot of monthly data for population size, prevalence and incidence of clinical tuberculosis in the population for a simulation run using base parameter files based on simulation output from *antithetic* run



Seasonal Pattern of Tuberculosis Infection

A comparison of the seasonal pattern of tuberculosis infection between data obtained from the longitudinal field study and monthly average incidence and prevalence figures of simulation output was done graphically as presented in figures 71a to f. Average monthly clinical tuberculosis prevalence and incidence based on the simulation runs broadly followed a similar seasonal pattern to that observed in the field study, bearing in mind that the field data covers two years and the simulated data is a mean for 28 years. Clinical prevalence was lowest during the autumn and winter while reaching peaks in spring-summer. Clinical incidence peaked during the spring months. The agreement between simulation output and field data is not as close when sub-grouped results for sex and age classes are considered separately, although the number of animals from which the field observations are derived is so small in many cases that no judgment can yet be made on the fit of the model to field data. In immature possums the model predicts similar prevalence as the field data so far shows. For mature possums and for both male and female possums combined across age groups, the model produces similar seasonal patterns but higher figures than were observed in the field study. Figure 71g presents the average temporal calendar year pattern of the proportion of dependent young possums which are infected with *Mycobacterium bovis.* This could not be measured in the field, and in the model it reflects the breeding success in adult female possums. Table 27 shows the summary statistics for each of the three different types of years and the two simulation runs. During the *original* run representing a scenario with endemic tuberculosis infection the prevalence of subclinical tuberculosis and clinical infection average around 20% and 8% respectively.

Figure 71a: Average monthly clinical tuberculosis prevalence (including standard deviation bars) over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

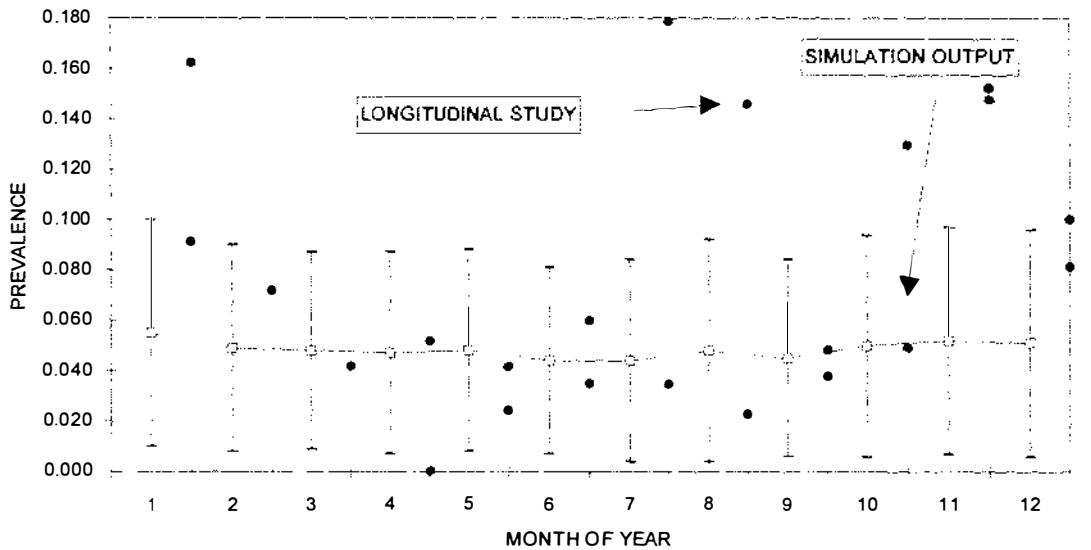


Figure 71b: Average monthly clinical tuberculosis incidence (including standard deviation bars) over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

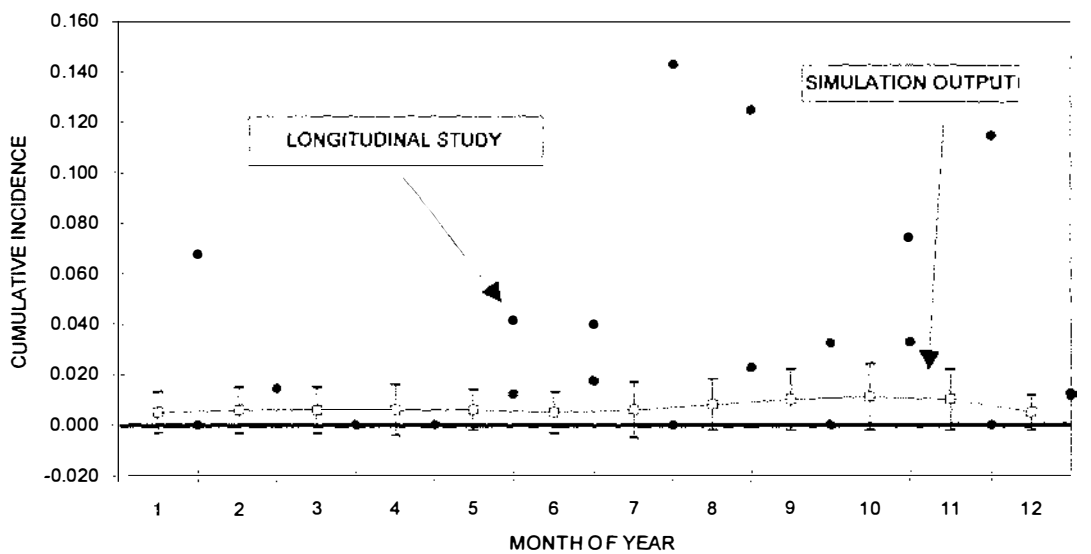


Figure 71c: Average monthly clinical tuberculosis prevalence (including standard deviation bars) in immature possums over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

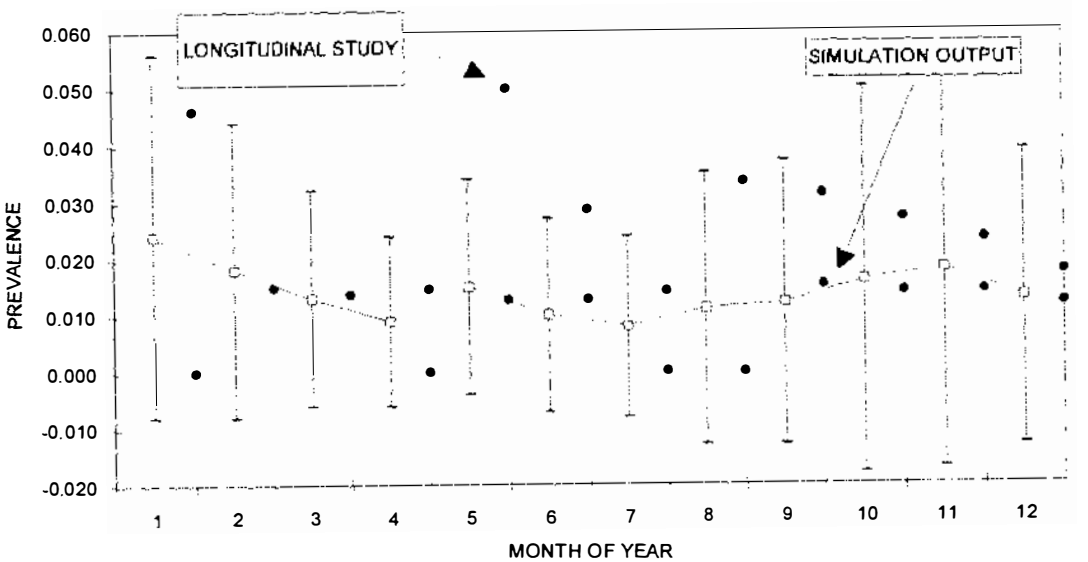


Figure 71d: Average monthly clinical tuberculosis prevalence (including standard deviation bars) in mature possums over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

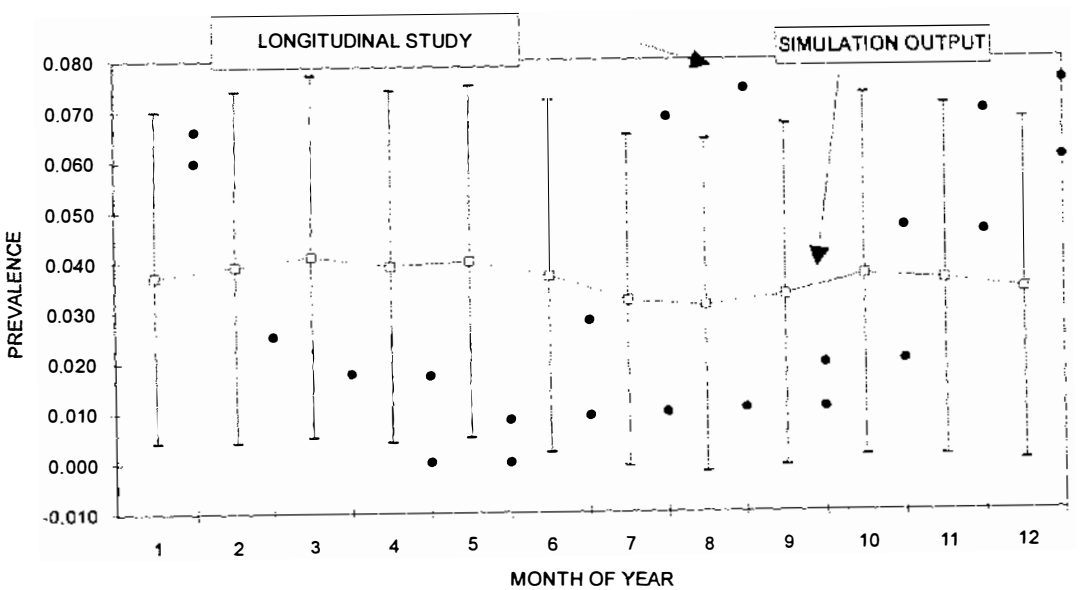


Figure 71e: Average monthly clinical tuberculosis prevalence (including standard deviation bars) in male possums over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

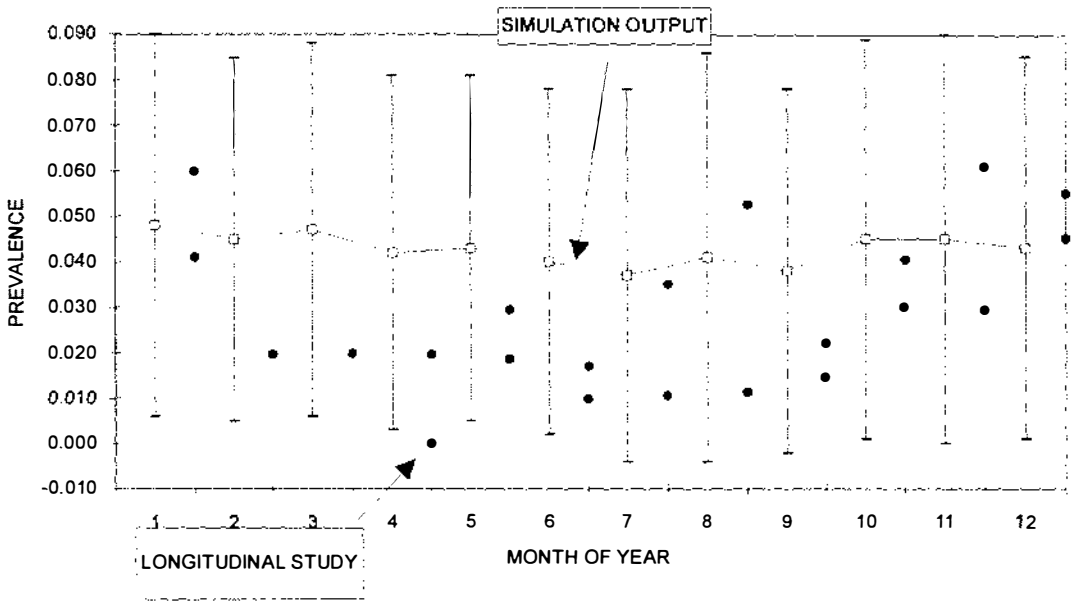


Figure 71f: Average monthly clinical tuberculosis prevalence (including standard deviation bars) in female possums over the course of a year for simulation output and data points obtained during the longitudinal study (*base* parameter files)

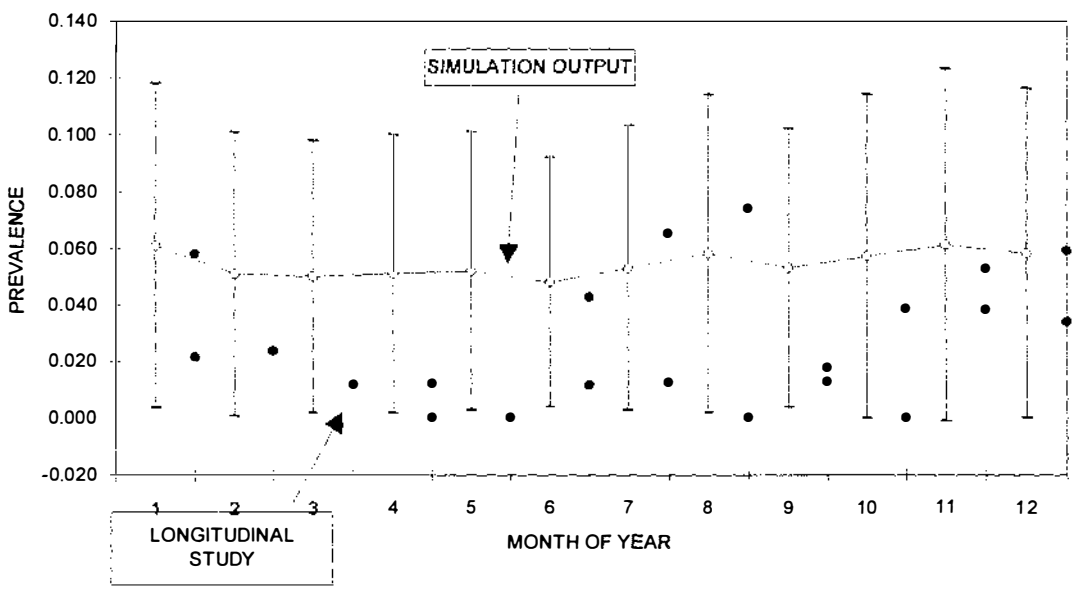


Figure 71g: Average monthly tuberculosis infection prevalence (including standard deviation bars) in dependent young possums over the course of a year for simulation output (*base* parameter files)

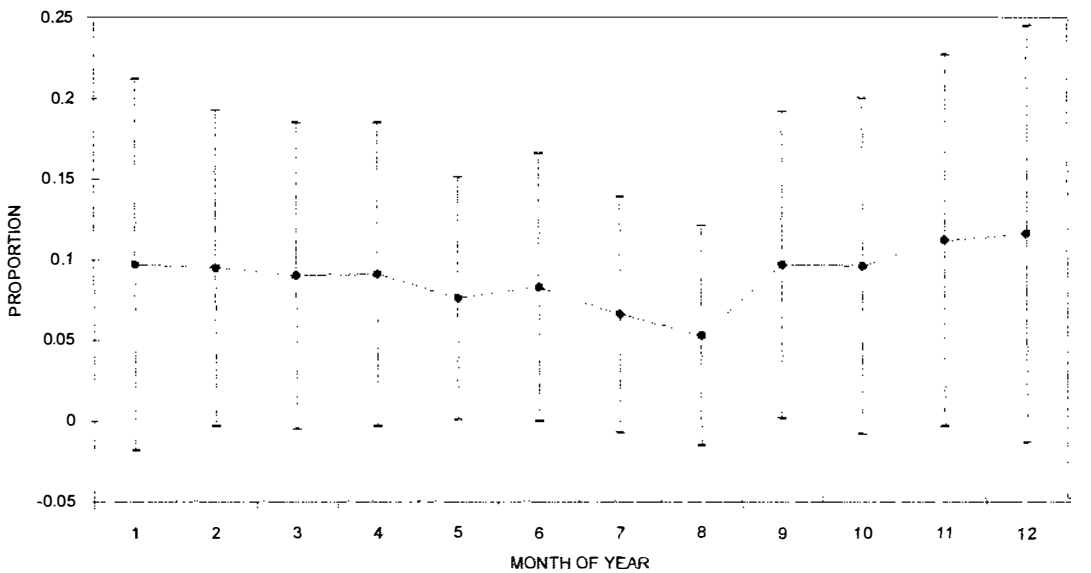


Table 27: Summary statistics of tuberculosis infection dynamics by simulation run and type of year using *base* parameter files

		SUBCLINICAL TB INCIDENCE		CLINICAL TB INCIDENCE		SUBCLINICAL TB PREVALENCE		CLINICAL TB PREVALENCE		
Run	Type of Year	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	N
original	g	0.033	0.027	0.007	0.008	0.195	0.092	0.078	0.037	96
original	a	0.035	0.025	0.011	0.011	0.223	0.067	0.083	0.031	120
original	b	0.028	0.021	0.015	0.013	0.192	0.059	0.075	0.023	112
antithetic	g	0.004	0.006	0.001	0.002	0.027	0.034	0.013	0.017	96
antithetic	a	0.006	0.014	0.002	0.005	0.040	0.059	0.014	0.024	120
antithetic	b	0.011	0.016	0.006	0.008	0.060	0.055	0.026	0.029	112
All		0.020	0.023	0.007	0.010	0.124	0.103	0.048	0.041	656

(g=good, a=average, b=bad)

Survival Analysis of Simulation Output

One of the valuable benefits expected to accrue from later use of the model is expected to be the capacity to predict total numbers of infected possums (both subclinical and clinical) under various circumstances, and to test hypotheses about the transition from subclinical to clinical diesease, as a guide to which transition pattern best explains the cumulative field data. At this stage comprehensive analyses of this kind are premature, but it was considered desirable to begin developing methods for analyzing time patterns for the transitions from uninfected to subclinical and subclinical to clinical states. Survival analysis provides a way of doing this

which does not suffer some of the biases of alternative methods, especially for comparing field and simulated data.

However standard methods of survival analysis require the assumption that all animals enter the study at the same time, clearly an assumption which cannot be strictly satisfied by either the field or the simulated data in this case. However extensions of the technique to deal with this difficulty are now becoming available, and in the following analysis Pollock *et al*'s (1989) extension of the Kaplan-Meier product limit estimator to allow staggered entry of animals was used to estimate the survival function for possums with subclinical and clinical tuberculosis (see figure 72a). In the current version of the model, no summaries were produced from the simulation runs which would have allowed to directly quantify mortality and births in the population. Therefore, it was not possible at this stage of model development to estimate a survival curve for possums free from tuberculosis infection. The combination of total possum numbers in the relevant population subgroups and number of incident and prevalent cases with subclinical and clinical tuberculosis was used to estimate the required figures for a survival analysis dealing only with diseased possums. Possums which disappeared from the population were treated as equivalent to deaths.

A worksheet for carrying out staggered entry survival analysis was obtained from Pollock and his co-workers, and used to perform the required calculations. In the analysis of survival of sub-clinically infected possums, transition to clinical disease status was considered a censoring event. Thus death or emigration alone was the outcome of interest. Median survival time to death for possums with subclinical and clinical tuberculosis was about 11 and 7 months respectively. The shape of the two survival curves was similar, reflecting the fact that tuberculosis was not the main cause of death in the population. At this stage there is likely to be substantial confounding in the shape of the curves, because subclinical animals would be younger than clinical cases, and would have quite a different mix of risk factors causing death in the model, quite apart from tuberculosis. However once the model is able to represent survival patterns of different cohorts and compare tuberculous and non-tuberculous animals within otherwise comparable cohorts, this mode of presentation should prove useful. Figure 27b shows the survival curve for onset of clinical disease as the outcome event. Median survival time is about 11-12 months which means that 50% of infected possums develop clinical disease within about a year from when they became infected. This suggests that tuberculosis has a long rather than a short time distribution, taking the assumptions currently built into the model.

At this stage the results are presented largely as an illustration of the potential of the technique. More detailed interpretation of the shapes of the curves will have to await more detailed definition of cohorts for analysis purposes.

Figure 72a: Survival of possums with subclinical and clinical tuberculosis (*base* parameter files)

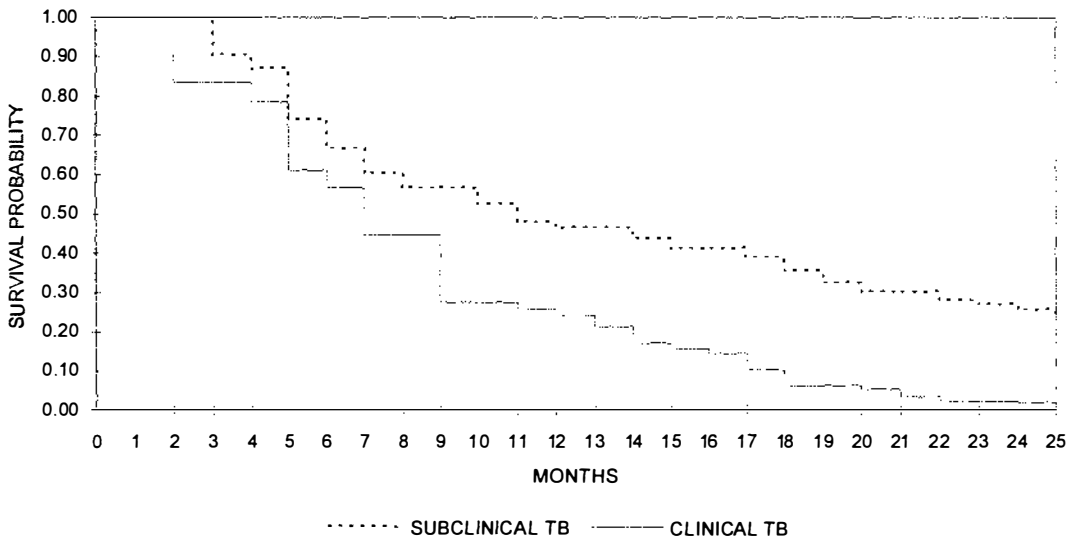
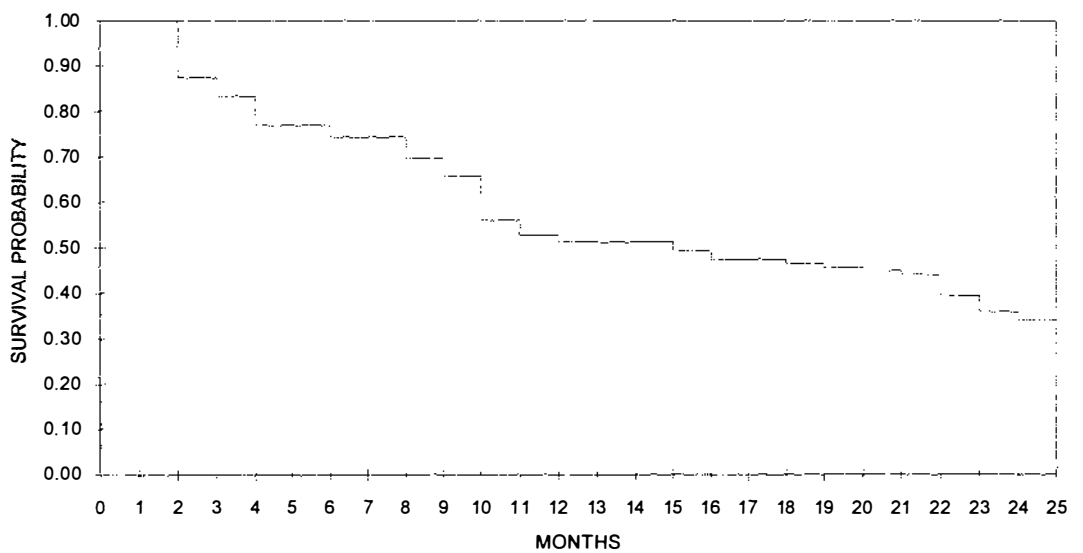


Figure 72b: Transition from subclinical to clinical tuberculosis (*base* parameter files)



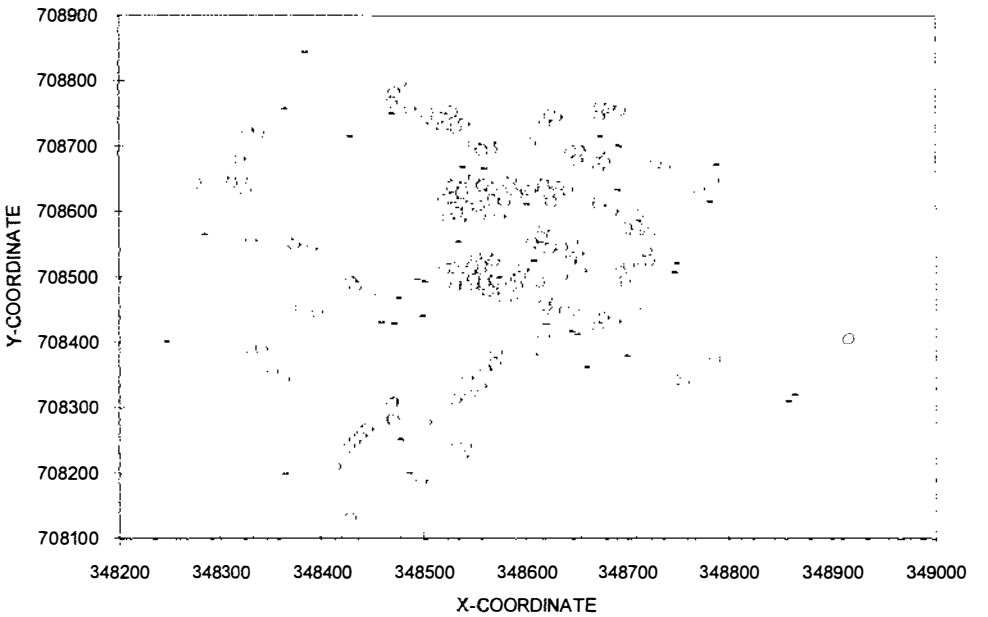
Spatio-Temporal Patterns of Tuberculosis Infection

Different aspects of the spatial distribution of den sites which had been occupied by possums considered in the model to be excreting *Mycobacterium bovis* (hereafter TB den sites) were analysed, much as this had been done for possums in the longitudinal study. This is used as a proxy variable for both direct den transmission and related mechanisms of transmission not

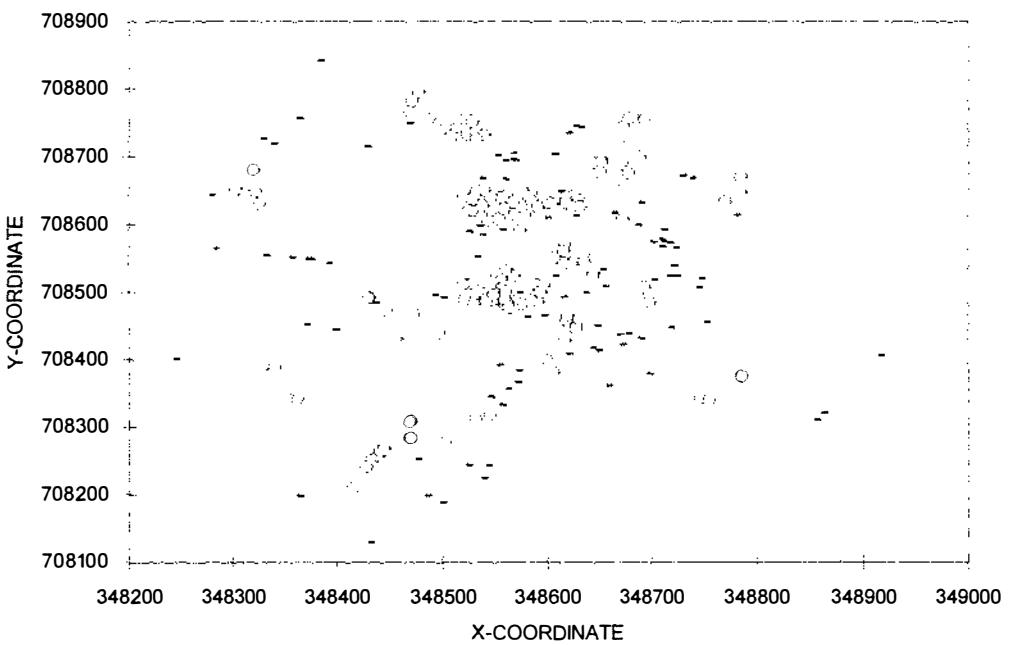
considered separately. The results of this preliminary analysis of the spatial dynamics of den site locations used by clinically tuberculous possums at this stage of model development should be seen as a reflection of the infection mechanisms built into the model. The following findings should be interpreted mainly as a means for understanding how the model handles these mechanisms which are responsible for the geographical component of the model.

A map of the cumulative spatial distribution of TB den sites is shown in figure 73a separately for the *original* and the *antithetic* run (circles represent TB den sites and dashes non-TB den sites). Using cumulative data for the whole simulation period of 28 years TB den sites appear to be spatially clustered. Figure 73b shows interpolated images of the cumulative frequency pattern of TB den sites as the third dimension over a map of all den site locations for the *original* and the *antithetic* run. Figure 73c presents a three dimensional image with the third dimension representing overall den site density per 20 m^2. Taking into account the frequency of use of particular den sites by possums with clinical tuberculosis a high concentration of such events is seen in one of the two areas with the highest den site density, but not in the other in both simulation runs. A time series plot describes the temporal pattern of the number of TB den sites compared with prevalence and incidence of clinical tuberculosis (see figure 73d). The number of TB den sites follows the temporal pattern of prevalence of clinical tuberculosis closely. Figures 73d and e suggest that during periods with higher prevalence the total number of individual TB den sites does not increase significantly, but the same dens are used more frequently by possums with clinical tuberculosis.

Figure 73a: Cumulative spatial distribution of den sites used by possums with clinical tuberculosis infection during the simulation run (*base* parameter files; *original* and *antithetic* run) overlaid on map of total den site locations (circles represent TB den sites and dashes non-TB den sites)

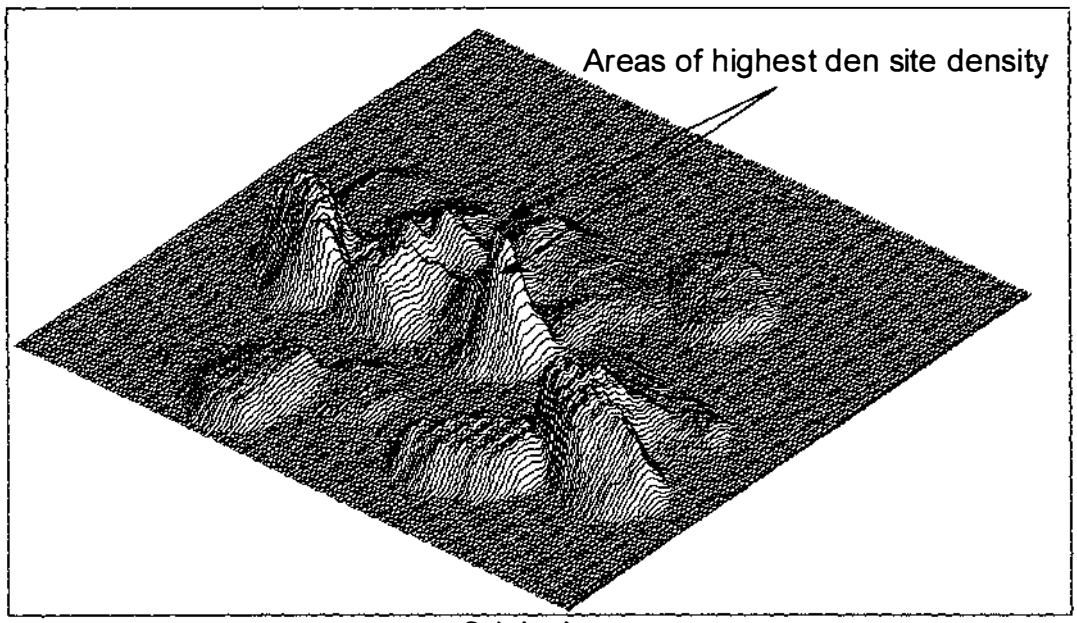


Data generated during *original run*

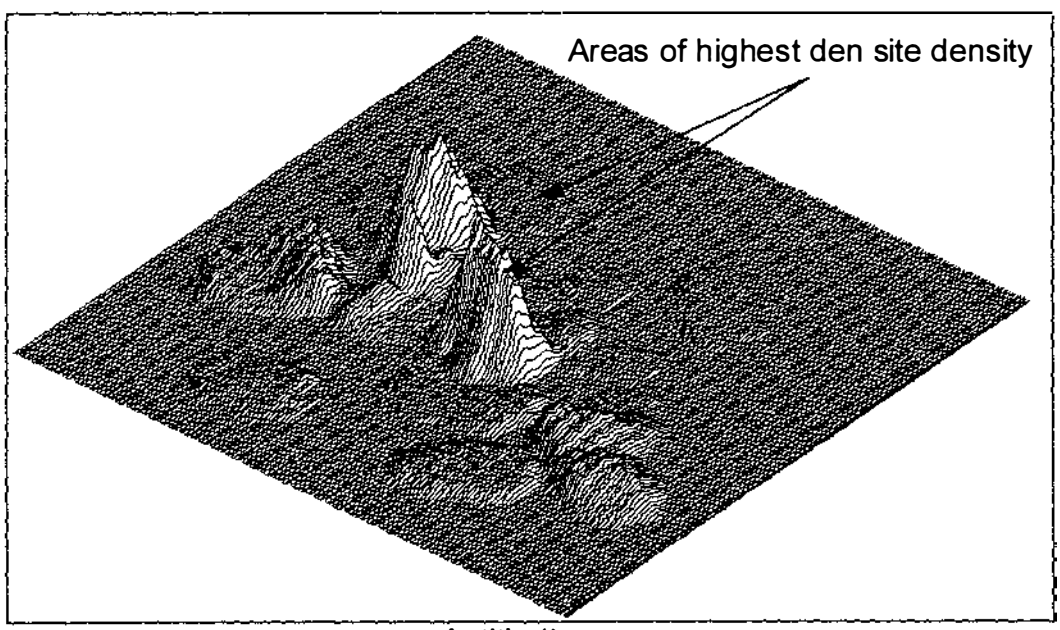


Data generated during *antithetic* run

Figure 73b: Cumulative spatial distribution of den sites used by possums with clinical tuberculosis infection during the two simulation runs (*original* and *antithetic* run) with height representing cumulative distribution of TB den sites

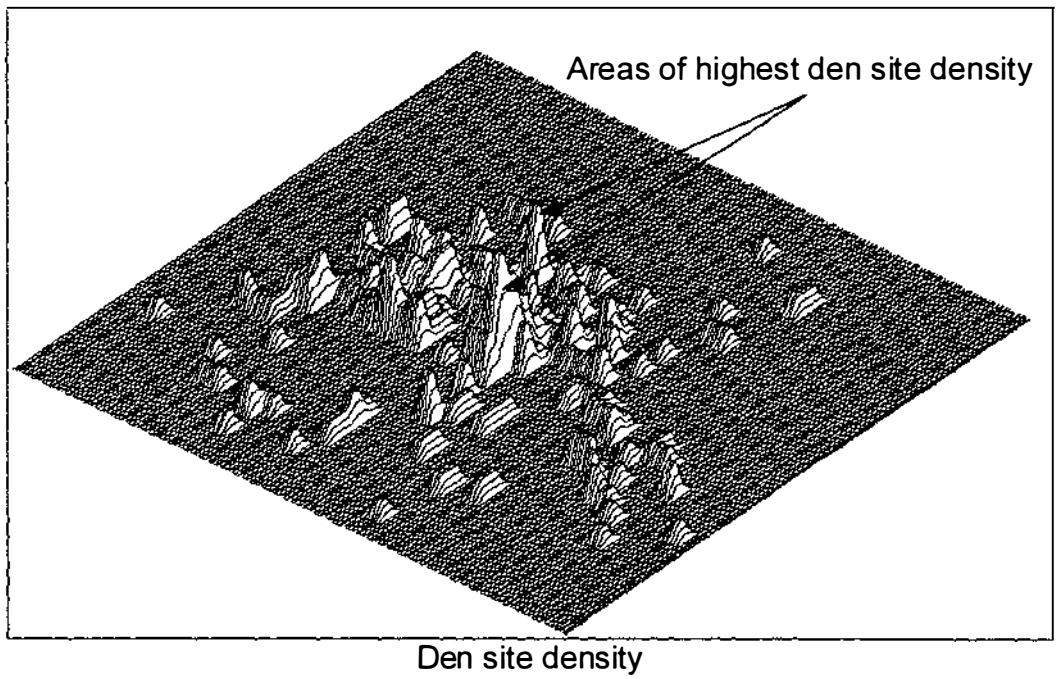


Original run



Antithetic run

Figure 73c: Density of all mapped den sites per 20m^2 used for simulation runs



Detailed Spatio-Temporal Patterns of Tuberculosis Infection

Figures 74a to f describe the spatio-temporal pattern of clinical tuberculosis infection during a simulation run using *standard* simulation conditions. Each of the 6 figures consists of a time series plot of population size, prevalence and incidence of clinical tuberculosis covering a period of 5 years and a series of 5 maps representing the locations of den sites which had been used by possum with clinical tuberculosis during a particular year.

During year 1 infection appeared to be randomly distributed within the area. This has to be expected because during program initialization den sites are randomly selected for each possum. Over the following years a relatively stable cluster of infection develops in one particular part of the area (centered around x-coordinate 348550 and y-coordinate 708500). This cluster focuses on one of the two areas with a particularly high density of den sites, and persists there in the long term despite the fact that the model does not incorporate any features which would inherently force this pattern on the distribution of disease. The existence of persistent geographical clustering at some sites is an emergent property of the disease process, given the epidemiological assumptions designed into the model on the basis of field findings. Another relatively stable cluster of infection developed around x-coordinate 348500 and y-coordinate 708750. This is an area of relatively low den site density. It is of note that various other clusters develop in other parts of the modelled surface over the course of the run, but disappear or evolve into new forms after varying time periods. These patterns are consistent with the evidence so far emerging from the field research. The higher resolution of the time series shows the expected positive correlation between incidence and prevalence of clinical tuberculosis. However visually, there does not appear to be any clear link between incidence

and prevalence of tuberculosis on one hand, and total possum population size on the other. Again, these model properties are consistent with field findings.

Figure 74a: Spatio-temporal pattern of clinical tuberculosis during year 1 to 5 of a simulation run (*original* run)

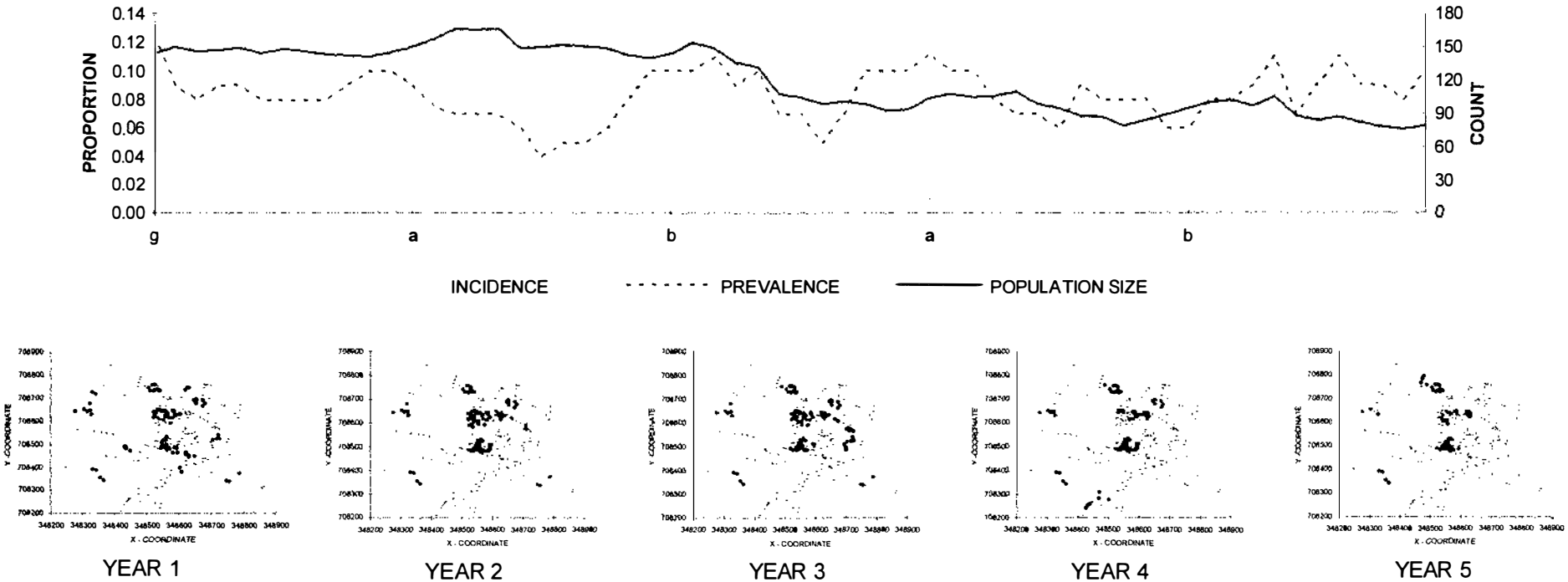


Figure 74b: Spatio-temporal pattern of clinical tuberculosis for years 6 to 10 of a simulation (*original* run)

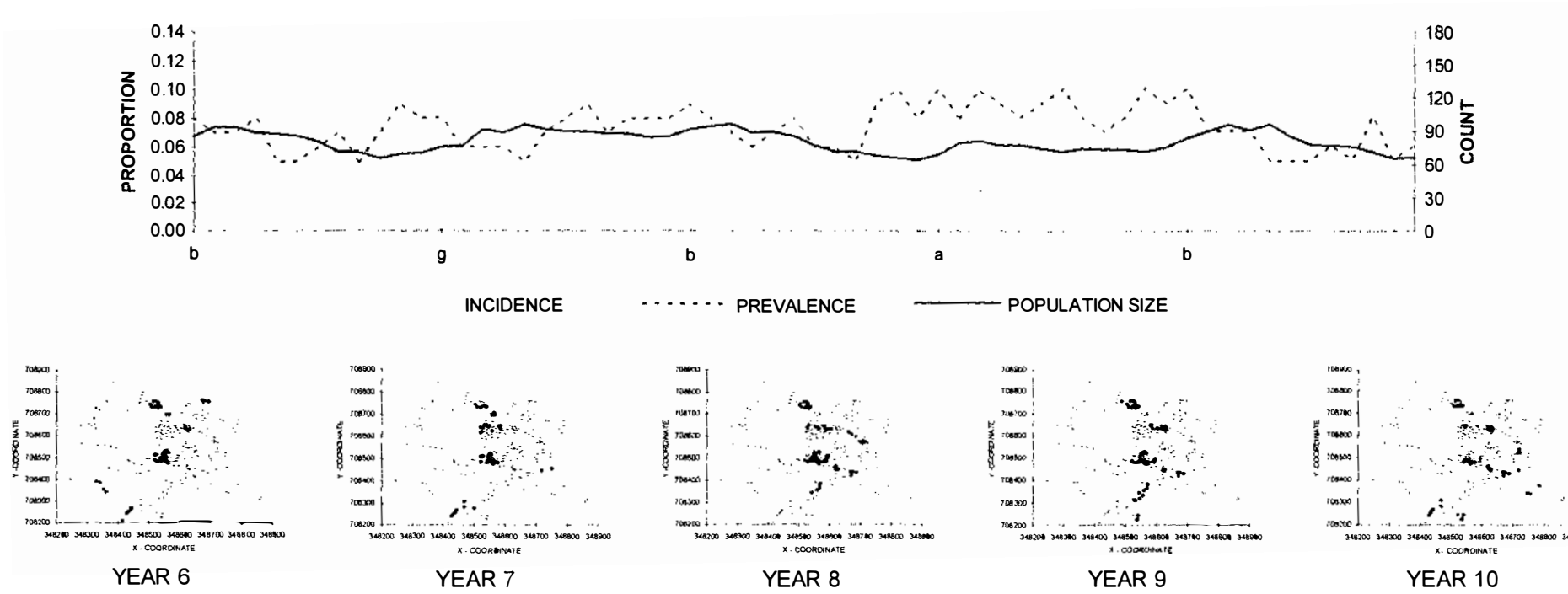


Figure 74d: Spatio-temporal pattern of clinical tuberculosis for years 11 to 15 of a simulation (o*riginal* run)

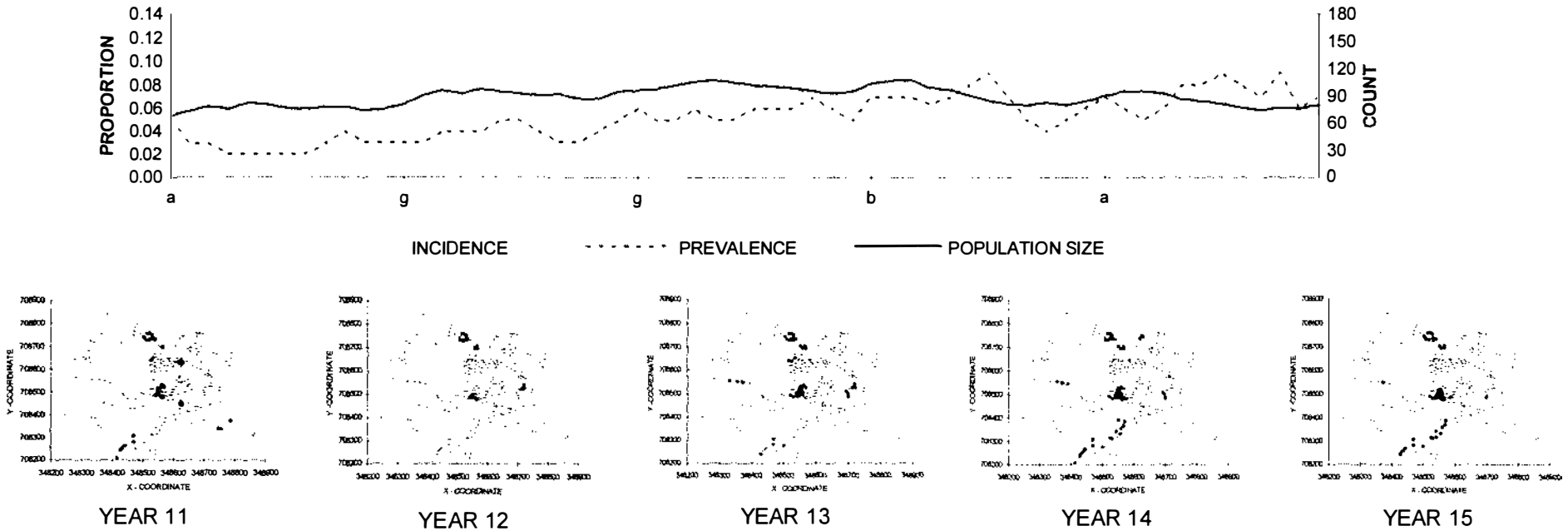
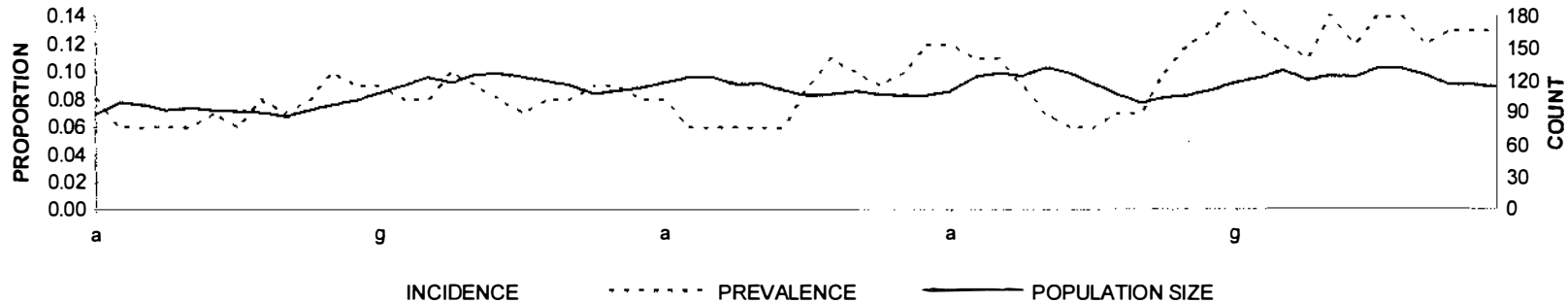
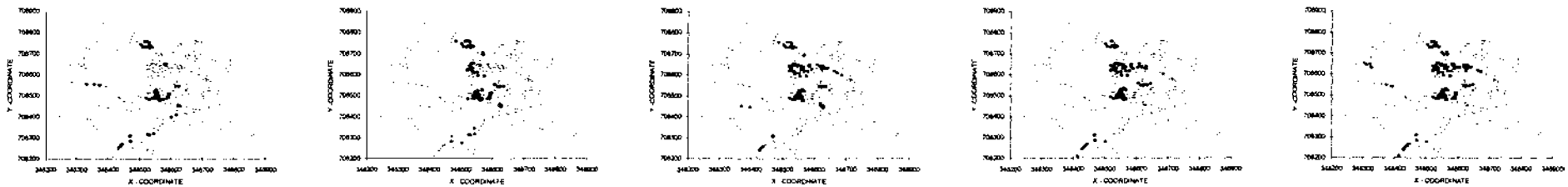


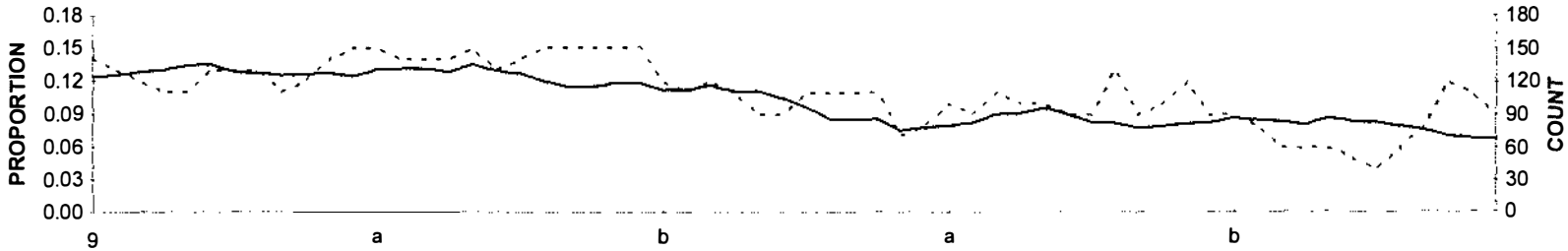
Figure 74e: Spatio-temporal pattern of clinical tuberculosis for years 16 to 20 of a simulation (o*riginal run*)

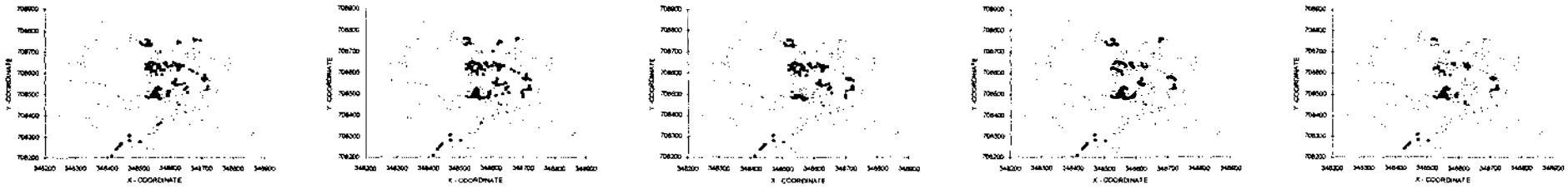




YEAR 16 YEAR 17 YEAR 18 YEAR 19 YEAR 20

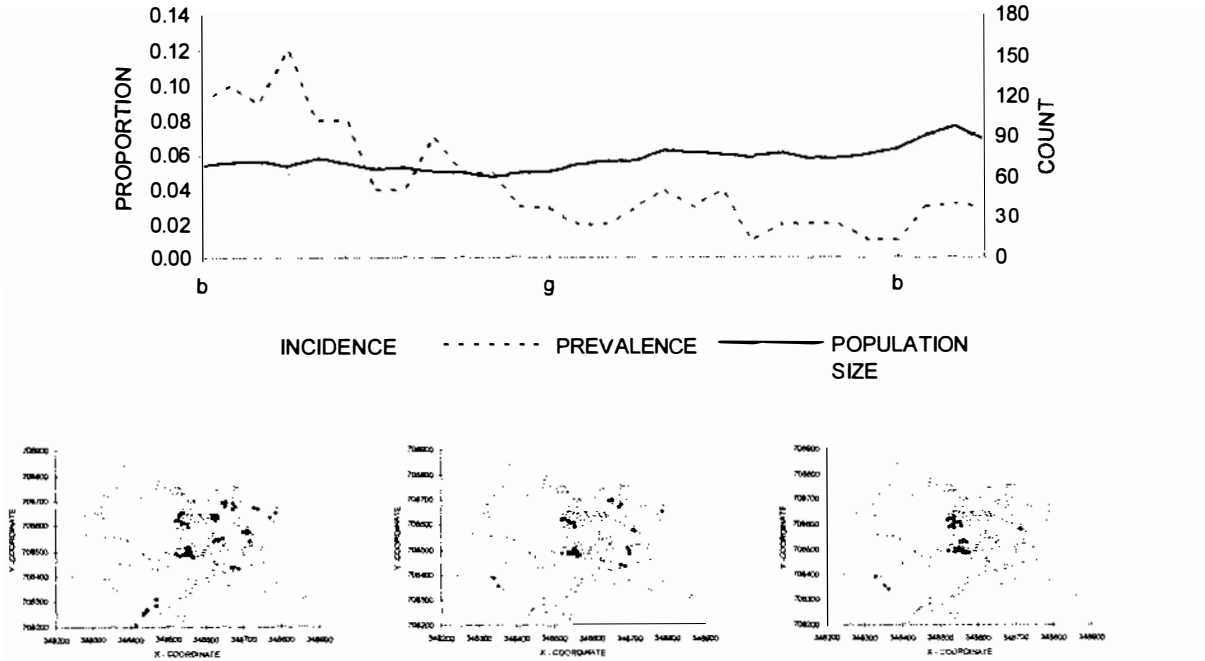
Figure 74f: Spatio-temporal pattern of clinical tuberculosis for years 21 to 25 of a simulation (o*riginal* run)





YEAR 21 YEAR 22 YEAR 23 YEAR 24 YEAR 25

Figure 74f: Spatio-temporal pattern of clinical tuberculosis for years 26 to 28 of a simulation (o*riginal* run)



Time-Series Analysis of Simulation Output

The analyses which were conducted in the preceding paragraphs did not take the temporal dependence structure of this kind of data into account. Time dependence is a consequence of the fact that processes within the model show contagious behaviour - what happened during the previous time interval influences what happens during the current interval. Statistically this produces autocorrelation between results for the various time periods, which therefore cannot be assumed to be independent estimates of the various outcome variables. Fishman and Kiviat (1967) pointed out that often simulation model users compute sample means and variances, ignoring the presence of autocorrelation; while others divide the sample record length into intervals which are assumed to remove the effect of major autocorrelation, such as that due to season of the year. Fishman and Kiviat doubt that the latter can be accomplished and suggest that by removing autocorrelation an important property of the process under study is being ignored. In their view the simulation analyst treats autocorrelation as a nuisance and tries to remove it, rather than to understand it. Fishman and Kiviat add that in studying a stochastic process the investigator is interested in the average level of some factor, the deviations from this level and the length of time these deviations last. In the study of a contagious process over time, autocorrelation (or contagiousness) is an important factor which should where possible be included into the analysis. Anderson *et al (*1984) used time series analysis to compare observed infectious disease cycle periods with those predicted from deterministic models.

Clinical tuberculosis prevalence can be considered as one of the most important variables in this analysis, both as a causal variable and an outcome variable. The presence of animals with clinical tuberculosis is essential to maintain the disease within the simulated population. The level of clinical tuberculosis at a particular point in time is the result of a complex web of interacting factors such as the probability of developing clinical disease for subclinically infected possums, the survival curve of clinically diseased animals and the overall level of infected possums in the population. These factors are in turn associated with many other effects such as number and density of susceptible possum in the population, the various transmission paths and their associated infection probabilities. These complex interactions result in direct or indirect effects which generate a dependence between factors that can be expressed as a lag structure. The temporal pattern of clinical TB prevalence is governed by a number of "emergent properties" of the model which can only be indirectly modified through changing the most influential model parameters. Clinical TB prevalence should also have a feedback effect on the incidence of subclinical TB. This means that the autocorrelational and crosscorrelational structure of the relevant factors has to be investigated.

It is the objective of the following time-series analysis to develop a procedural approach which can in later stages of the modelling process be used to understand the temporal pattern of clinical tuberculosis prevalence and its association with other disease and population

parameters. In this case an exploratory analysis was carried out on findings which were recorded during the *original* and *antithetic* simulation run using the base parameter files.

Time-Series Analysis in Time Domain

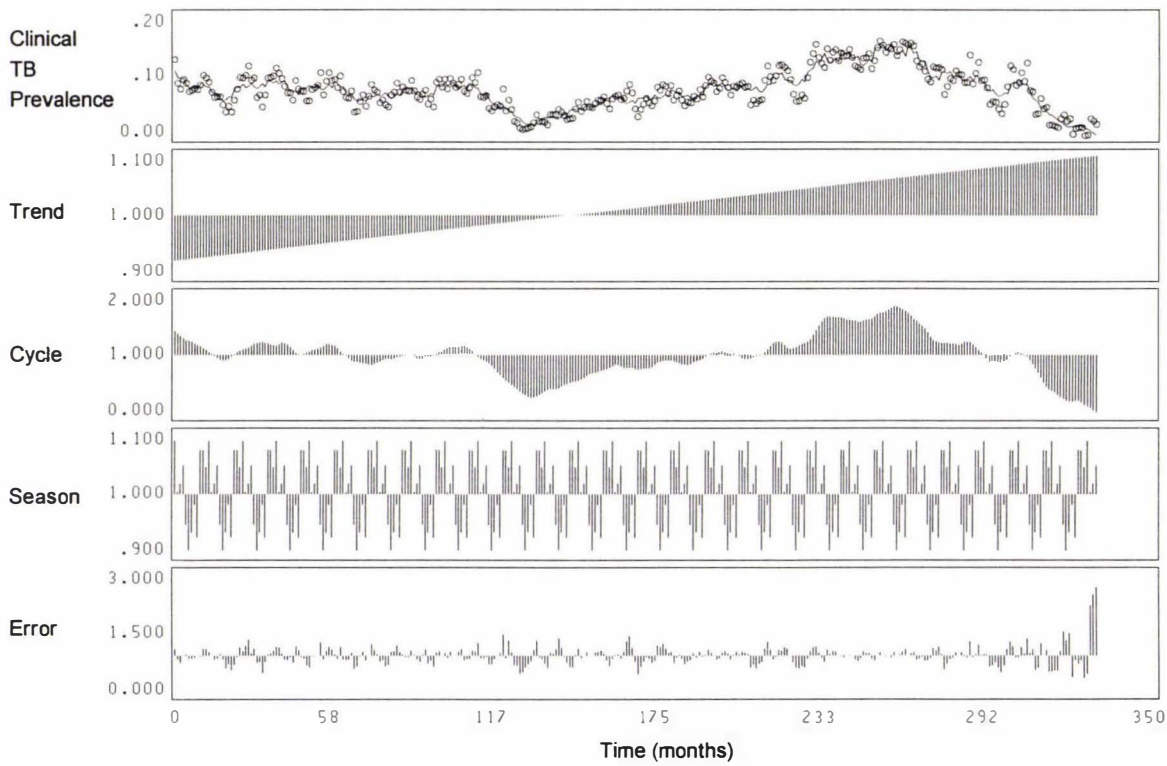
A time series is a set of ordered observations. It is assumed that it is the realization of some underlying stochastic process. Time series analysis involves development of a model of this stochastic process based on the realization which was generated by the same process. A typical time-series may be composed of four parts: a trend or long-term movement, oscillations about the trend (of greater or lesser regularity), a seasonal effect and a random, unsystematic or irregular component. When seasonal variation and trend have been removed from a series, it will generally consist of fluctuations which may (or may not) show some regularity to them, produced by underlying biological processes. One of the objectives of time-series analysis is to determine if there are systematic components in this residual series which can be represented by a statistical model, or if it is only white noise (random fluctuation; Kendall *et al* 1983).

As a first step a classical decomposition of the variable clinical TB prevalence into a long-range trend, a seasonality, a cycle and a random component is done using the statistical software NCSS (NCSS, Kaysville, Utah, U.S.A.). A disadvantage of this technique is that personal judgment must be employed to decide when and how a cycle component is required for forecasting. The structure of the decomposition model estimated for clinical tuberculosis prevalence, based on simulation output for the *original run* of the *base* parameter set files, is presented in table 28. The results suggest that prevalence increases slowly during the simulation period (a positive trend). During the spring-summer months prevalence is above average and reaches minima during winter months (seasonality). The model does fit the data fairly well with an R^2 of 0.84. Figure 75 displays a time series plot of the original data (fitted values as a line, original data as circles), as well as the trend, cycle, seasonal and random component.

Table 28: Classical decomposition model for clinical tuberculosis prevalence based on simulation output from *original* run using *base* parameter set files

MEAN Prevalence	0.079	Rsquared	0.84
TREND	0.93 + 0.00049 * time	FORECAST STD	0.00122
SEASONALITY	COMPONENT RATIOS		
Jan	1.08		
Feb	1.00		
Mar	1.02		
Apr	1.04		
May	0.95		
Jun	0.91		
Jul	0.94		
Aug	0.98		
Sep	0.93		
Oct	1.07		
Nov	1.07		
Dec	1.04		

Figure 75: Time series decomposition plot of clinical tuberculosis prevalence (*base* parameter files)



Time-Series Analysis in Frequency Domain

The autocorrelation function which is the basis of time-series analysis in the time domain has been described as the natural tool for considering the evolution of a process through time. The spectral density function is the complementary tool for analysis in the frequency domain

(Chatfield 1989). Fishman writes that the autocorrelation function provides information about the extent of correlation between events in a series as a function of their time separation, whereas the spectrum identifies cyclic phenomena and the extent of regularity (Fishman 1973). Hugh-Jones and Tinline (1976) used spectral analysis to estimate the incubation period of disease during a foot and mouth disease epidemic in the United Kingdom in 1967-68. Emanuel *et al* (1978) applied spectral analysis techniques to output produced by a stochastic model of forest stand dynamics and emphasized the advantages of using spectral analysis for detecting and understanding subtle differences in the dynamics of time series data.

For the analysis of the simulation data spectral analysis was used to compare spectral patterns between time series of variables derived from simulation output. The analyses were done using Statistica/W version 4.3 (StatSoft™ software, Tulsa, Oklahoma, U.S.A.). The spectral estimates were smoothed using the Hamming. The series were adjusted for trend and seasonality. Chatfield writes that if present, trend or seasonal variation are likely to dominate the results of spectral analysis. Trend will produce a peak at zero frequency. Seasonal variation results in peaks at the seasonal frequency and at integer multiples of the seasonal frequency (harmonics; Chatfield 1989).

The spectral density describes the degree to which cycles at different frequencies contribute to the variance of a series. If all frequencies contribute equally, the spectrum will be flat and the series is considered to be a realization of a white noise process. The frequency ω presented in the spectrograms can be converted into a cyclic period of months by taking the product between ω and the number of observations in the total time series. Figure 76a indicates a strong similarity in cycling pattern between clinical and subclinical TB prevalence. The smaller peak at a period of 6 months for clinical prevalence reflects a cycle repeating itself at 6 month intervals. The spectral pattern for the measures of clinical and subclinical disease incidence are distinctively different from the pattern for the prevalence measures. Clinical TB incidence has peaks at 12 month intervals which are caused by the seasonality of the occurrence of transitions from subclinical to clinical disease status, which is produced inherently by the monthly variation in model parameters. The spectrum for subclinical disease incidence resembles a white noise series (see figure 76b). This suggests that with the current parameter settings the two seasonal disease transmission mechanisms in the model, pseudo-vertical and mating transmission, do not have a dominant effect on the temporal pattern of transmission. The spectra for the population size are dominated by seasonality (see figure 76c).

Figure 76a: Spectrogram for subclinical and clinical TB prevalence

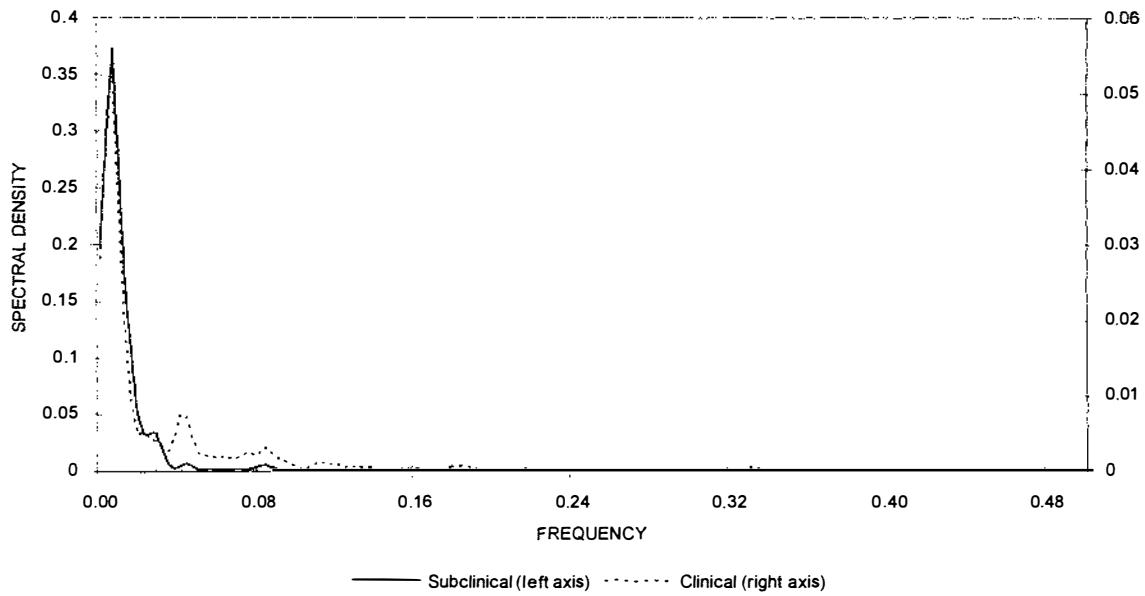


Figure 76b: Spectrogram for subclinical and clinical TB incidence

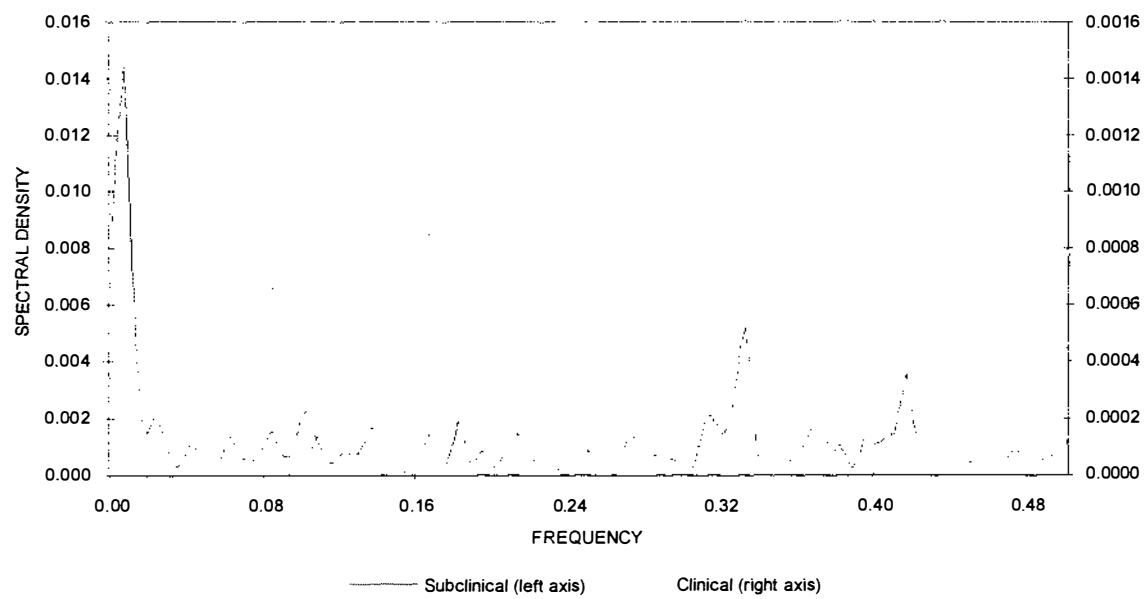
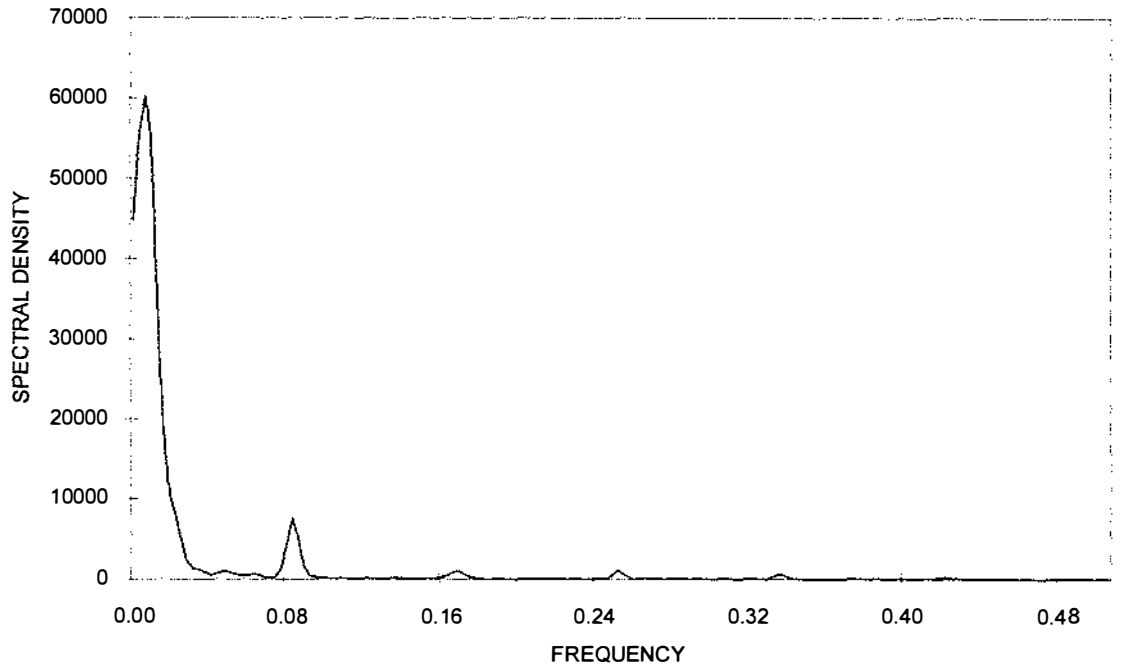


Figure 76c: Spectrogram for population size



Comparison of Antithetic Pairs Approach and Mean of Random Runs Approach

A series of simulation runs were conducted to test the effectiveness of the antithetic pairs approach in achieving a reduction in variance compared with the alternative strategy of summarizing the results from 5 and 10 runs using independent random numbers. The simulation model was run 10 times with independent random numbers (first set of values) and each run was repeated using a set of antithetic random numbers (second set of results). For each data point (= status of population at the end of a simulated month) the results for the *original* run and its *antithetic* counterpart were averaged resulting in a third set of values called the average of antithetic pairs. The simulations were based on two scenarios. Scenario no.1 was based on repeating the simulation runs using the *base* start population and the base parameter set files. Scenario no.2 was based on a start population which was 25% the size of the *base* start population and the *base* parameter set files were adjusted by removing clinical tuberculosis in immigrating possums. This last scenario was used as it was likely to achieve eradication of tuberculosis infection. The simulation results would then allow to measure the impact of these methods on a measure such as time to extinction of tuberculosis infection. For both simulation scenarios the model was run over a period of 10000 days for each of the individual simulations.

Model output from scenario no.1 was analysed by summarizing data for population size and prevalence of clinical tuberculosis for the whole simulation period, for each month of the year and for the first 24 months of the simulation runs. Scenario no.2 was used to analyse time

to extinction of clinical tuberculosis as the time from the beginning of the simulation until the disappearance of clinical tuberculosis from the simulated population.

Effect on population size

Analysis of simulation output for population size does not show a statistical difference between the three different types of output using data from the first five and from all ten runs (see table 29, figures 77a, b and c). The summary statistics indicate that the standard deviation for the average of the antithetic pairs is generally smaller than for sets of *original* and *antithetic* runs. Therefore for this variable there does seem to be some gain in using the option of variance reduction through averaging of antithetic pairs.

Table 29: Summary statistics for population size based on simulation output for the three different methods of treatment of random numbers (using 5 and 10 runs)

METHOD	MEAN	S.D.	Runs, Observations
INDEPENDENT RANDOM NUMBERS	131.55	27.15	10, 3280
ANTITHETIC RANDOM NUMBERS	130.58	27.33	10, 3280
AVERAGE OF ANTITHETIC PAIRS	131.07	25.17	10, 3280
INDEPENDENT RANDOM NUMBERS	128.73	25.71	5, 1640
ANTITHETIC RANDOM NUMBERS	130.56	27.58	5, 1640
AVERAGE OF ANTITHETIC PAIRS	129.65	24.94	5, 1640

Figure 77a: Error bar chart for population size (incl. standard deviation) based on 10 *original* and 10 *antithetic* runs and 10 averages of antithetic pairs from the 10 simulation runs over the period of a simulation year

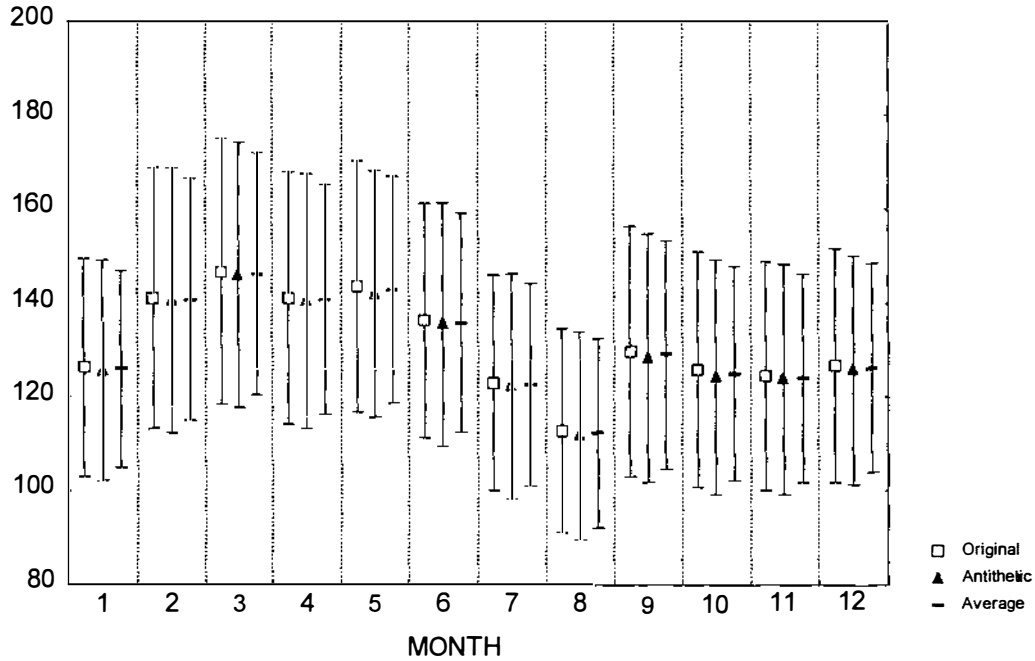


Figure 77b: Box-and-whisker plots for population size based on 10 independent runs, 10 antithetic runs and averages of antithetic pairs from 10 simulation runs for the whole simulation period of 10000 days

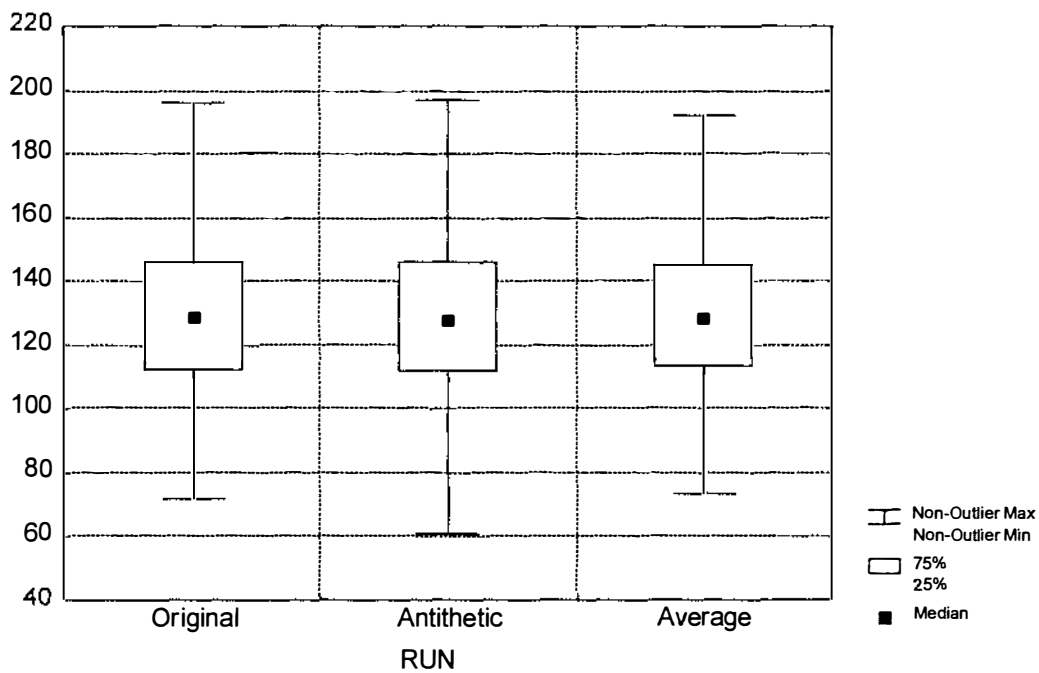
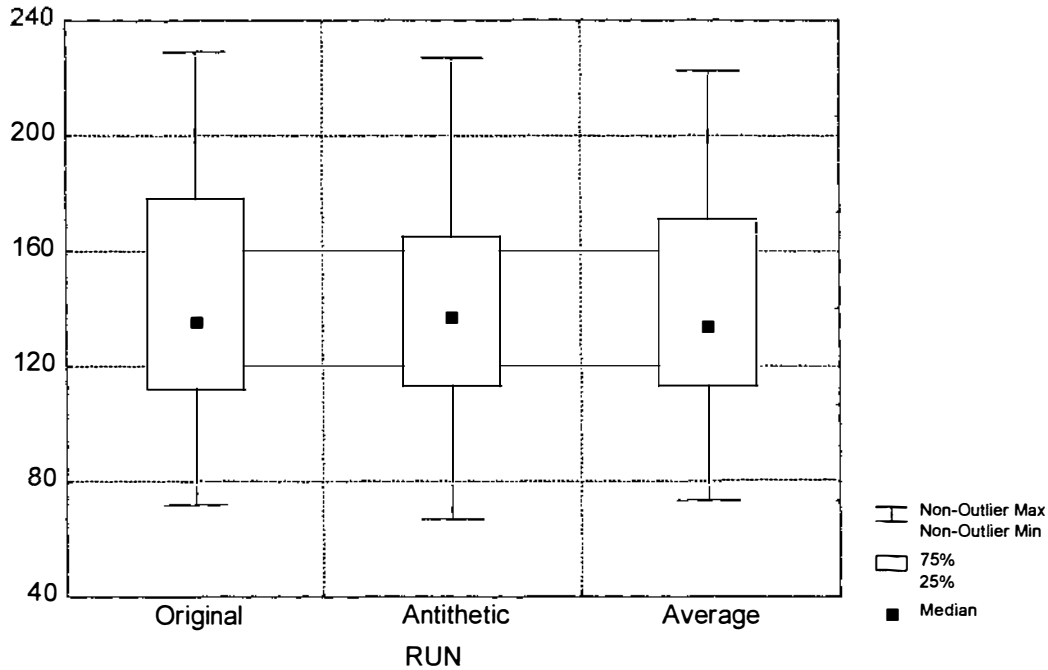


Figure 77c: Box-and-whisker plots for population size based on 5 independent runs, 5 antithetic runs and 5 averages of antithetic pairs for the whole simulation period of 10000 days



Effect on Prevalence of Clinical Tuberculosis Infection

Analysis of simulation output for clinical tuberculosis prevalence does show a reduction in variance when comparing the average of antithetic pairs with the two other sets of random numbers (see table 30). The summary time plots for the months of a year and the box plots for the whole simulation periods show that the distribution of values based on averages between antithetic pairs show less spread than the other two sets of runs based on independent random numbers and the antithetic runs (see figures 78 a,b,c and d). This demonstrates that the use of antithetic pairs may be useful if a reduction in variance is required.

Table 30: Summary statistics for clinical tuberculosis prevalence based on simulation output for the three different methods of treatment of random numbers (using 5 and 10 runs)

METHOD	MEAN	S.D.	Runs, Observations
INDEPENDENT RANDOM NUMBERS	0.065	0.034	10, 3280
ANTITHETIC RANDOM NUMBERS	0.057	0.032	10, 3280
AVERAGE OF ANTITHETIC PAIRS	0.061	0.027	10, 3280
INDEPENDENT RANDOM NUMBERS	0.064	0.035	5, 1640
ANTITHETIC RANDOM NUMBERS	0.061	0.034	5, 1640
AVERAGE OF ANTITHETIC PAIRS	0.062	0.029	5, 1640

Figure 78a: Error bar chart for clinical tuberculosis prevalence (incl. standard deviation) based on 10 independent runs and 10 averages of antithetic pairs over the period of a simulation year

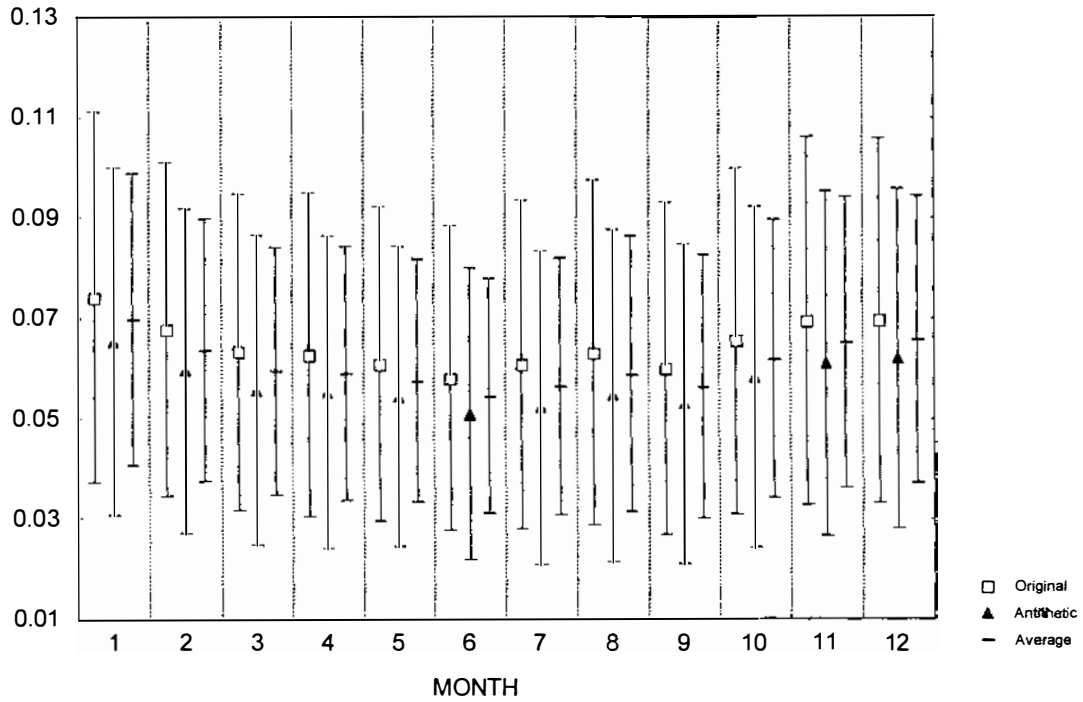


Figure 78b: Error bar chart for clinical tuberculosis prevalence (incl. standard deviation) based on 10 independent runs and 10 average of antithetic pairs during the first 24 months of the simulation runs

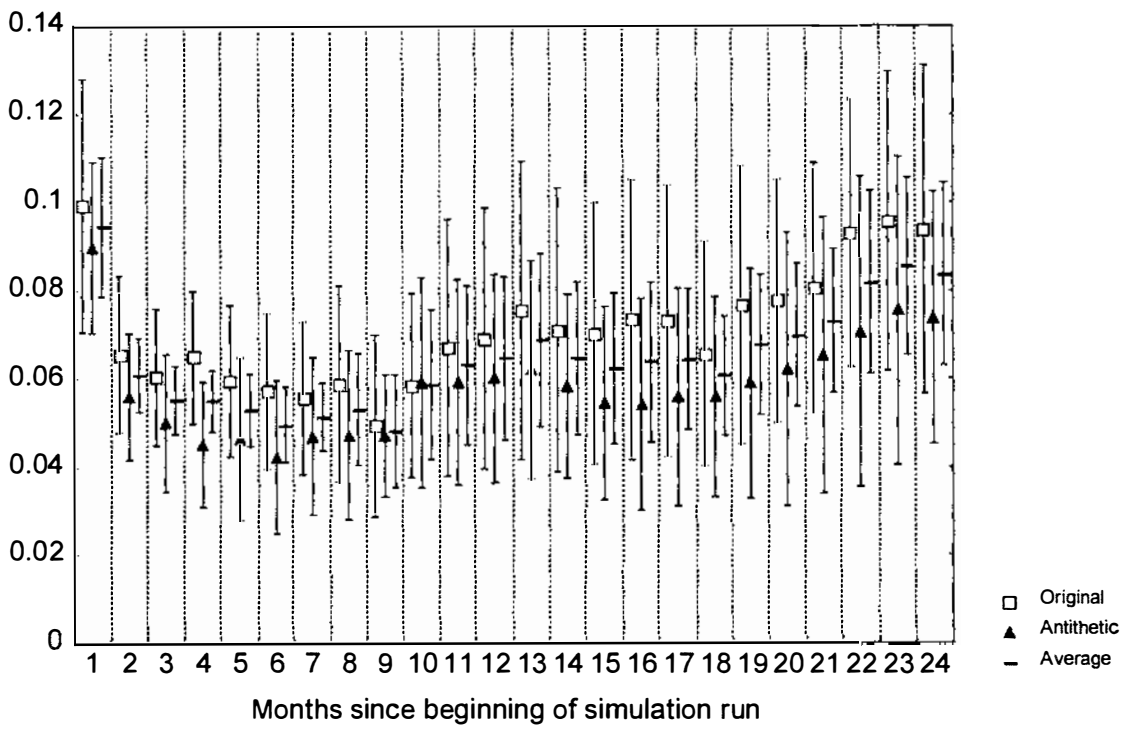


Figure 78c: Box-and-whisker plots for clinical tuberculosis prevalence based on 10 independent runs, 10 antithetic runs and 10 averages of antithetic pairs for the whole simulation period of 10000 days

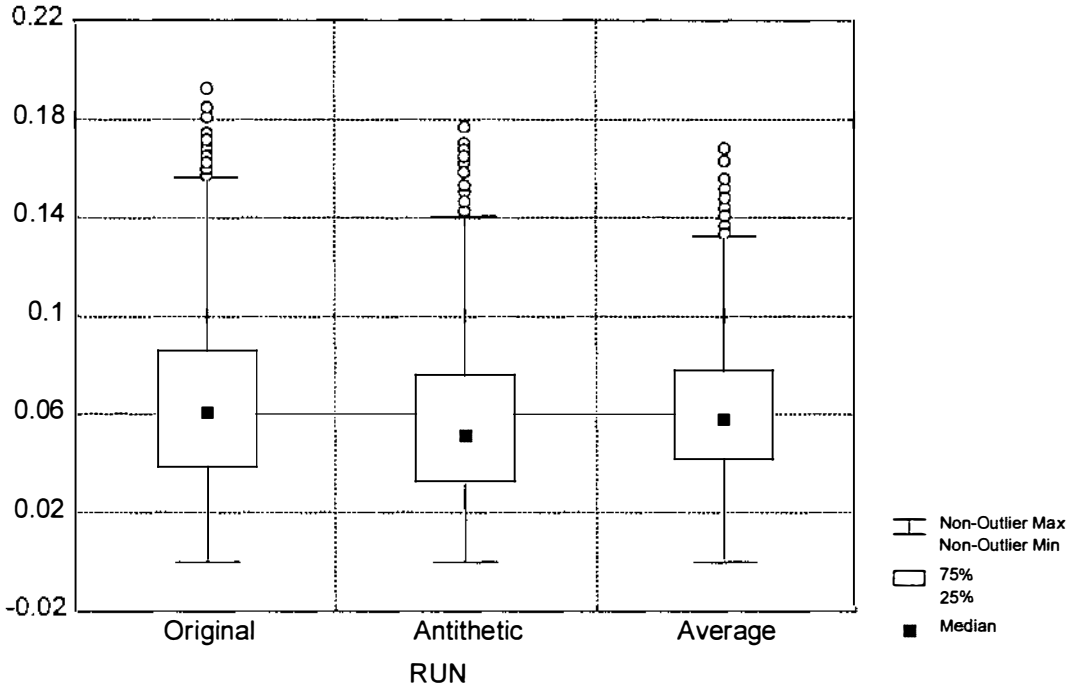
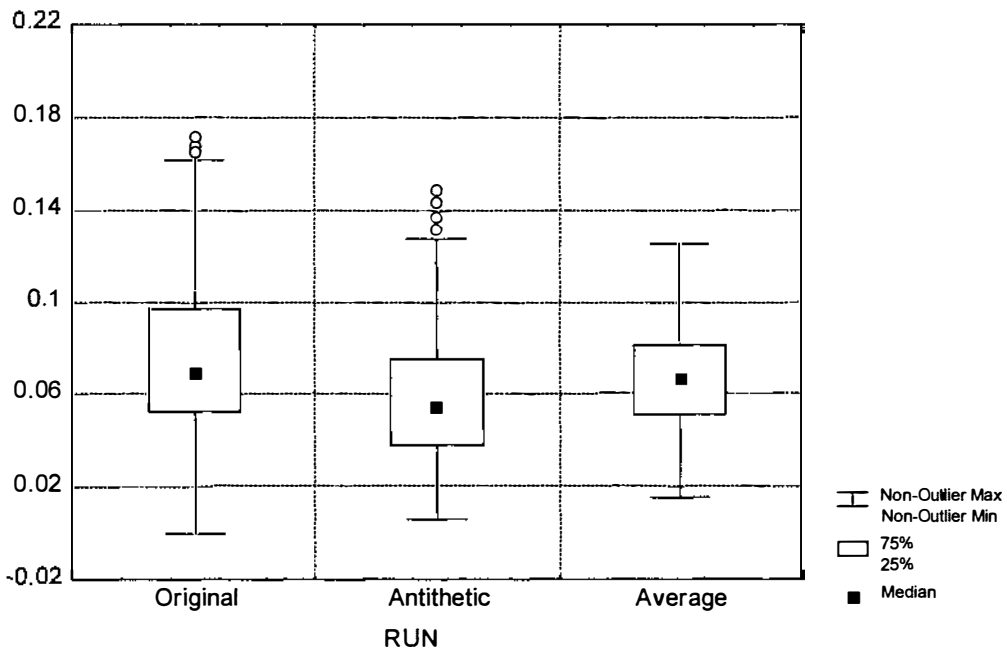


Figure 78d: Box-and-whisker plots for clinical tuberculosis prevalence based on 5 independent runs, 5 antithetic runs and 5 averages of antithetic pairs for the whole simulation period of 10000 days



Effect on Time to Extinction of Clinical Tuberculosis

Simulation output from scenario no.2 was used to analyze the parameter "time to extinction of clinical tuberculosis". Survival analysis was used to compare the different simulation runs between the three methods (10 runs each; see figure 79a). The survival plot shows that the survivorship curve for model output based on independent random numbers was similar to the curve based on data for averages of antithetic pairs. The data was then stratified into two samples of 5 runs each. The resulting survival plots indicate that the shape of the curves representing the probability of extinction varies considerably between the two sets of 5 simulation runs, but is very similar for the set of 10 runs (see figures 79 a and b). The data suggests that median survival time is about 28 months which means that there in 50% of simulation runs clinical disease had disappeared by 24 months. It is not useful to use antithetic variates for estimation of this parameter as it would tend to overestimate time to extinction.

Figure 79a: Survival plot of time to extinction of clinical tuberculosis based on 10 independent runs, 10 antithetic runs and 10 averages of antithetic pairs for the whole simulation period of 328 months

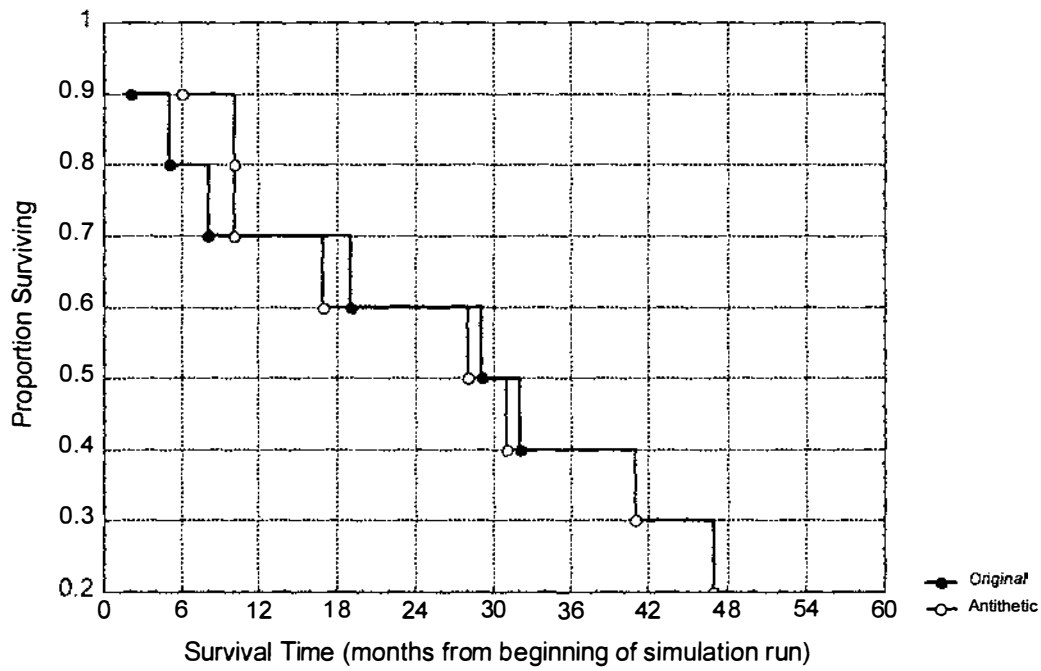
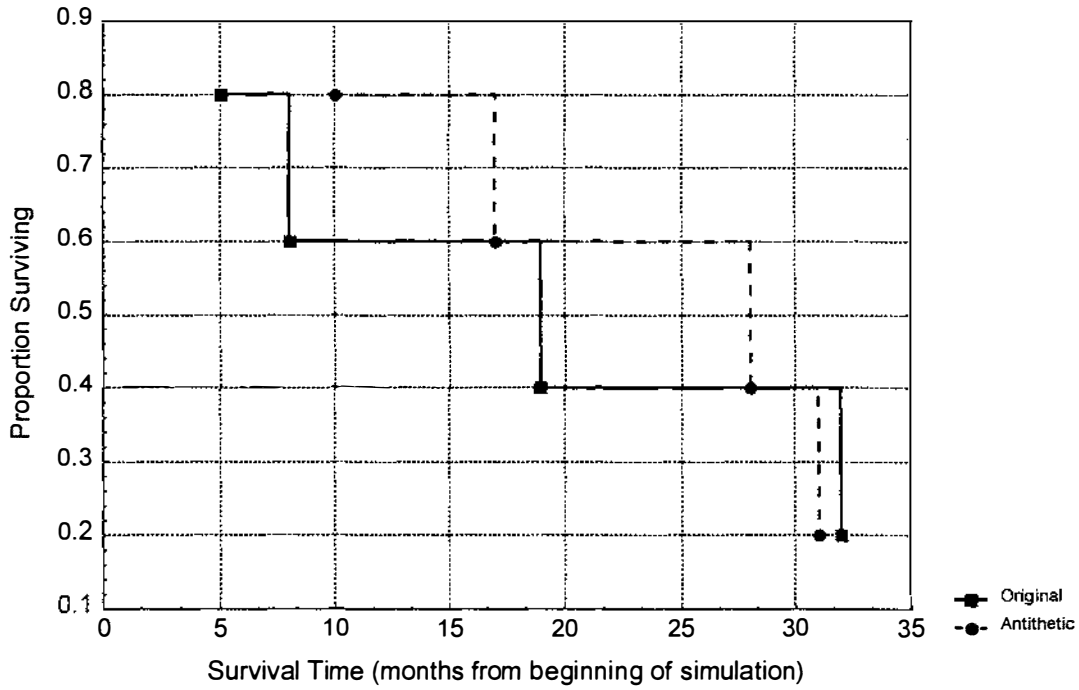


Figure 79b: Survival plot of time to extinction of clinical tuberculosis based on *first* set of 5 independent runs, 5 antithetic runs and 5 averages of antithetic pairs for the whole simulation period of 328 months



Sensitivity Analysis

Before a model can be used for experimentation it is necessary to conduct a sensitivity analysis of parameters which are considered important. This process will identify parameters which have a significant influence on the outcome of a simulation run. Accurate estimates of such parameters are required in order to produce valid simulation results.

Sensitivity analysis for this model focused on the main factors which influence tuberculosis infection dynamics. These include the four transmission mechanisms and three spatial parameters related to transmission. Model runs were compared with a *baseline* simulation scenario which was based on *base* parameter settings but excluded tuberculosis in immigrating possums. This approach was considered appropriate as the presence of tuberculosis infection in immigrants might obscure the results of the sensitivity analysis.

Baseline Simulation without Clinical TB in Immigrants

This simulation scenario was based on *base* parameter settings, but without the presence of clinical tuberculosis in immigrating possums.

Figure 80 shows time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Table 31 lists the summary statistics for the two simulation runs.

Figure 80: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs used as *baseline* simulation scenario for sensitivity analysis

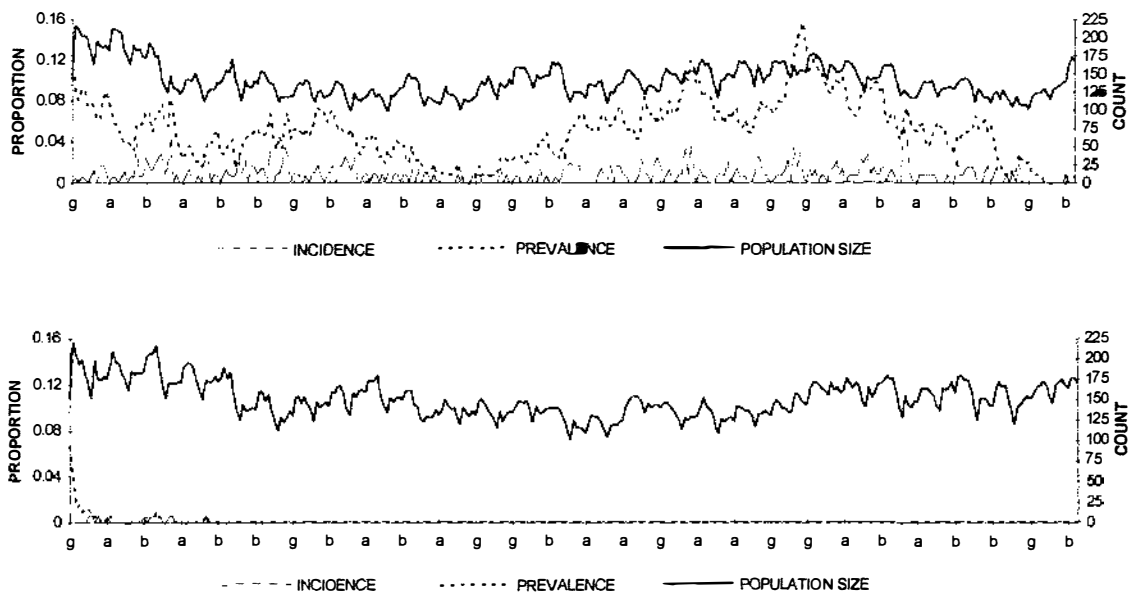


Table 31: Summary of simulation output used as *baseline* simulation scenario for sensitivity analysis

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	140.3	138	132	22.6	97	216
	antithetic	151.0	148	140	21.9	101	221
CLINICAL INCIDENCE	*original*	0.008	0.007	0.000	0.009	0.000	0.055
	antithetic	0.000	0	0.000	0.001	0.000	0.007
CLINICAL PREVALENCE	*original*	0.051	0.051	0.008	0.030	0.000	0.156
	antithetic	0.001	0.000	0.000	0.005	0.000	0.066

Effect of Spatial Parameters on Infection Dynamics

The influence of the settings for the three parameters "Radius of Area around an infected Den with increased Risk of Infection", "Maximum Travel Distance for Mating" and "Maximum Search Distance for Den" on infection dynamics was tested by conducting additional runs for each parameter with different parameter settings. No tuberculosis infection was allowed in immigrants to ensure that it did not obscure the effect of varying the above parameters.

Radius of Area around an Infected Den with Increased Risk of Infection

The setting for the radius around an infected den with increased risk of infection was 50m for the *base* parameter files. Additional runs using settings of 25m and 75m were conducted to test the sensitivity of the model to changes in values of this parameter.

Figures 81a and b show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Tables 32a and b list the summary statistics for the two simulation runs.

From the results of these simulation runs it can be concluded that a change in the radius of the area with an increased risk of infection around an infected den is of critical importance for the model infection dynamics. A radius of 25m did not allow sufficient contacts between infectious and susceptible possums for the disease to reach an endemic state in the population. Using a radius of 75m resulted in both runs reaching an endemic state of tuberculosis infection, even without immigration of possums with clinical tuberculosis.

Figure 81a: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 25m radius of increased risk of infection around infected dens

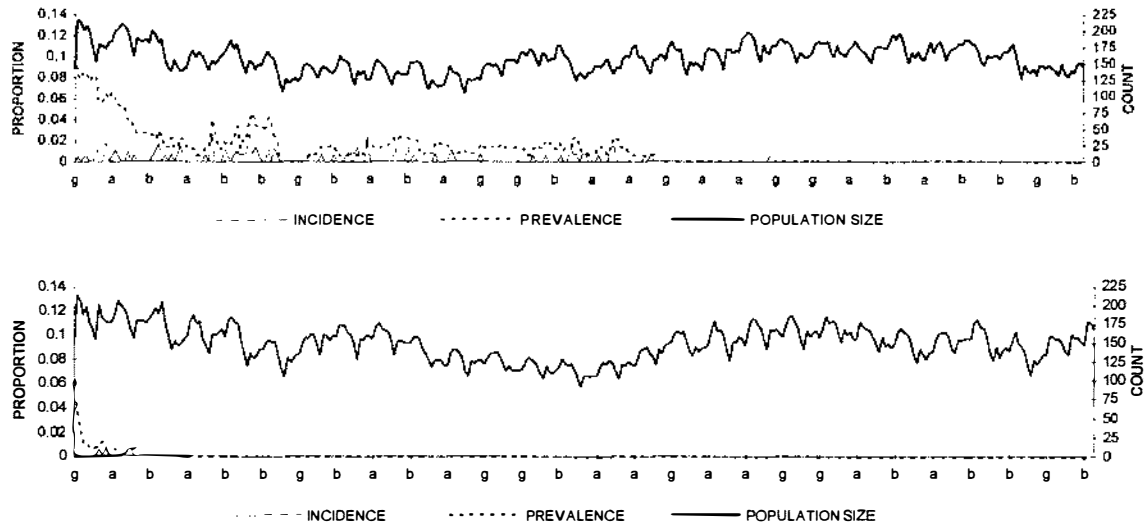


Table 32a: Summary of simulation output for simulation scenario testing the effect of a 25m radius of increased risk of infection around infected dens

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	157.4	157	151	21.0	105	218
	antithetic	149.9	152	156	22.8	92	214
CLINICAL INCIDENCE	*original*	0.002	0.000	0.000	0.004	0.000	0.026
	antithetic	0.000	0	0.000	0.001	0.000	0.006
CLINICAL PREVALENCE	*original*	0.011	0.006	0.000	0.017	0.000	0.119
	antithetic	0.001	0.000	0.000	0.004	0.000	0.060

Figure 81b: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 75m radius of increased risk of infection around infected dens

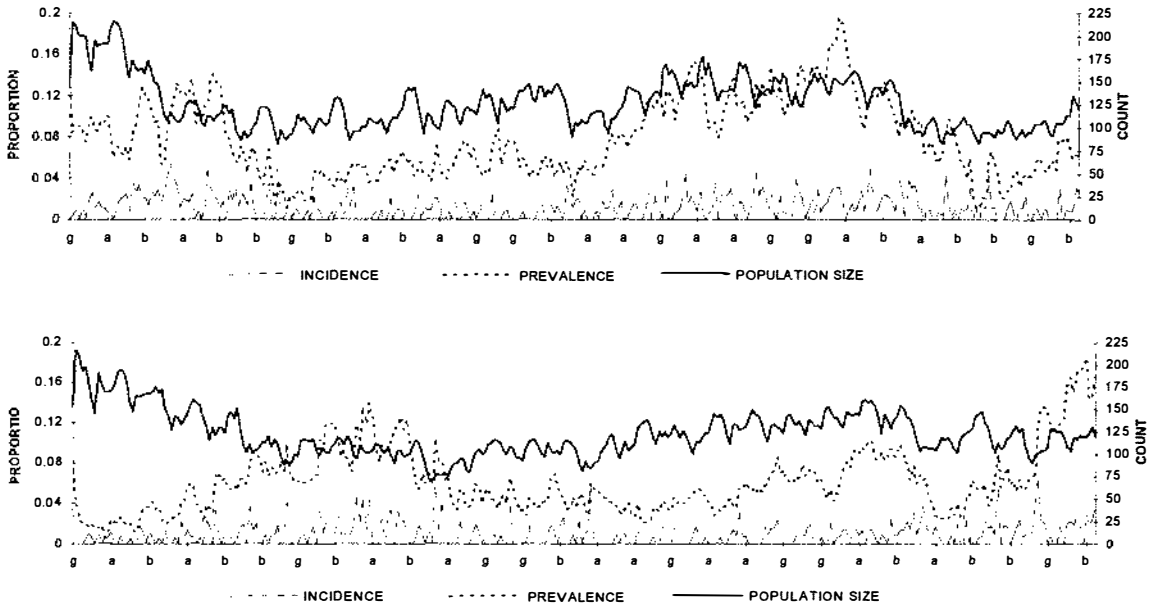


Table 32b: Summary of simulation output for simulation scenario testing the effect of a 75m radius of increased risk of infection around infected dens

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	126.4	122	105	27.8	81	216
	antithetic	122.5	118	101	25.1	68	217
CLINICAL INCIDENCE	*original*	0.012	0.010	0.000	0.012	0.000	0.054
	antithetic	0.010	0.008	0.000	0.012	0.000	0.067
CLINICAL PREVALENCE	*original*	0.078	0.070	0.058	0.037	0.011	0.195
	antithetic	0.062	0.056	0.054	0.033	0.006	0.185

Maximum Mating Travel Distance

In the *base* parameter files the parameter "Maximum Mating Travel Distance" is set to 100m. For the following simulations the parameter was changed to 75m and 125m.

Figures 82a and b show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Tables 33a and b list the summary statistics for the two simulation runs.

The results of these simulations suggest that the model is only moderately sensitive to changes in mating travel distance. This suggests that individual female possums need only a small number of mating contacts until they become pregnant and therefore would not have to go as far as the mating travel distance would allow.

Figure 82a: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 75m mating travel distance

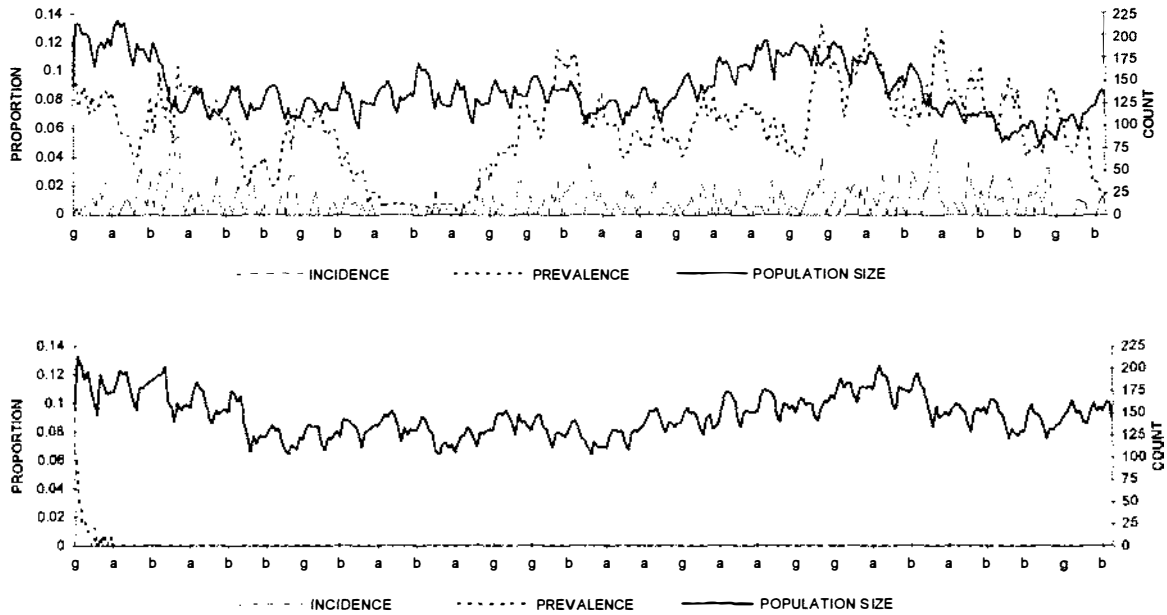


Table 33a: Summary of simulation output for simulation scenario testing the effect of a 75m mating travel distance

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	139.2	134	143	29.2	77	217
	antithetic	146.9	146	150	22.8	102	213
CLINICAL INCIDENCE	*original*	0.009	0.007	0.000	0.011	0.000	0.055
	antithetic	0.000	0	0.000	0.001	0.000	0.006
CLINICAL PREVALENCE	*original*	0.061	0.064	0.008	0.029	0.000	0.132
	antithetic	0.001	0	0.000	0.005	0.000	0.066

Figure 82b: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 125m mating travel distance

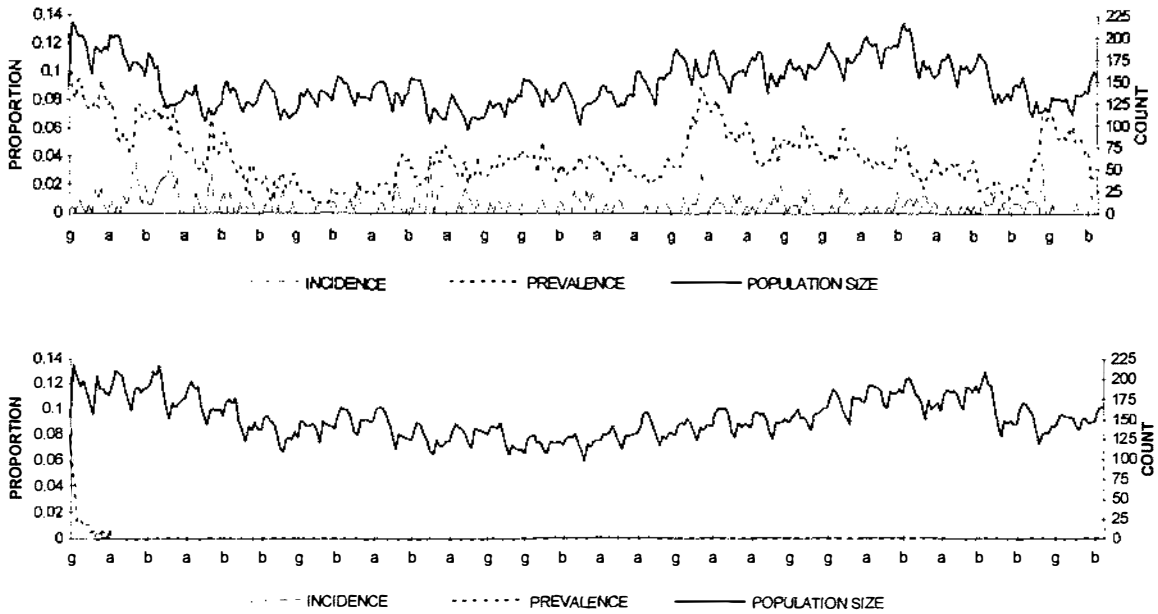


Table 33b: Summary of simulation output for simulation scenario testing the effect of a 125m mating travel distance

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	147.9	145	135	25.8	94	217
	antithetic	151.7	147	146	25.3	97	218
CLINICAL INCIDENCE	*original*	0.006	0.000	0.000	0.007	0.000	0.041
	antithetic	0.000	0	0.000	0.001	0.000	0.006
CLINICAL PREVALENCE	*original*	0.039	0.036	0.026	0.019	0.007	0.125
	antithetic	0.001	0	0.000	0.005	0.000	0.066

Maximum Search Distance for a Den

The importance of the parameter "Maximum Search Distance for a Den" was tested using two simulation scenarios, one with a 75m and the other with a 125m search distance. This parameter is set to 100m in the *base* parameter file.

Figures 83a and b show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Tables 34a and b list the summary statistics for the two simulation runs.

The results of these two simulation scenarios indicate that there is some sensitivity towards changes in den search distance. With a reduced den search distance disease incidence and prevalence are both lower compared with the *baseline* scenario, whereas they are slightly higher when the search distance is increased. This indicates that an increase in a possum's "den range" results in an increased risk of infectious contacts. The change in risk becomes smaller with increasing search distance until reaches an equilibrium.

Figure 83a: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 75m den search distance

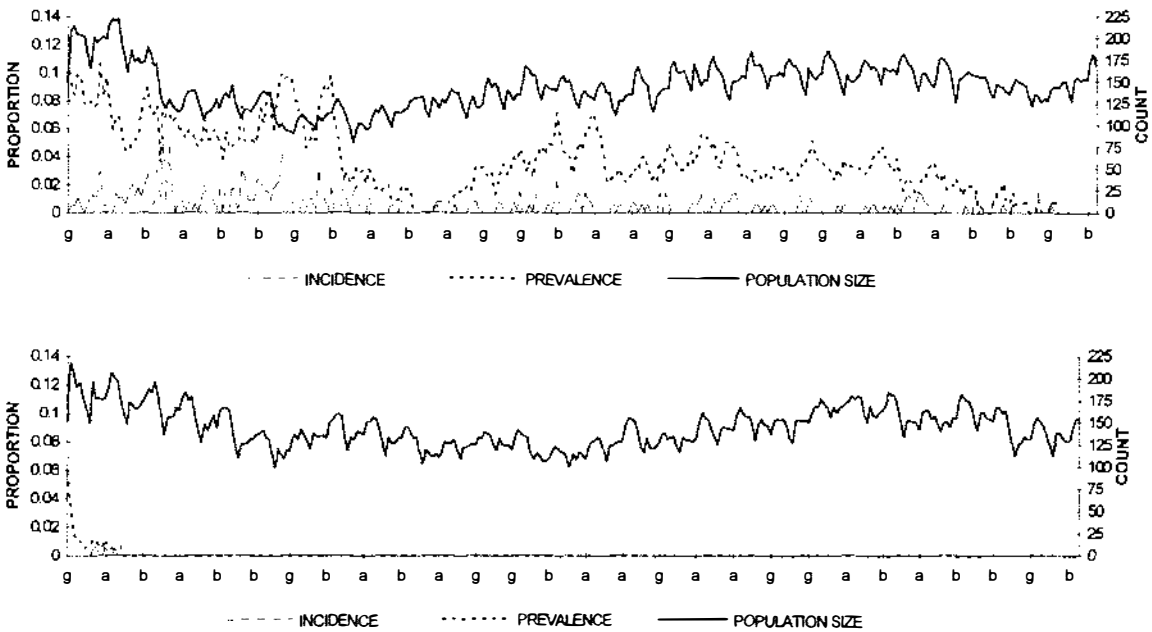


Table 34a: Summary of simulation output for simulation scenario testing the effect of a 75m den search distance

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	original	144.4	143	139	25.8	80	222
	antithetic	144.6	142	133	22.0	99	219
CLINICAL INCIDENCE	original	0.005	0.000	0.000	0.008	0.000	0.050
	antithetic	0.000	0	0.000	0.001	0.000	0.006
CLINICAL PREVALENCE	original	0.037	0.032	0.000	0.025	0.000	0.125
	antithetic	0.001	0.000	0.000	0.004	0.000	0.059

Figure 83b: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs testing the effect of a 125m den search distance

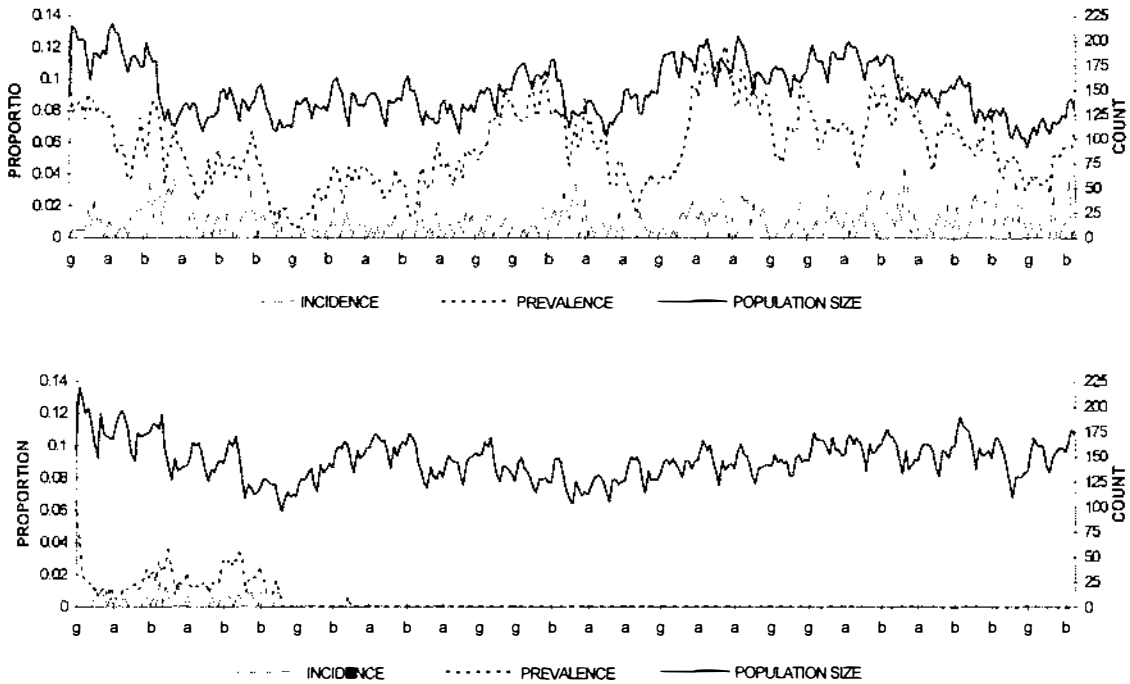


Table 34b: Summary of simulation output for simulation scenario testing the effect of a 125m den search distance

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	149.4	145	143	26.7	91	216
	antithetic	146.7	146	146	20.0	95	219
CLINICAL INCIDENCE	*original*	0.009	0.007	0.000	0.009	0.000	0.044
	antithetic	0.001	0.000	0.000	0.002	0.000	0.017
CLINICAL PREVALENCE	*original*	0.058	0.056	0.034	0.024	0.007	0.121
	antithetic	0.004	0.000	0.000	0.008	0.000	0.065

Transmission Mechanisms for Mycobacterium bovis infection

Each of the four transmission mechanisms for *Mycobacterium bovis* infection represented in the model was disabled in separate simulation scenarios.

Figures 84a to d show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Tables 35a to d list the summary statistics for the two simulation runs.

The results of these four simulation scenarios demonstrate that given the current model structure and parameter settings the most important transmission mechanism is spatial proximity. Pseudo-vertical transmission ranks second, transmission during mating third and transmission through infected den sites seems to be relatively unimportant. It is interesting that by removing den site transmission infection, unexpectedly infection becomes endemic during the *antithetic* run. This may be attributable to stochastic effects which result in an increase of transmission during the first two years of the simulation. This in turn allows a prevalence sufficient for maintenance of disease to build up. The model is very sensitive to the infection prevalence early in the run, if there is no immigration of infected animals.

Figure 84a: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs with the transmission mechanism in den sites disabled

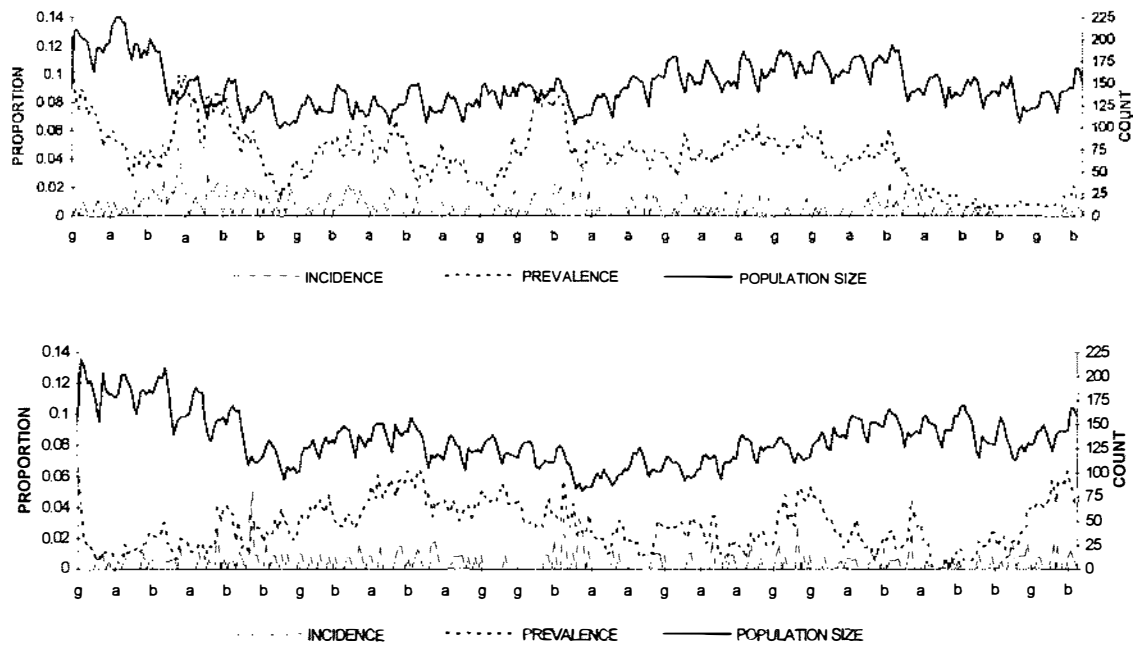


Table 35a: Summary of simulation output for simulation scenario testing the effect of disabling transmission through infected den sites

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	147.0	143	117	25.5	99	227
	antithetic	134.2	131	143	26.2	81	218
CLINICAL INCIDENCE	*original*	0.006	0.005	0.000	0.007	0.000	0.048
	antithetic	0.004	0.000	0.000	0.006	0.000	0.050
CLINICAL PREVALENCE	*original*	0.042	0.043	0.007	0.021	0.000	0.125
	antithetic	0.028	0.027	0.019	0.015	0.000	0.065

Figure 84b: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs with transmission through spatial proximity disabled

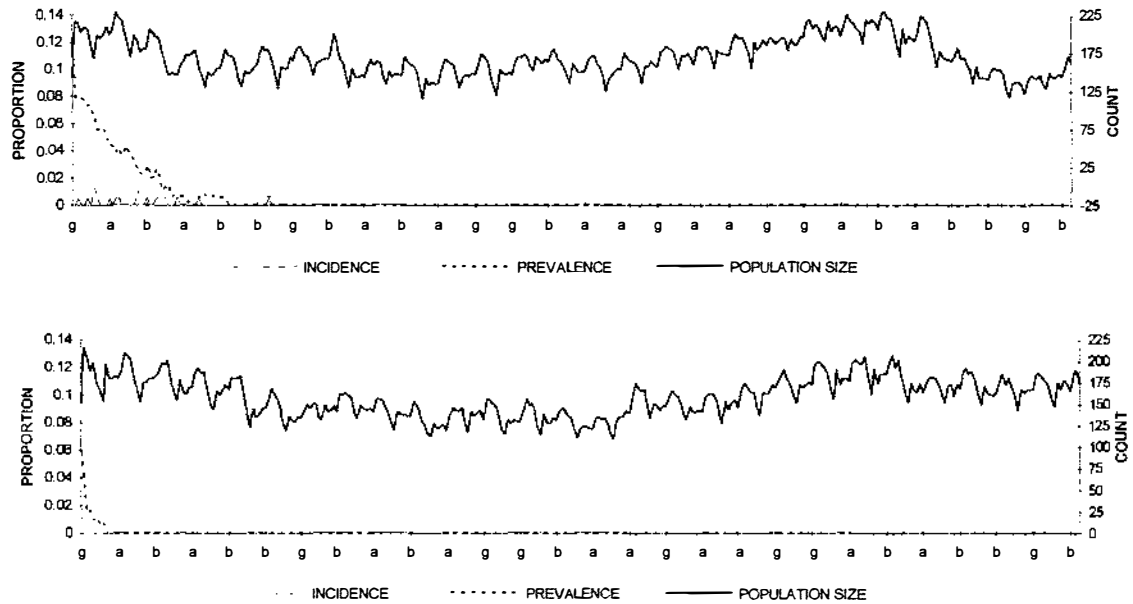


Table 35b: Summary of simulation output for simulation scenario testing the effect of disabling transmission through spatial proximity

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	169.2	166	147	24.8	115	230
	antithetic	158.0	159	161	22.6	109	216
CLINICAL INCIDENCE	*original*	0.000	0.000	0.000	0.001	0.000	0.013
	antithetic	0.000	0.000	0.000	0.000	0.000	0.000
CLINICAL PREVALENCE	*original*	0.005	0.000	0.000	0.015	0.000	0.119
	antithetic	0.001	0	0.000	0.005	0.000	0.066

Figure 84c: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs with transmission through mating contact disabled

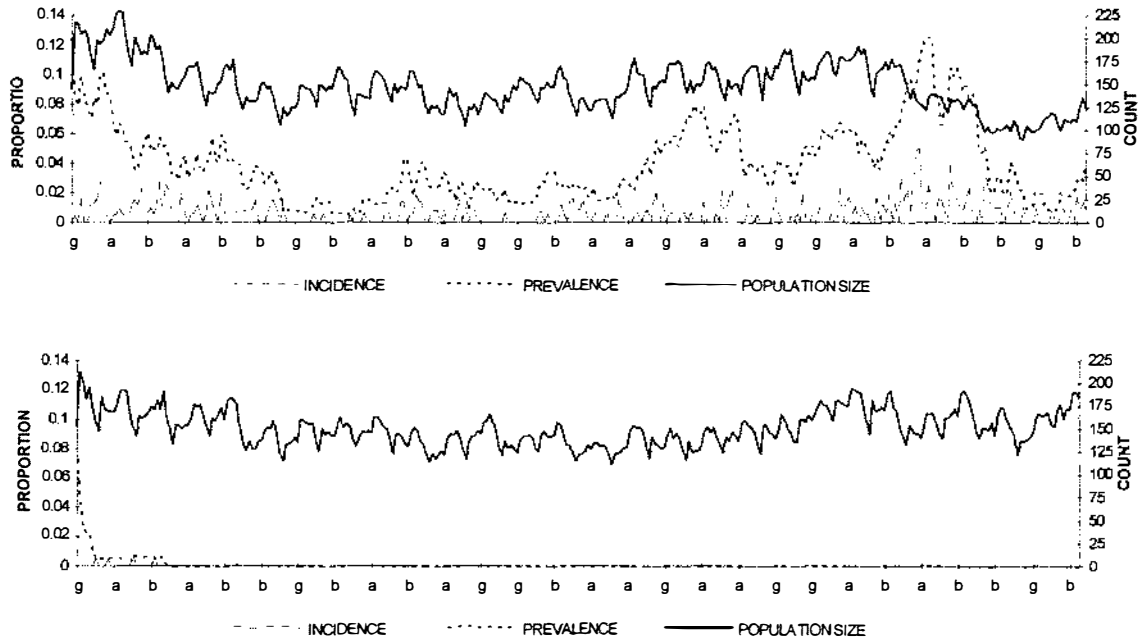


Table 35c: Summary of simulation output for simulation scenario testing the effect of disabling transmission through mating contact

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*Original*	147.7	146	145	26.7	89	229
	Antithetic	151.0	149	152	19.5	110	213
CLINICAL INCIDENCE	*Original*	0.007	0.006	0.000	0.008	0.000	0.050
	Antithetic	0.000	0	0.000	0.001	0.000	0.007
CLINICAL PREVALENCE	*Original*	0.040	0.034	0.022	0.026	0.000	0.125
	Antithetic	0.001	0.000	0.000	0.005	0.000	0.072

Figure 84d: Time plots of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* runs with pseudo-vertical transmission disabled

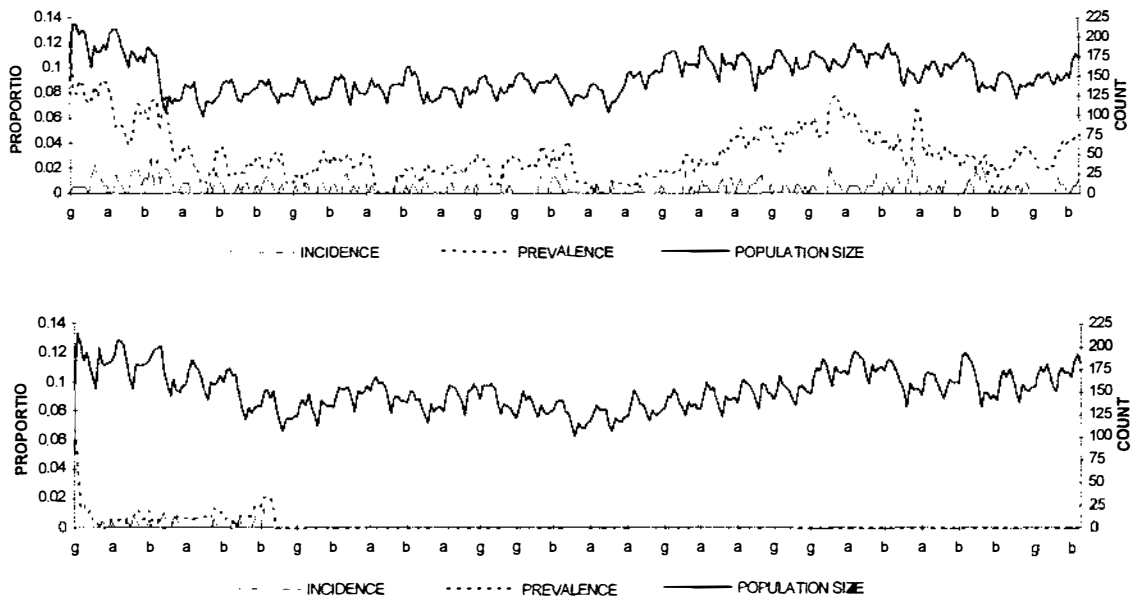


Table 35d: Summary of simulation output for simulation scenario testing the effect of disabling pseudo-vertical transmission

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*Original*	149.6	147	136	23.0	98	217
	Antithetic	152.1	151	151	21.7	100	215
CLINICAL INCIDENCE	*Original*	0.004	0.000	0.000	0.006	0.000	0.030
	Antithetic	0.000	0.000	0.000	0.002	0.000	0.013
CLINICAL PREVALENCE	*Original*	0.031	0.027	0.017	0.020	0.000	0.118
	Antithetic	0.002	0.000	0.000	0.006	0.000	0.066

Statistical Comparison of Simulations Runs

The mean values for populations size and clinical tuberculosis prevalence combining data from the *original* and the antithetic run were compared between the *baseline* scenario and each of the sensitivity analysis scenarios. Scheffé's test statistic for comparison of means was used to test the difference between means for statistical significance.

Table 36 presents the results of this comparison. Figures 85a and b show box-and-whisker plots for population size and clinical tuberculosis prevalence for each of the sensitivity analysis simulation scenarios.

Model output for population size and clinical tuberculosis prevalence is very sensitive to changes in the size of the area with increased risk of infection and removing transmission through spatial proximity altogether. The other parameters which were tested in this sensitivity analysis did not have a statistically significant impact on population size. The remaining three transmission paths did have minor effects on the level of clinical tuberculosis prevalence.

Table 36: Statistical comparison of sensitivity analysis scenarios with the *baseline* simulation scenario using Scheffé's test combining data from *original* and *antithetic* run

Simulation Scenario	*Population Size*			*Clinical TB Prevalence*			
	Mean	**S.D.**	**p-value**	**Mean**	**S.D.**	**p-value**	**N**
baseline	145.6	22.8		0.0257	0.0329		656
buffer 25m	153.6	22.2	0.0001	0.0059	0.0137	0.0000	656
buffer 75m	124.4	26.5	0.0000	0.0699	0.0360	0.0000	656
den travel 125m	148.1	23.5	0.9722	0.0306	0.0326	0.3707	656
den travel 75m	144.5	23.9	0.9999	0.01889	0.0256	0.0184	656
mating travel 125m	149.8	25.6	0.4679	0.0199	0.0239	0.1067	656
mating travel 75m	143.1	26.4	0.9649	0.0308	0.0366	0.2964	656
no buffer transmission	163.6	24.3	0.0000	0.0026	0.0115	0.0000	656
no den site transmission	140.6	26.6	0.1821	0.0248	0.0196	0.0001	656
no mating contact transmission	149.3	23.3	0.6639	0.0204	0.0270	0.2335	656
no vertical transmission	150.9	22.4	0.1260	0.0166	0.0209	0.0000	656
all	146.7	26.0		0.0251	0.0317		7216

Figure 85a: Box-and-whisker plots for population size based on combined simulation output from *original* and *antithetic* runs for each of the sensitivity analysis scenarios

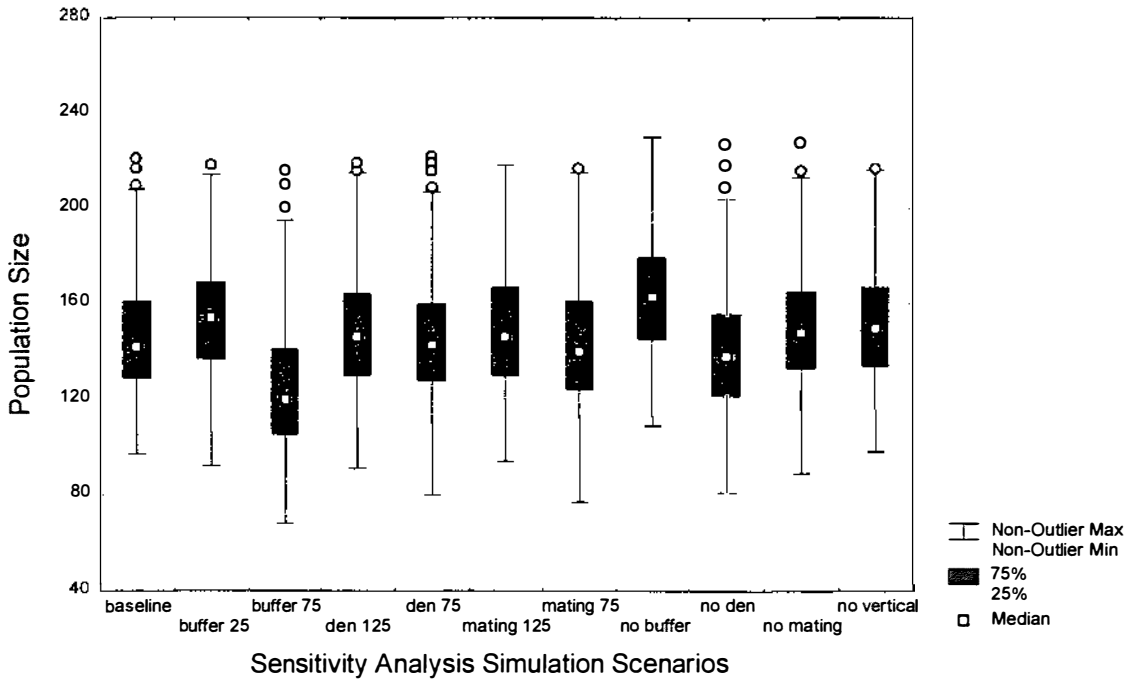
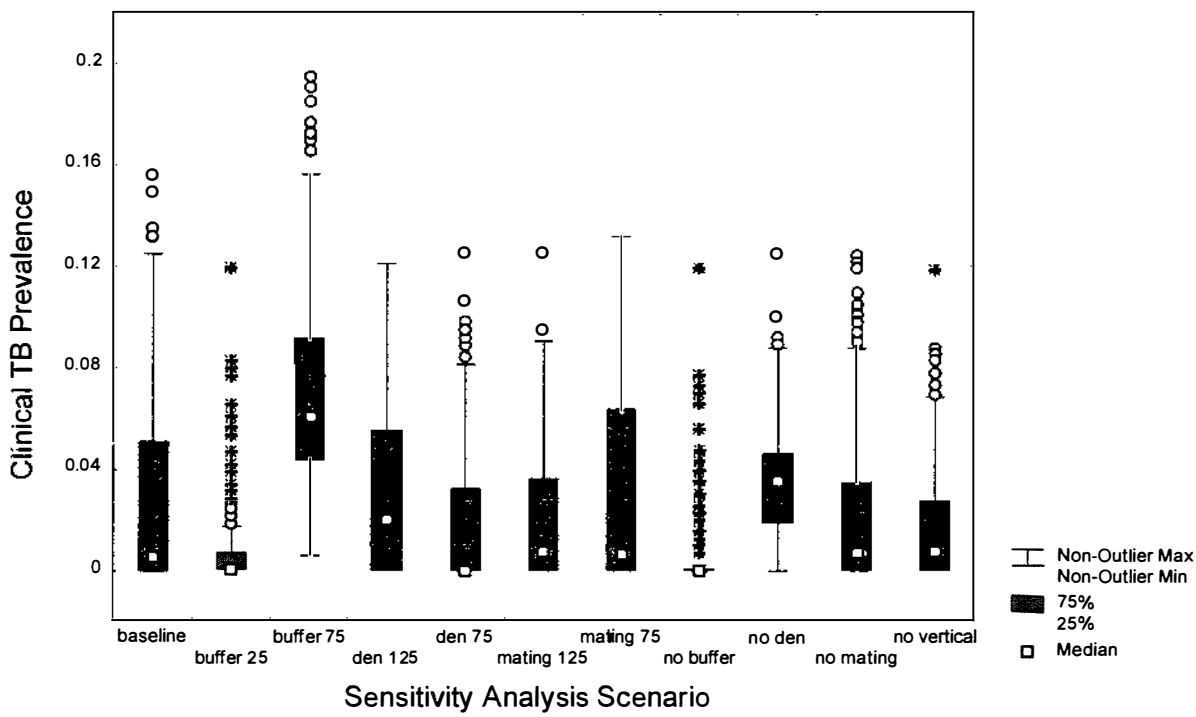


Figure 85b: Box-and-whisker plots for clinical tuberculosis prevalence based on combined simulation output from *original* and *antithetic* runs for each of the sensitivity analysis scenarios



Model Experimentation

Variations of the *base* parameter set files and other model input such as the *start* population and den site map have been used to conduct a sensitivity analysis of some model parameters. This analysis can be considered as model experimentation, although because the model is only at an early stage of development they are not intended as true experimentation but rather as explorations of the effects of varying control parameters of the model outside the range for which data from the longitudinal study was available. The investigations were also designed to begin to incorporate into the model the influence of dispersal between areas of infected possums.

A small number of scenarios are tested for their effect on the dynamics of tuberculosis infection. These scenarios allow inferences regarding the sensitivity of the model to changes in parameters, particularly those which would be most important in representing the effects of disease control options on the dynamics of tuberculosis infection in a possum population. For each scenario a simulation run consisting of *original* and *antithetic* runs is conducted over a period of 10000 days. In this context antithetic variates are not used to reduce variance. Instead, data from both, the *original* and the *antithetic* run, is presented to give an indication of the possible variation between runs. The variance between simulation scenarios is reduced by using common random numbers for all runs. The common random number seed for each of the following simulation runs is 269551662 which had also been used for examination of model behaviour under *standard* conditions.

As discussed in the analysis of model behaviour under *standard* conditions the *original* run represents a situation where the disease remains endemic within the model population, whereas the *antithetic* run is standing for a situation where disease disappears and is then reintroduced towards the end of the simulation run. Therefore, both runs represent two different scenarios which are likely to respond differently to controlled changes in parameters.

Simulation Run using Base Parameter Files without Immigration

This simulation scenario examines the behaviour of the model using *standard* conditions, except for removing immigration. This simulation should provide insight into the recovery mechanisms of the model.

Figure 86 show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* run. Table 37 list the summary statistics for the two simulation runs.

This simulation represents a scenario where mortality is only compensated by local reproduction. Given this situation population size averages at about 100 possums. This is slightly lower than the average of about 130 possums reached in the simulations using standard conditions which included immigration. In the *original* run tuberculosis infection is able to persist within the population whereas it disappears soon after beginning of the simulation in the *antithetic* run.

These results suggest that excluding immigration from the model does not change major aspects of model behaviour compared with the simulation under *standard* conditions. Local reproduction appears to balance mortality and emigration.

Figure 86: Time plot of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* run testing the effect of removing immigration

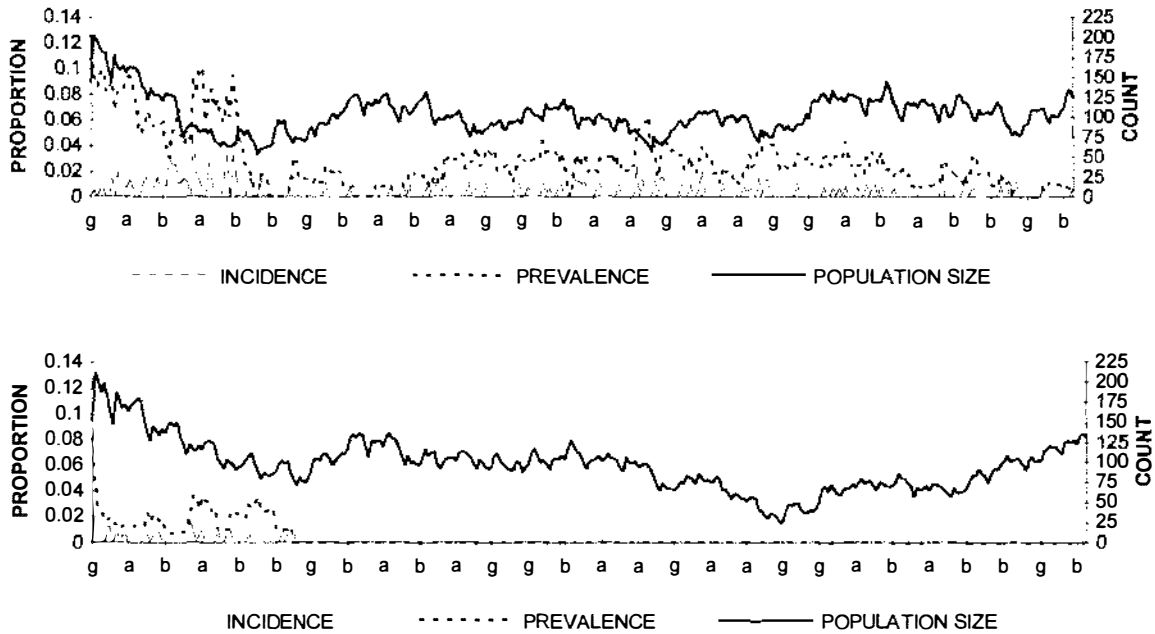


Table 37: Summary of simulation output for model testing the effect of removing immigration

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	102.9	101	101	24.1	52	202
	antithetic	96.8	99	101	32.0	24	214
CLINICAL INCIDENCE	*original*	0.004	0.000	0.000	0.007	0.000	0.048
	antithetic	0.001	0.000	0.000	0.002	0.000	0.016
CLINICAL PREVALENCE	*original*	0.028	0.024	0.000	0.023	0.000	0.125
	antithetic	0.004	0.000	0.000	0.009	0.000	0.072

Single Reduction in Population Size without Immigration

The effect of a single reduction in population size in the absence of immigration on population dynamics is tested by reducing the size of the population which is read into the model at the beginning of the simulation run, and then allowing it to recover. The population is reduced to 25% of the *standard* population, which represents a high intensity possum

control operation. No immigration occurs. Therefore all population recovery is by local breeding.

Figure 87 show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output from the two simulation runs. Table 38 list the summary statistics for both runs.

After a reduction of the *start* population to 25% the model population needs about 6 years to get back to average levels of about 100 animals. Tuberculosis infection disappears on average after 1-2 years during both runs. From this simulation scenario it appears that in order to eliminate infection with a single population reduction either the level of residual infection has to be low or the time until population recovery has to be prolonged.

Figure 87: Time plot of prevalence and incidence of clinical tuberculosis and population size for simulation output from *original* and *antithetic* run testing the effect of a single reduction in population density to 25% without immigration

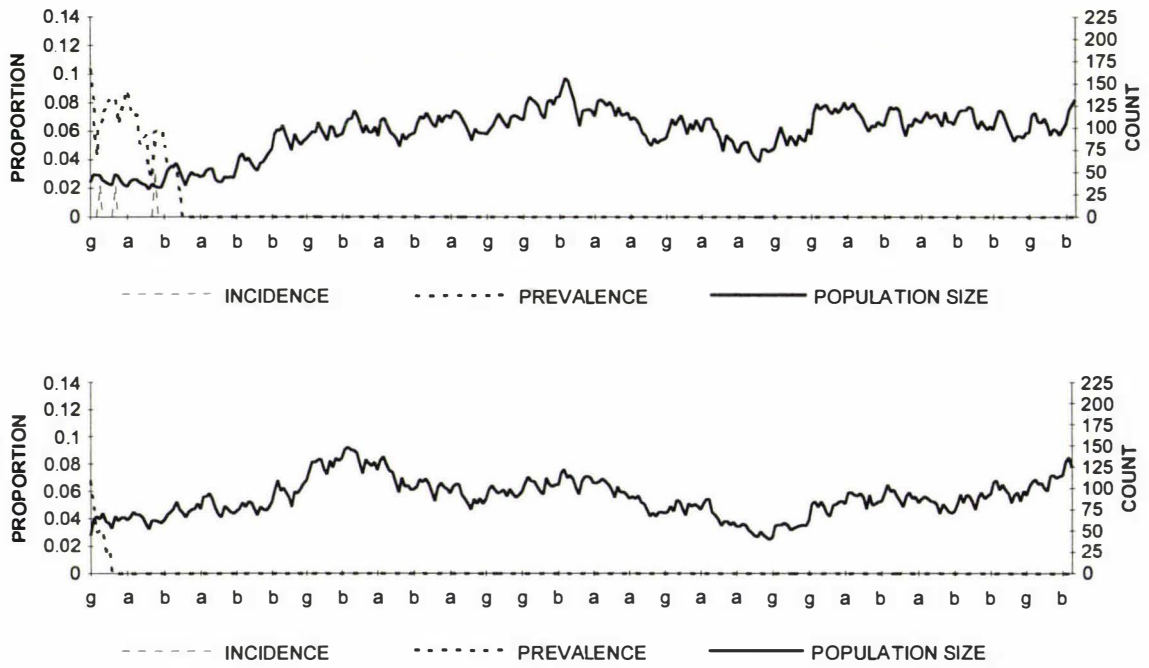


Table 38: Summary of simulation output for model testing the effect of a single reduction in population density to 25% without immigration

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	94.1	100	92	26.8	31	156
	antithetic	88.8	88	82	21.9	40	148
CLINICAL INCIDENCE	*original*	0.000	0	0.000	0.002	0.000	0.030
	antithetic	0	0	0	0	0	0
CLINICAL PREVALENCE	*original*	0.006	0.000	0.000	0.019	0.000	0.103
	antithetic	0.001	0	0	0.005	0.000	0.067

Single Reduction in Population Size in the Presence of Immigration Free from Clinical Tuberculosis

This simulation scenario investigates the effect of a single reduction in population to 25% of the size of the *standard* population, in the presence of immigrants which are free from clinical tuberculosis infection, on tuberculosis infection and population dynamics.

Figure 88 show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and *antithetic* runs. Table 39 list the summary statistics for the two simulation runs.

Given a reduction of the *start* population to 25% and immigration free from clinical tuberculosis, the model population requires about 3 to 4 years to get back to average levels of about 110 to 130 animals. During the *original* run when the population recovers over a shorter time period tuberculosis infection remains endemic in the population. In the *antithetic* run tuberculosis disappears during the first year.

Maintenance of infection during the *original* run is likely to be the result of two factors. The level of infection at the start of the simulation is slightly higher and population recovery occurs faster than in the *antithetic run.*

Figure 88: Time plot of results of *original* and *antithetic* run for prevalence and incidence of clinical tuberculosis and population size for a simulation scenario testing the effect of a single reduction in population density to 25% in the presence of immigration free from clinical tuberculosis

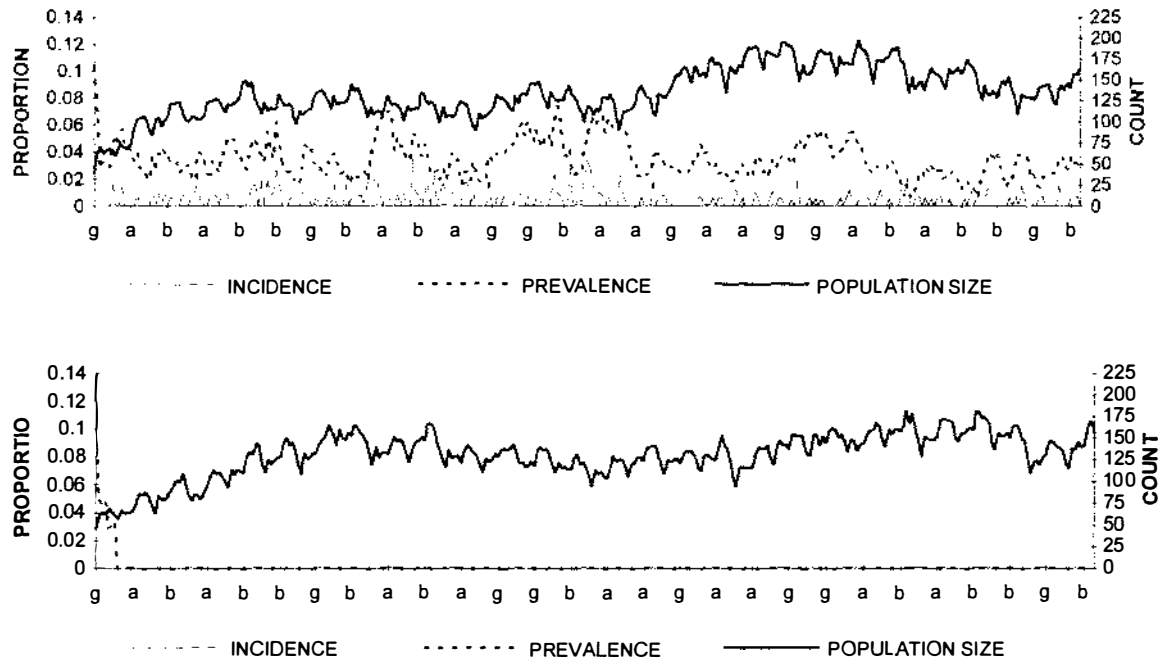


Table 39: Summary of simulation output for model testing the effect of a single reduction in population density to 25% in the presence of immigration free from clinical tuberculosis

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	133.8	130	133	28.9	39	198
	antithetic	130.0	133	128	25.7	46	181
CLINICAL INCIDENCE	*original*	0.005	0.000	0.000	0.007	0.000	0.036
	antithetic	0.000	0.000	0.000	0.000	0.000	0.000
CLINICAL PREVALENCE	*original*	0.035	0.033	0.038	0.014	0.006	0.103
	antithetic	0.001	0	0.000	0.007	0.000	0.087

Single Reduction in Population Size with Clinical Tuberculosis in Immigrants

This simulation scenario tests the effect of a single reduction in population size, in the presence of clinical tuberculosis infection in immigrants, on tuberculosis infection and population dynamics. The population is reduced to 25%, which represents a high intensity possum control operation. Prevalence of clinical tuberculosis in immigrating possums is 5%.

Figure 89 show time plots for prevalence and incidence of clinical tuberculosis and population size based on simulation output for the *original* and the *antithetic* run. Table 40 list the summary statistics for the two simulation runs.

Given a scenario involving a reduction of the *start* population to 25% and the presence of clinical tuberculosis in immigrants the model population requires about 3 to 4 years to reach average levels of about 110 to 130 animals. During the *original* run when population recovery occurs more quickly and tuberculosis infection remains endemic in the population. In the *antithetic* run tuberculosis disappears during the first year and multiple introductions through infected immigrants over the next 15 years are unsuccessful until the disease resurges during the final 8 months of the simulation.

The results of these two simulation runs suggest that given a sufficiently low tuberculosis prevalence after population reduction, tuberculosis infection will disappear and it is quite difficult for infection to restablish in the population through introduction from diseased immigrants.

Figure 89: Time plot of results *of original* and *antithetic* run for prevalence and incidence of clinical tuberculosis and population size for a simulation scenario testing the effect of a single reduction in population density to 25% in the presence of 5% clinical tuberculosis prevalence in the immigrants

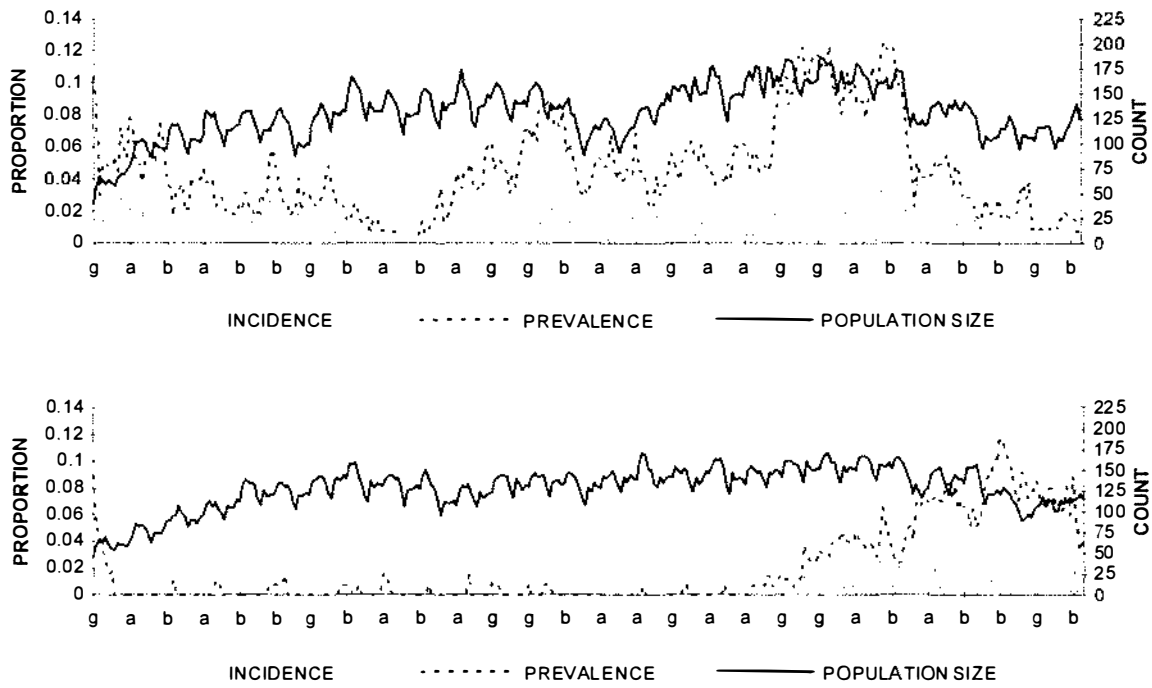


Table 40: Summary of simulation output for model testing the effect of a single reduction in population density to 25% in the presence of 5% clinical tuberculosis prevalence in immigrants

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	130.9	132	140	26.8	39	187
	antithetic	127.7	133	114	25.2	45	171
CLINICAL INCIDENCE	*original*	0.005	0.000	0.000	0.007	0.000	0.042
	antithetic	0.003	0.000	0.000	0.007	0.000	0.046
CLINICAL PREVALENCE	*original*	0.046	0.041	0.023	0.029	0.000	0.125
	antithetic	0.019	0.000	0.000	0.029	0.000	0.116

Permanent Reduction in Den Site Density in the Absence of Immigration

The importance of the factors controlling the spatial component in this simulation model is tested by reducing the number of available den sites by 50%. This was done in a random fashion by generating a uniformly distributed random number between 0 and 1 for each den site using the computer spreadsheet software package Microsoft EXCEL for Windows

version 4.0. Dens were excluded if the random number exceeded 0.5. *Base* parameter set files were used in this simulation scenario, except for disabling the immigration mechanism.

A time plot for prevalence and incidence of clinical tuberculosis and population size, based on simulation output from both runs for the scenario testing the effect of a reduction in den site density, is presented in figure 90. Table 41 show the summary statistics for the two simulation runs.

Reduction in den site density by 50% results in a reduction of population size to about 90 animals. This figure is slightly lower than the population size of about 100 reached in the simulation scenario where the full number of dens had been used but immigration was absent (see table 37). The model requires about 2 years to reach the population levels which the simulated habitat is capable of carrying. Tuberculosis infection disappears from the population within a period of 5 to 10 years. These results suggest that the model population density was too low to maintain endemic disease levels in the simulated population.

Figure 90: Time plot of prevalence and incidence of clinical tuberculosis and population size based on *original* and *antithetic* run for simulation output showing the effects of a permanent reduction in den site density in the absence of immigration

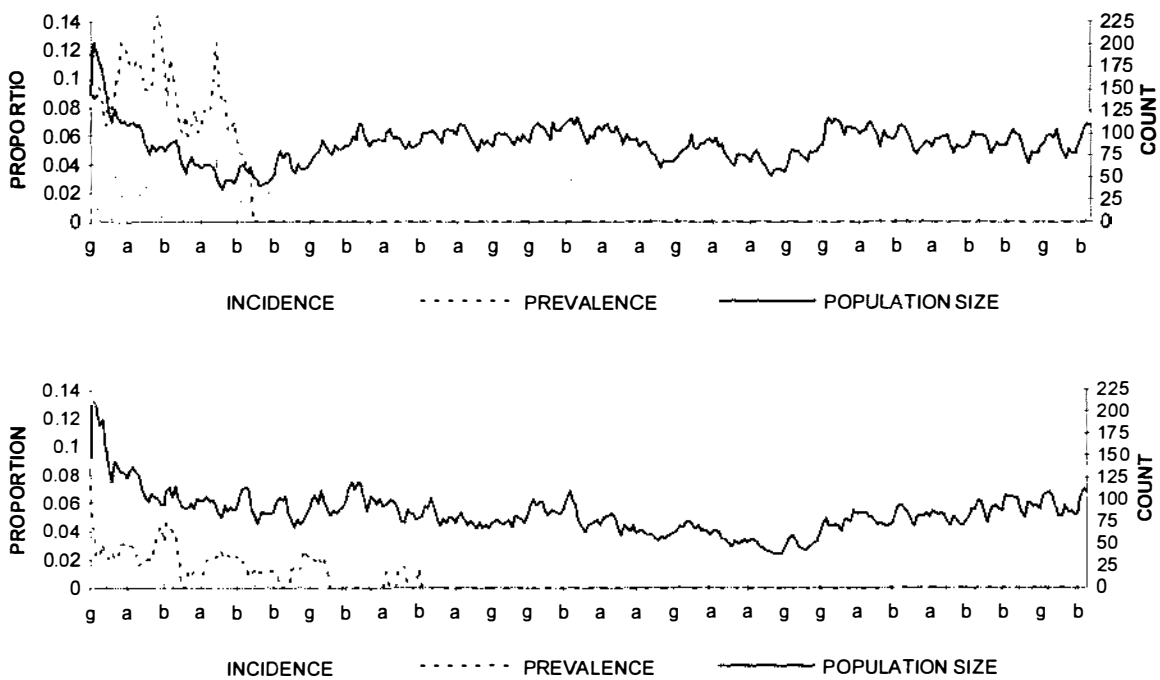


Table 41: Summary of simulation output for model testing the effect of a permanent reduction in den site density in the absence of immigration

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	88.3	89	87	21.2	36	202
	antithetic	84.6	83	85	23.9	38	212
CLINICAL INCIDENCE	*original*	0.002	0.000	0.000	0.009	0.000	0.074
	antithetic	0.001	0.000	0.000	0.003	0.000	0.021
CLINICAL PREVALENCE	*original*	0.014	0.000	0.000	0.034	0.000	0.145
	antithetic	0.005	0.000	0.000	0.010	0.000	0.072

Permanent Reduction in Den Site Density in the Presence of Immigrants free from Clinical Tuberculosis

The objective of this simulation scenario was to examine the effect of a reduction in den site density on infection and population dynamics in a situation where immigrants do not carry infection with *Mycobacterium bovis*.

A time plot for prevalence and incidence of clinical tuberculosis and population size based on simulation output from both runs for the scenario testing the effect of a reduction in den site density is presented in figure 91. Table 42 show the summary statistics for the two simulation runs.

Reduction in den site density by 50% results in a reduction of population size to about 105 animals. The model requires about 2 to 3 years to reach the population levels which the simulated habitat is capable of carrying. The combination of sufficient residual infection and number of susceptible animals could explain why tuberculosis infection does not disappear from the population in the *original* run.

Figure 91: Time plot of prevalence and incidence of clinical tuberculosis and population size based on *original* and *antithetic* run for simulation output showing the effects of a permanent reduction in den site density in the presence of immigration free from clinical tuberculosis

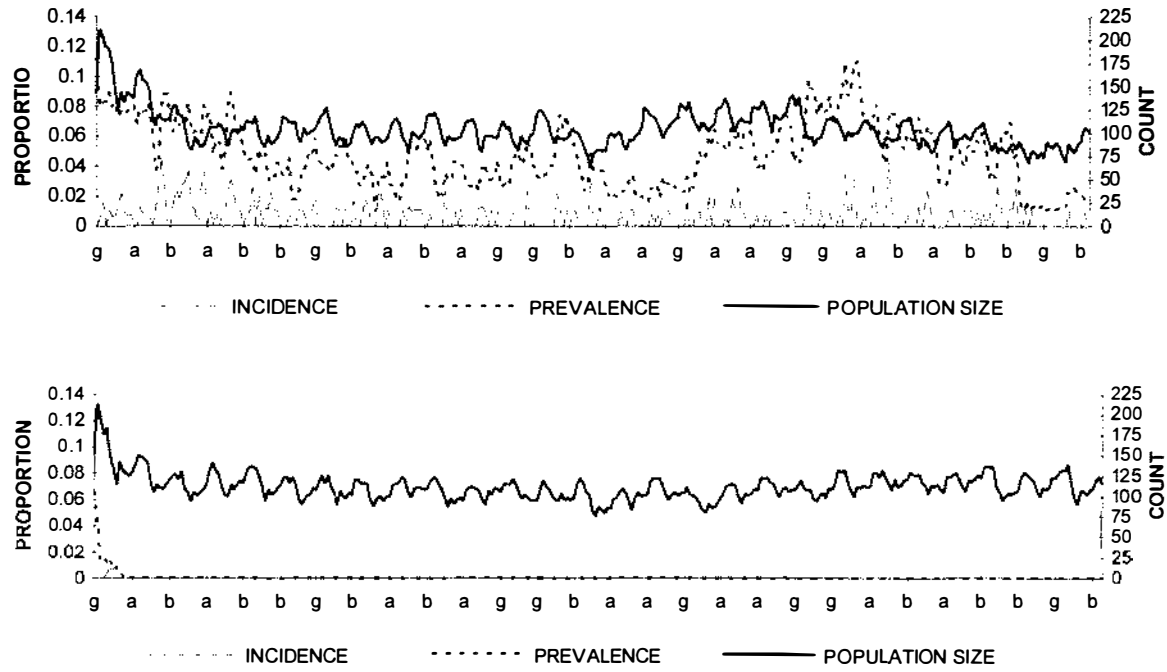


Table 42: Summary of simulation output for model testing effect of permanent reduction in den site density in the presence of immigration free from clinical tuberculosis

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	original	104.9	101	97	20.1	63	211
	antithetic	112.6	111	115	16.2	76	214
CLINICAL INCIDENCE	original	0.007	0.000	0.000	0.009	0.000	0.045
	antithetic	0.000	0	0.000	0.001	0.000	0.009
CLINICAL PREVALENCE	original	0.048	0.045	0.063	0.022	0.010	0.112
	antithetic	0.001	0	0.000	0.005	0.000	0.066

Permanent Reduction in Den Site Density with Clinical Tuberculosis in Immigrants

In this simulation scenario the number of den sites was permanently reduced to 50% and *base* parameter settings were used to run the simulation.

A time plot for prevalence and incidence of clinical tuberculosis and population size based on simulation output from both runs for the scenario testing the effect of a reduction in den site density is presented in figure 92. Table 43 show the summary statistics for the two simulation runs.

Reduction in den site density by 50% in the presence of immigration results in a reduction of population size to about 100 animals. This figure is similar to the population size reached when using the full number of dens, but removing immigration from the simulation. It appears that if the number of dens is reduced then disease transmission occurs more readily, so that the disease can persist in both runs. A likely explanation for this result is that given the lower number of total available den sites possums are more likely to share dens sequentially and therefore to become infected. It is notable that in this *antithetic* run which in all other scenarios resulted in disease disappearing shortly after the start of the simulation, disease was able to recover from very low levels to reach an endemic state within a few simulated years.

Figure 92: Time plot of prevalence and incidence of clinical tuberculosis and population size based on *original* and *antithetic* run for simulation output showing the effects of a permanent reduction in den site density, in the presence of immigration with 5% clinical tuberculosis prevalence

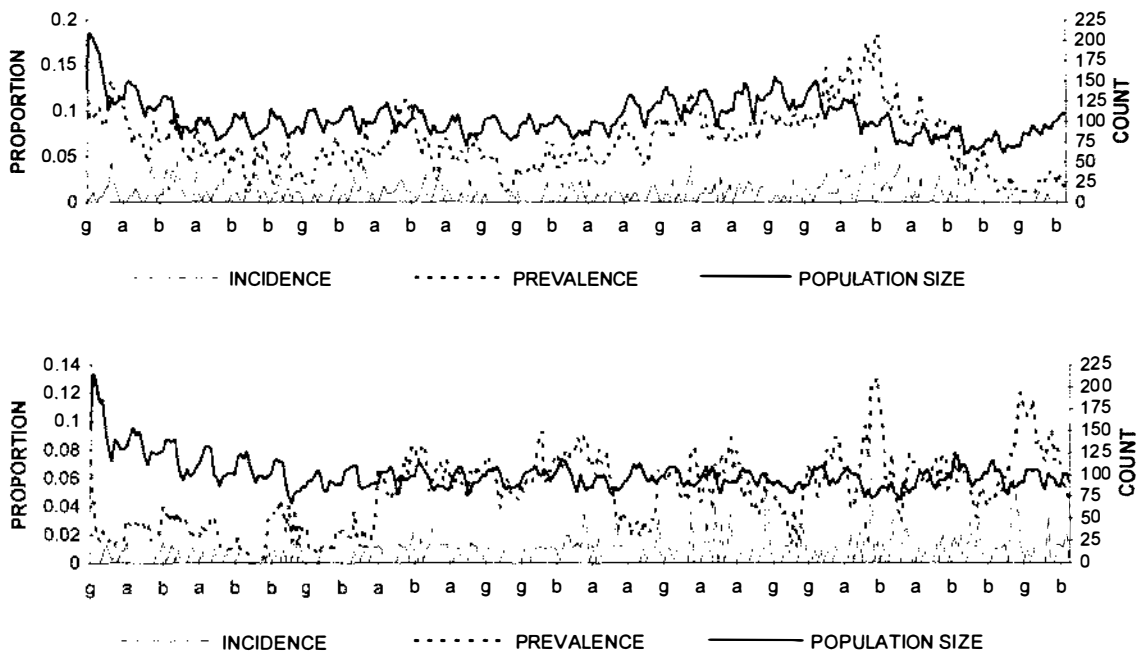


Table 43: Summary of simulation output for model testing effect of permanent reduction in den site density, in the presence of immigration with 5% clinical tuberculosis prevalence

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	103.2	99	82	22.8	60	209
	antithetic	100.5	98	100	18.5	67	215
CLINICAL INCIDENCE	*original*	0.010	0.008	0.000	0.012	0.000	0.062
	antithetic	0.007	0.000	0.000	0.010	0.000	0.063
CLINICAL PREVALENCE	*original*	0.066	0.063	0.080	0.033	0.009	0.183
	antithetic	0.050	0.051	0.057	0.026	0.000	0.130

Repeated Population Reduction at Three - Yearly Intervals in the Presence of Clinical Tuberculosis in Immigrants

The effectiveness of a repeated reduction in population size is tested for a possum population with endemic tuberculosis infection and clinical tuberculosis in immigrants (*base* parameter set files). The simulation runs are conducted by replacing the *base* parameter set file at predetermined intervals (*Control interval*) for a specific duration (*Control duration*) with a *control* parameter file, which adjusts the survival parameters to simulate the effect of a possum control mechanism. After each control operation the control parameter file is replaced with the standard parameter file until the next control operation.

A time plot for prevalence and incidence of clinical tuberculosis and population size based on simulation output from both runs for the scenario testing the effect of a reduction in den site density is presented in figure 93. Table 44 show the summary statistics for the two simulation runs.

Repeated reductions in population density at three yearly intervals result in considerable variation in population size between about 30 and more than 100 animals. The population takes about 3 years to recover to the level it had before the last reduction in population size. It appears that in both runs tuberculosis infection could not maintain itself in an endemic state without introductions through infected immigrants.

Figure 93: Time plot of prevalence and incidence of clinical tuberculosis and population size based on *original* and *antithetic* run for simulation output showing the effects of repeated population control operations at 3 yearly intervals in the presence of 5% clinical tuberculosis infection in immigrants

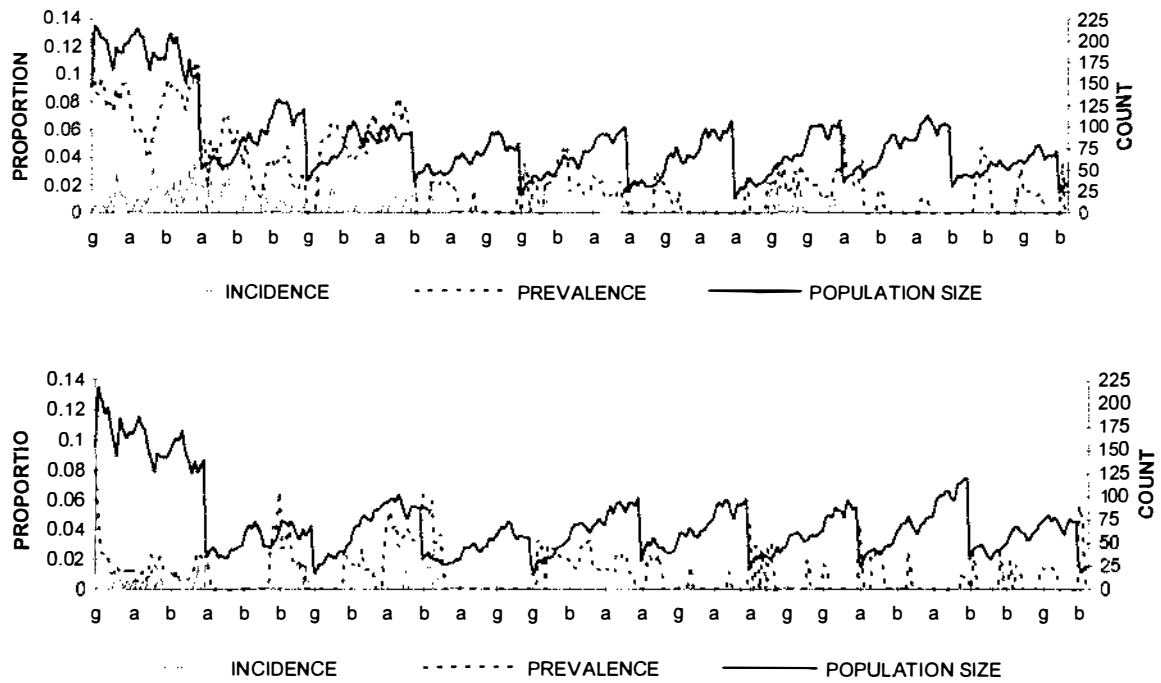


Table 44: Summary of simulation output for model testing effect of repeated population control operations at 3 yearly intervals in the presence of 5% clinical tuberculosis in immigrants

	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	81.5	69	90	43.9	16	216
	antithetic	70.7	63	46	38.3	15	218
CLINICAL INCIDENCE	*original*	0.003	0.000	0.000	0.008	0.000	0.051
	antithetic	0.001	0.000	0.000	0.004	0.000	0.032
CLINICAL PREVALENCE	*original*	0.025	0.018	0.000	0.028	0.000	0.124
	antithetic	0.010	0.000	0.000	0.014	0.000	0.078

Repeated Control Operations at Six - Yearly Intervals in the Presence of Clinical Tuberculosis in Immigrants

This simulation scenario represents the effect of a repeated reduction in population size at six yearly intervals.

A time plot for prevalence and incidence of clinical tuberculosis and population size based on simulation output from both runs for the scenario testing the effect of a reduction in

den site density is presented in figure 94. Table 45 show the summary statistics for the two simulation runs.

The period of six years between population reduction is sufficient for the population to recover to levels of more than 110 possums which is similar to the population size without any population control. Tuberculosis infection remains endemic in both runs. In fact, it appears that disease levels peak shortly after each control operation.

Figure 94: Time plot of prevalence and incidence of clinical tuberculosis and population size based on *original* and *antithetic* run for simulation output showing the effects of repeated population control operations at 6 yearly intervals in the presence of 5% clinical tuberculosis infection in immigrants

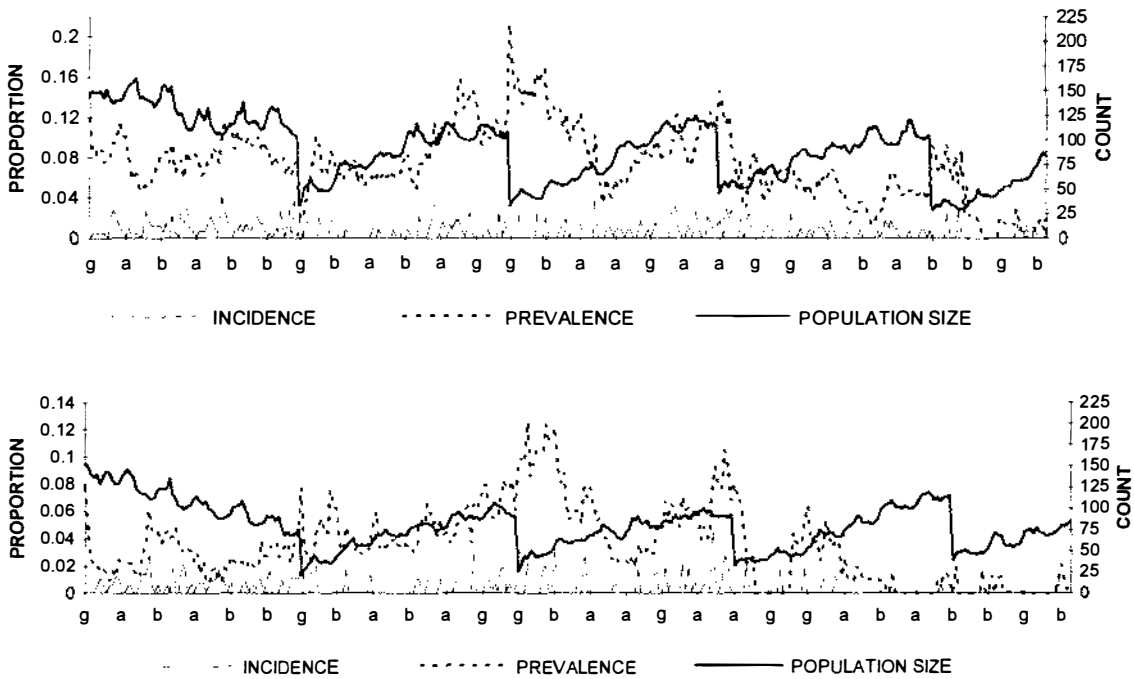


Table 45: Summary of simulation output for model testing effect of repeated population control operations at 6 yearly intervals in the presence of 5% clinical tuberculosis in immigrants

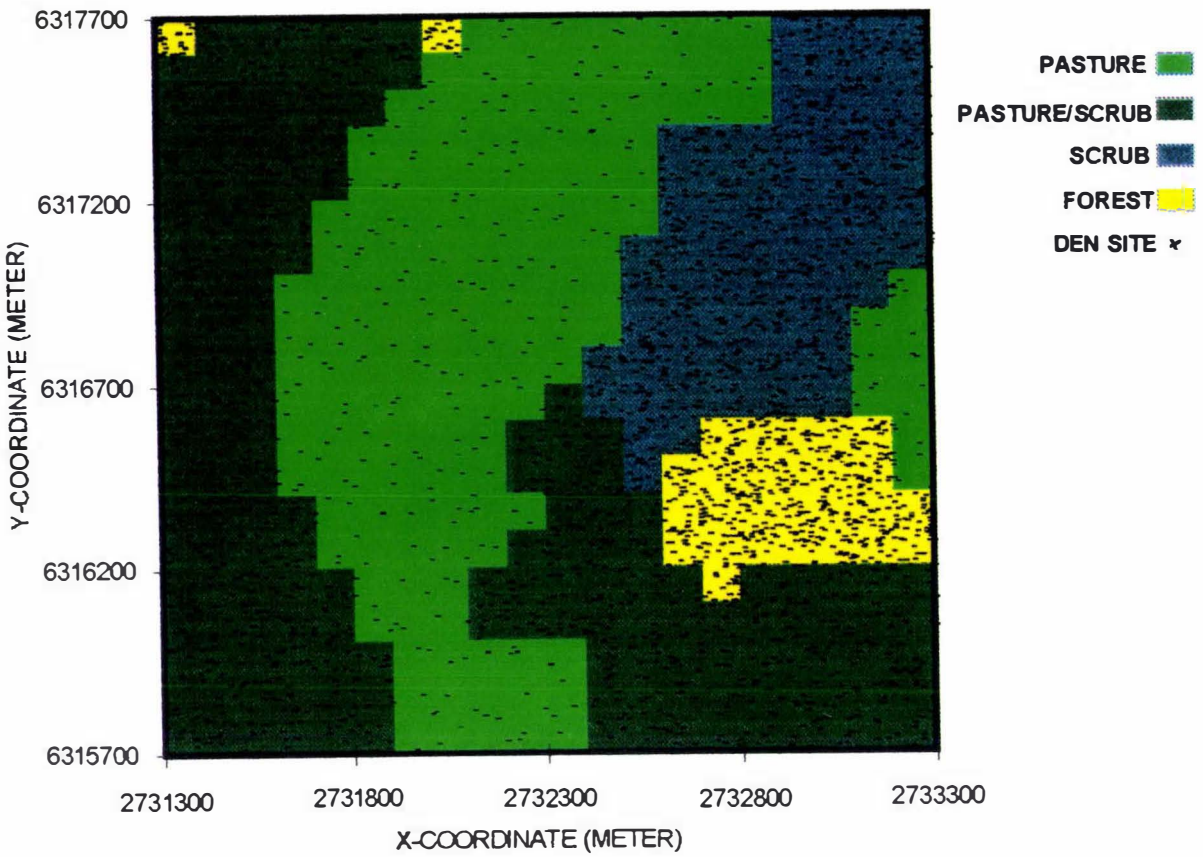
	Run	Mean	Median	Mode	Standard Deviation	Minimum	Maximum
POPULATION SIZE	*original*	111.7	116	141	42.0	33	216
	antithetic	97.5	97	114	35.4	26	214
CLINICAL INCIDENCE	*original*	0.007	0.000	0.000	0.009	0.000	0.041
	antithetic	0.004	0.000	0.000	0.008	0.000	0.045
CLINICAL PREVALENCE	*original*	0.074	0.074	0.000	0.036	0.000	0.211
	antithetic	0.035	0.032	0.000	0.028	0.000	0.125

Simulation over a 400 Hectare Area

A simulation over a 400 hectare area was conducted to test the model's capacity for performing large scale simulation experiments. An area in the southern Waikato region was selected as the hypothetical site for the experiment. It is an area within the tuberculosis case-control study area discussed elsewhere in this thesis. The location of the area is defined by the following NZ map grid coordinates: x_{min} = 2731300, x_{max} = 2733300, y_{min} = 6315700 and y_{max} = 6317700. Digital vegetation data for the area was available from New Zealand Land Resource Inventory (Hunter and Blaschke 1986). The data was stored as a polygon coverage in PC Arc/Info vector format. Vegetation cover classification as defined by NZLRI was recategorized into 4 major classes according to expected densities of possum den sites. Vegetation class 1 included all types which were predominantly pasture, class 2 consisted of map units covered by a mixture of pasture and scrubland, class 3 was assigned to map units dominated by scrubland and class 4 represented a dominance of forest cover. The required area was extracted from the vegetation coverage and converted into ERDAS raster format with 100m cell height and width using PC-Arc/INFO module POLYGRID. This data was imported into the geographic information system computer software IDRISI version 4.1. The data file storing the cell ids which represent the four different vegetation classes was then imported into the computer spreadsheet software Microsoft EXCEL for Windows version 4.0 in order to produce variables describing the coordinate boundaries of each cell in the raster image. The data was then imported into the database management software Borland Paradox 4.0. A program written in the Paradox Application Language was used to generate the required number of dens per cell and random x,y coordinates within the boundary limits of each cell. This whole procedure can be much simplified by writing a Pascal program which accesses IDRISI images directly and generates the spatially stratified random coordinates of the den locations. For this simulation exercise it was assumed that vegetation class 1 (pasture) would provide 2 den sites, vegetation class 2 (pasture/scrub) 6 den sites, vegetation class 3 (scrubland) 13 den sites and vegetation class 4 (forest) 26 den sites per $100m^2$. These estimates are based on the number of den sites which were identified during the first 22 months of the longitudinal study (254 dens over 20 hectares) and the assumption that the longitudinal study area can be classified as predominantly scrubland.

The figures probably will have to be revised as soon as more accurate information on den site concentrations in different habtitat types is available. It is emphasized that den site location as used in the simulation model should probably be interpreted in a more abstract sense as center of possums' night activity areas, and den site density could represent resource availability. For this simulation experiment a map with 2972 den site locations randomly distributed within each $100m^2$ cell of the simulation area was produced using the process described above (see figure 95).

Figure 95: Rasterized map of major vegetation cover classes in simulation area and locations of random den sites

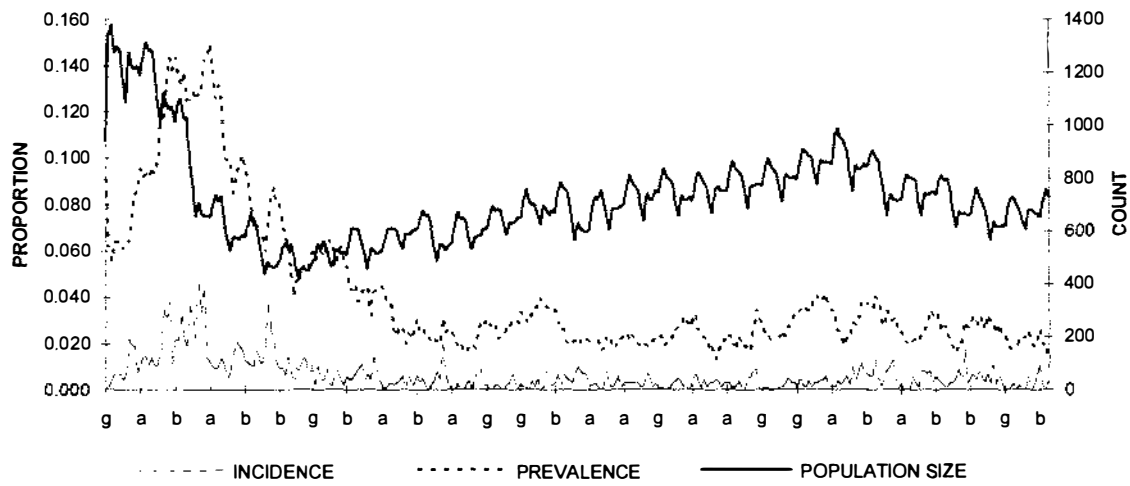


A simulation consisting of an *original* and *antithetic* run was conducted for a duration of 10000 days. A start population of 1000 possums was used for the simulation. The age and sex structure of the population was taken from the longitudinal study population. It was generated using a procedure for random number generation in Microsoft EXCEL for Windows 4.0. It was decided to run the simulation assuming that immigrants would not carry *Mycobacterium bovis* infection. This would ensure that random introductions of disease did not disturb emergence of spatial patterns of infection within the population. During a number of preliminary runs it was found that using the *base* parameter settings, but disabling infection in immigrants, it was not possible to maintain endemic infection within the simulated population. To ensure maintenance of infection at reasonable levels it was decided to increase the probabilities assigned to each of the transmission mechanisms for the purposes of this exploratory simulation exercise. Both the probability of infection through spatial and through temporal proximity, were increased from 0.0011 to 0.01 and the probability of infection during mating from 0.0056 to 0.50.

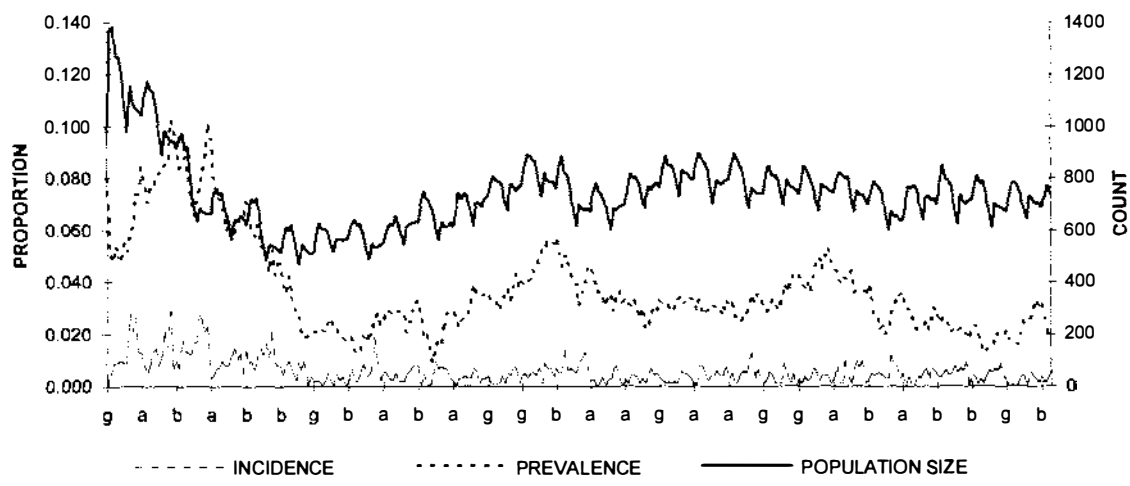
The results of this simulation are presented in the following figures. As the objective of this simulation experiment was only to assess the model's capacity for simulating larger geographical areas, no detailed statistical analyses were conducted. Figure 96a shows the time plots of incidence and prevalence of clinical TB as well as of population size for the *original*

and the *antithetic* simulation run. Infection was present in the population during the whole simulation period of 28 years during the *original* run stabilizing at a level of 2% to 3% clinical tuberculosis prevalence and after the period of transition of about 10 years reached up to about 5% during the *antithetic* run. The spatial and temporal pattern of TB den site locations during this simulation period is represented by the sequence of maps in figures 96c and d. The maps show that the disease maintained itself in a main cluster of infection in forest habitat. Occasionally infection spread across to scrub and pasture/scrub habitat, but only very rarely on to pasture. Figure 96e displays the locations of den sites used by possums with clinical tuberculosis cumulative for the years 10 to 28 of the *original* simulation run. Infection was clustered mainly within areas of highest concentration of den sites (e.g. forest and scrubland).

Figure 96a: Time plots of incidence/prevalence of clinical tuberculosis and population size for both simulation runs over 400 hectare area



***original* run**



***antithetic* run**

Figure 96b: Locations of den sites used by possums with clinical tuberculosis for years 1 to 12 for o*riginal* run over 400 hectare area

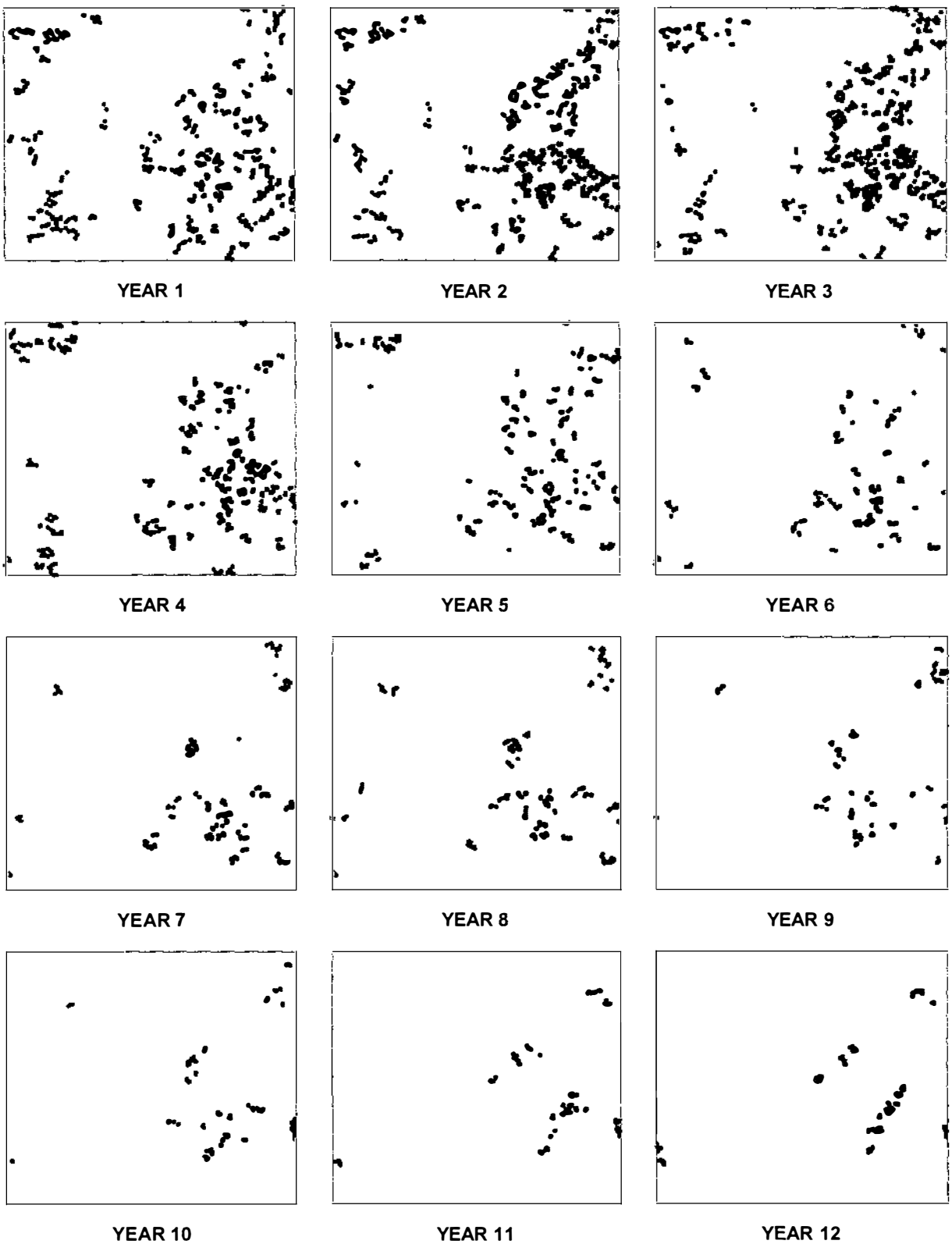


Figure 96c: Locations of den sites used by possums with clinical tuberculosis for years 13 to 24 for o*riginal* run over 400 hectare area

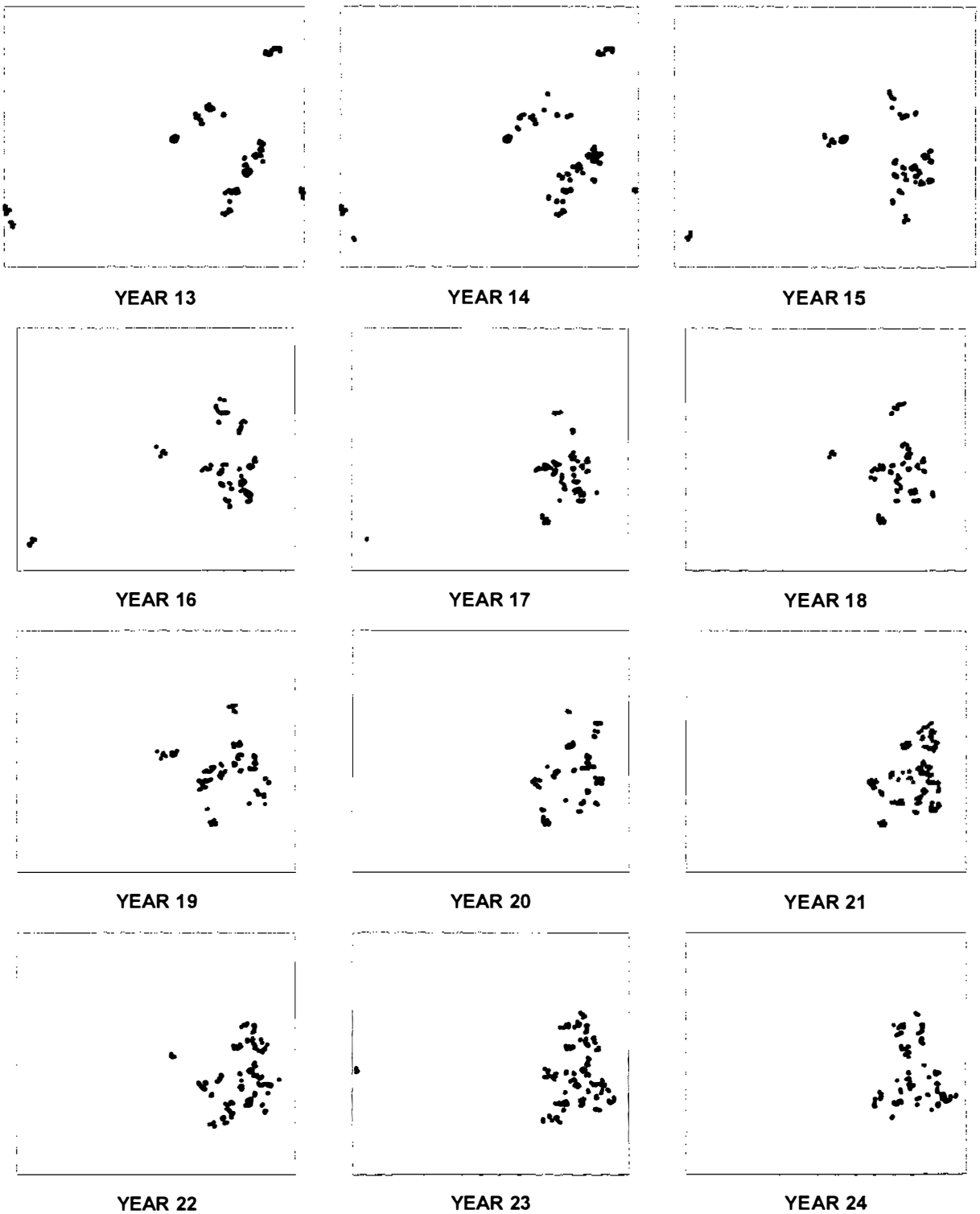


Figure 96d: Locations of den sites used by possums with clinical tuberculosis for years 25 to 28 for *original* run over 400 hectare area

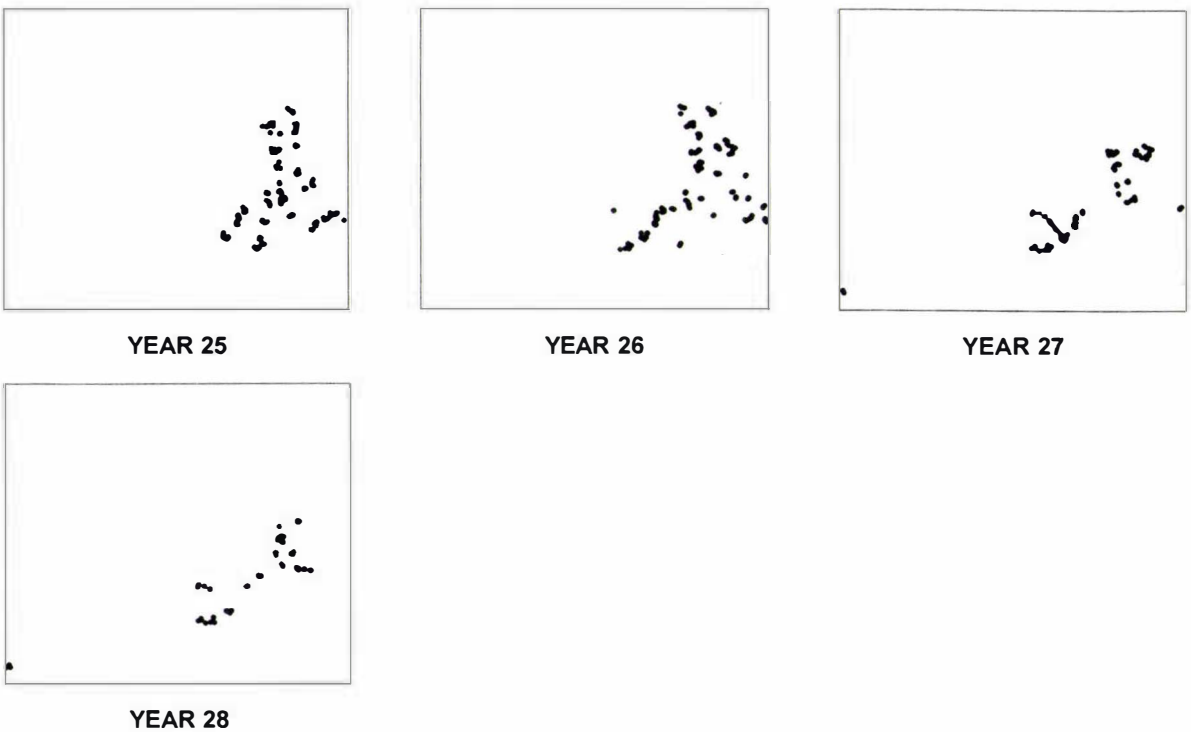
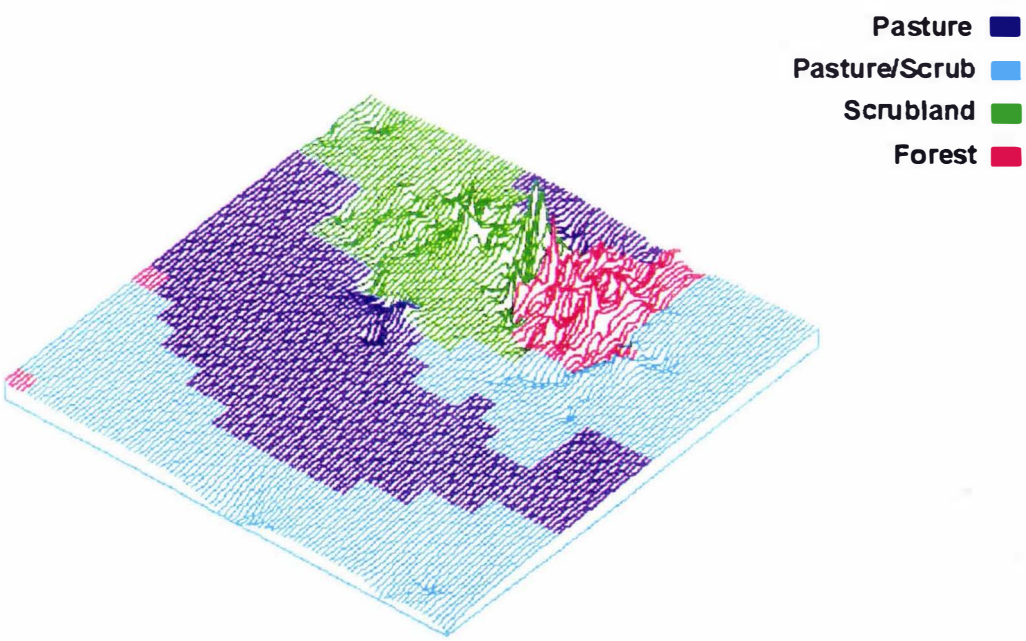


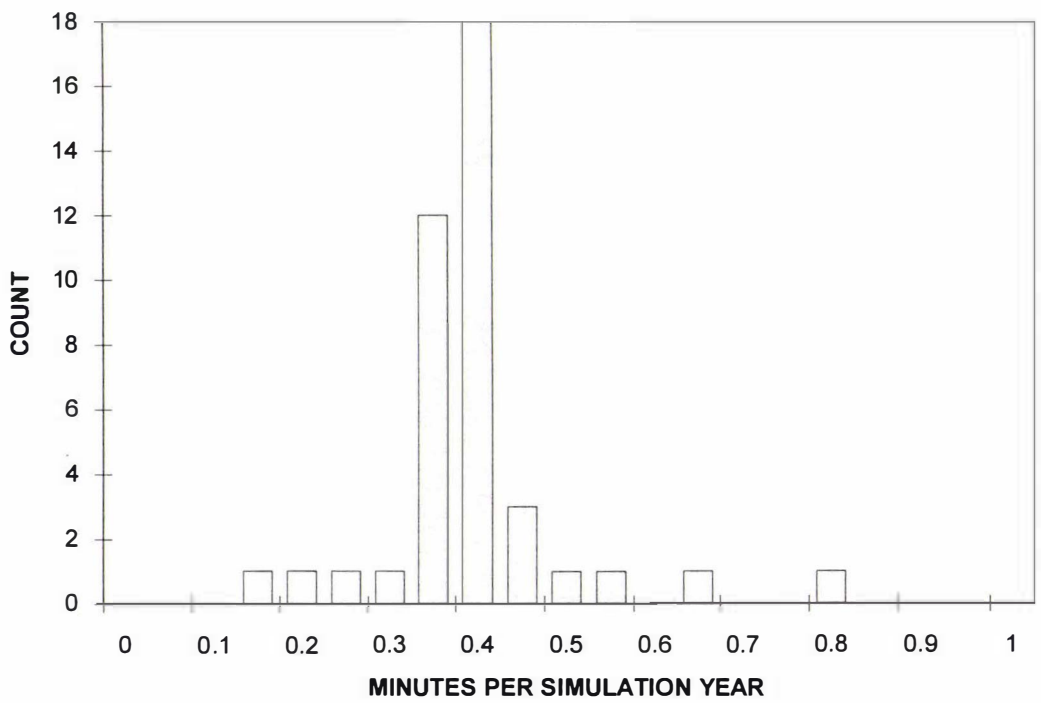
Figure 96e: Digital elevation model with height representing frequency with which clinically tuberculous possums use den sites and shades of grey representing vegetation type based on cumulative TB den site locations used between years 10 and 28 of the *original* run of the simulation over 400 hectare area



Model Performance

The performance of the simulation model was measured using the minutes required to simulate a model year as an indicator. The simulation runs were conducted on a personal computer with a 80486DX2 microprocessor running at 66 megahertz as the central processing unit, equipped with 16 megabyte of random access memory (RAM). Microsoft Windows for Workgroups Version 3.11 was running in "enhanced mode" which allows the simulation of several instances of the model concurrently. Data on 45 simulations is included in the following results. Figure 97 shows the distribution of the time in minutes real time required to simulate one year simulation time. On average it took about 10 minutes to simulate 10000 days for population similar to the size of the possum population from the longitudinal study. During the simulation over the large 400ha area simulation time was about 111 minutes. It took about a minute to simulate one simulation model year.

Figure 97: Histogram of time required to simulate one year of simulation time based on data from simulation runs over the 21ha longitudinal study area



DISCUSSION

Comparison of Model Output and Field Data

The results of the analysis of output produced by this simulation model suggest that it provides a reasonable initial approximation to the spatial and temporal epidemiology of *Mycobacterium bovis* infection in possum populations. There are some limitations however. These include possum survival estimates and immigration of possums. Information which will generate better estimates for these parameters will be available at the end of 1994, once the longitudinal study is terminated. At that time it will also be necessary to review the estimates used for various probabilities which govern transmission of infection and transition from subclinical to clinical disease, in the light of data covering the full study period.

The structure of the model represents the author's current understanding of the important processes in the epidemiology of tuberculosis infection in possum populations. The model is flexible and sufficiently general that it can be applied to different locations and populations by adjusting the input parameters and input data files. It is also possible to use the model to simulate the dynamics of *Mycobacterium bovis* over areas larger than the longitudinal study area. The model is programmed as an application running under Microsoft Windows (version 3.0 and higher) which allows simulation of meta populations only limited by available computer memory. A dispersal module will have to be added to the model in order to simulate this aspect of possum ecology, which was not important for simulation of a local population. Due to the modular structure of the model this can be achieved quite easily.

Given the current model structure this model can be used to simulate the dynamics of any disease which has a stable spatial component in its epidemiology (such as the den locations of possums in this simulation exercise), and which has only limited social group relationships. The parameter and data files which are used to initialize and control model operation would have to be adjusted accordingly. By appending a module representing social group behaviour, the model could be extended to allow simulation of the epidemiology of other broadly similar diseases such as tuberculosis in badgers and rabies in various wildlife populations.

The model calibration and the subsequent validation process which has led to the final parameter set file (*base* parameter files) have demonstrated the inadequacy of some of the data which is currently available. During the model experimentation process it became clear that the survival and immigration parameters currently used require better estimates. However, it is unlikely that this will change the dynamics of the disease in the local population significantly. The mechanisms which are implemented in the model appear to include most of the processes which are necessary to model the disease and population dynamics in a possum population. The spatial component of the model represents a new approach to spatial disease modelling. It allows mechanisms which require a spatial dimension such as reproduction and disease transmission to be represented realistically. One of the major shortcomings of

deterministic modelling approaches is that it is relatively difficult to represent heterogeneity (Anderson *et al* 1991). In this simulation model, both spatial and other types of heterogeneity are represented through the mechanisms built into the model.

The objective of this modelling exercise was to gain further insight into the epidemiology of *Mycobacterium bovis* infection in possum populations and to test the effectiveness of possible options for controlling tuberculosis.

Because only a limited amount of field data is currently available, it was not possible to perform an extensive validation of the model results. The parameter settings which were developed based on the results of the longitudinal study resulted in population sizes which were lower than those found in the field. This suggests that either the estimates for the parameters controlling population processes were incorrect or that there was a significant *'edge effect'* which resulted in inflated field population estimates. The main factors influencing population dynamics are reproduction, survival and immigration. The yearly pattern for proportion of adult females with pouch young showed large discrepancies when compared with the field data. It appeared that in general too many females had a pouch young. This proportion of females with a dependent young represents an emergent property of the model, because it depends on male-female contact patterns and is only indirectly controlled in the model as currently designed. During the longitudinal study only relatively unreliable information about the duration of the dependency relationship between mother and young could be collected, so that it was not possible to obtain better estimates for this model parameter. The possible impact of incorrect estimates for this parameter has to be taken into account during the later consideration of the effectiveness of different control strategies.

Comparison between field data and simulation output shows that the model represents survival in clinically diseased possums reasonably well. This finding suggests that the disease dynamics produced by the model are a realistic representation of the field situation. As more field data from the longitudinal study becomes available the agreement between model output and field results will have to be reassessed. The model produces a spatially and temporally clustered pattern of den sites which appears to be relatively stable in space. This behaviour is consistent with the design, as transmission mechanisms built into the model have a strong spatial component. It is somewhat surprising though that the model can produce a stable spatial infection cluster at some sites over many years. Although this phenomenon was exhibited in an area with high den site density it is not only dependent on den site density, because another area with similarly high den site density did not produce a permanent infection cluster. It is likely that persistence of the cluster arose out of the combination of ample den sites in the vicinity plus a substantial number of possums becoming infected. Once a "critical mass" of infected possums and den sites was established, it would be possible for it to persist over many years due to the nature of transmission mechanisms in the model.

The results of the time series analysis do not indicate the presence of long-term cycling patterns in any of the disease or population parameters examined. Most parameters did show a strong seasonality, except for subclinical tuberculosis incidence. Anderson and Trewhella (1985) developed a stochastic version of a deterministic model for tuberculosis in badgers. Simulating population and infection dynamics for an area of 100km^2 and 100 years, their model produced a 10 to 15 year period between peaks in badger density and infection prevalence. In this possum tuberculosis simulation model, seasonal effects clearly dominated infection as well as population dynamics. The short life expectancy of possums compared with badgers probably explains the difference in cyclicity.

The results of spectral analysis further demonstrate the difference between incidence and prevalence patterns as produced by the simulation model. Incidence of infection with *Mycobacterium bovis* does not appear to be seasonal at all in the model. Of the three disease transmission mechanisms built into the model, only the one which is related to mating would be able to produce a biannual effect. The other two mechanisms do not depend directly on season of the year. Prevalence of clinically and subclinically tuberculous possums is seasonal. It is interesting that the seasonal variation in the prevalence of infectious animals does not produce seasonality of incidence of infection, under the assumptions currently built into the model.

Evaluation of Disease Control Options with the Current Model

The current model is still in a relatively early stage of development. The assumptions used for developing the model and the parameters used for running the simulations require further refinement and validation against field data. Therefore, the results of these preliminary model experiments have to be interpreted very cautiously, since a number of features of the model require refinement, and the parameters are as yet very provisional. The objective of the model experiments is to test the behavioural characteristics of the *model*, not to predict real-world results.

The simulation runs were conducted using the same random number streams as had been used for the *main* run using *standard* conditions. It is therefore expected that in a comparison with the *main* run most changes in model behaviour after adjusting model input are likely to be a direct effect of these adjustments, rather than random variation.

The standard method for disease control in wildlife populations is by directly killing animals. In the case of the possum in New Zealand this is done mainly by poisoning with compound 1080 (sodium monofluoroacetate). The effect of such poisoning operations on a possum population was implemented in this simulation model by reducing the population size at the start of the simulation run and by changing survival estimates for short periods at different intervals during a simulation run. Empirical data on the effectiveness of 1080 aerial poisoning from 11 control operations during the 1980s suggests that on average a 71% reduction in population size can be expected, with 8 operations achieving a kill between 50%

and 80% (Batcheler and Cowan 1988). In the analysis of the current model the effect of a reduction in population size by 75% was tested when applied singly or repeatedly. Single control was evaluated using scenarios without immigration, with tuberculosis-free immigration and with tuberculous immigrants. Assuming no immigration a single reduction in population size by 75% did successfully remove infection from the population during both simulation runs. In the presence of tuberculosis-free immigration a single reduction in population size did remove infection from the population in the *original* run, but not in the *antithetic* run. Comparing simulation output from both runs it appears that the effectiveness of control depended on the level of residual infection in the population and the time required for population recovery. In the current model both effects are subject to stochastic variation. If immigrants were assumed to have a 5% tuberculosis prevalence, disease remained endemic in the *original* run and disappeared in the *antithetic* run, but was reintroduced towards the end of the simulation. Hence, model behaviour was similar to the behaviour during the *main* simulation exercise under *standard* conditions. The simulation model was then used to test the effect of repeated 75% population reduction in the presence of 5% clinical tuberculosis prevalence in immigrants at three - and at six- yearly intervals. Repeated population reduction had been recommended by Barlow (1991) as being able to reduce possum densities by at least 90% in 8 years and eventually eliminate TB. Given a 75% population reduction at three year intervals disease was present in the population most of the time, but appeared to be largely dependent on frequent disease introduction through infected immigrants. Repeated population reduction at 6 yearly intervals seemed, somewhat surprisingly, to result in an increase in disease prevalence during both runs. Prevalence levels peaked shortly after disease control. This finding could be explained by the fact that infected immigrants constitute a larger proportion in the reduced population after control has been applied. These results suggest that prevalence levels in immigrants were a major factor in determining effectiveness of control. If there is a 5% clinical prevalence among immigrants, the success of applying control through population reduction is extremely uncertain. It should be noted that 5% is a high prevalence in immigrants, and likely to be the maximum found in the field, not an average figure.

The process of establishment of infection in a population free from infection, through introduction by infected immigrants, was indirectly tested. During the runs where infection had disappeared after population reduction with a 5% prevalence in immigrants it took about 15 years of repeated attempts until infection could be reliably re-established within the population.

The effect of a 50% reduction in den site density on the persistence of *Mycobacterium bovis* infection within a local possum population produced some rather surprising results. In the absence of immigration infection disappeared in both runs after about 5 to 9 years. In the presence of disease-free immigration infection remains endemic in the *original* run and disappeared in the *antithetic* run. With 5% clinical disease present in immigrating possums both simulation runs resulted in a situation of stable disease levels averaging 5% to 6%. Given

the reduced den site density and a relatively large number of possums per available den (100 possums per 125 dens compared with 130 possums per 250 dens in *main* run) it appears that it was easier for infected immigrants to introduce infection into the population. Caughley (1977) writes that where possible, attempts at lowering population densities should be aimed towards manipulating the habitat, because this method has only a few of the drawbacks of direct control or control by pathogens. As the current model is still in a preliminary phase of model development results of this simulation should not be used to assess the effectiveness of habitat manipulation for controlling bovine tuberculosis in possum populations. It is likely that the settings for the population density-dependent mortality mechanism in this simulation require some adjustment which would then result in a reduction of population size.

Control of reproduction was difficult to test with the model at this stage. It has been suggested that methods of sterilization which affect social behaviour have the potential for a population to become destabilized (Bomford 1990). Barlow (1991) evaluated the effect of permanent sterilisation of females. He found that if 70% of females could be sterilised, this would still have much less effect on the population of TB possums than does a 70% kill. The disease could be eliminated by repeating the operation at 6-yearly intervals.

The results of the modelling experiments described in this thesis suffer from 2 major shortcomings. One is the quality of the information on immigration and survival used to derive the respective model parameters and the other is the small size of the area covered by the main simulation experiment. It was demonstrated that this model can be used to simulate the disease dynamics over areas of at least 400 hectares and that the simulation model can be adjusted to the local conditions by generating den site maps based on existing vegetation information in digital form. The simulation exercise over the large area did show that the disease occurrence becomes strongly clustered within habitat types of high den site density. The model is still at a relatively early stage of development and it should therefore not yet be used for recommendation of optimal possum population control strategies.

Improvements on the Current Model

In the analysis of simulation output for this particular model, the data produced during the transient phase at the beginning of the simulation run should be discarded. At the beginning of the simulation den sites and clinical tuberculosis are randomly allocated to possums in the simulated population. During the transient phase population size stabilizes, possums establish a "den range" and tuberculosis infection clusters develop. Depending on the size of the population and the area used during the simulation this period is likely to vary. For the small-scale simulation the transient period was about 3 years and for the large-scale simulation about 10 years. Given varying transient periods it may be necessary to subjectively assess the duration of transition for each simulation exercise based on examination of a time plot of population and disease dynamics as well as a map of locations used by tuberculous possums.

The use of antithetic random numbers for variance reduction has been investigated during this simulation exercise. A reduction of variance appears appropriate for continuous variables such as population size. These techniques are not appropriate if the outcome variable relates to an extinction process such as presence/absence of disease in a population. An interpretation of simulation output based on the average of the antithetic pairs of a variable measuring presence/absence of disease is not possible. An objective of the simulation should be rather to come up with a probability of extinction which can only be derived on the basis of a minimum of 5 to 10 simulations based on independent random numbers. Hence, during future simulation exercises with the current model the output from between 5 and 10 independent simulation runs should be used as the basis for analysis.

The sensitivity analysis has shown that given the current parameter settings the transmission mechanism representing spatial proximity controls the infection dynamics in a simulation run. Transmission during the mating process is only of minor importance. Current understanding of the epidemiology of *Mycobacterium bovis* in possum populations suggests that transmission during mating has a more substantial influence on infection dynamics. During the simulations, possums seem to use only a relatively small number of dens. This area needs some further comparison with field data. It will be necessary to build a mechanism into the model allowing storage of den site codes used by individual possums in a file on disk. Using this simulation output the actual area covered by the dens used by individual possums can be estimated and compared with field data.

During most of the simulation runs, a 5% prevalence of clinical tuberculosis in immigrants was assumed. This figure will require some further sensitivity analysis. It is difficult to validate this information using field data, but 5% is almost certainly at the top end of the true range which occurs in the field. In the current model immigrants can only be introduced as susceptible or clinically diseased animals. It seems appropriate to also introduce subclinically diseased immigrants. It will be very difficult to obtain field data on the proportion of subclinically diseased possums.

Improvements will have to be made to the user interface of the model. The management of distances between den sites is currently implemented in a very inefficient way as a file must be stored on disk for each den, containing the distances to the dens within a given radius. When using large numbers of dens in a simulation, currently several thousand small files have to be stored in a directory on disk. The distances should be stored in a single file and accessed through a indexing system. A module allowing the creation of random den site locations from a vegetation map also has to be added to the model.

Next Stage of Model Development

The current model is part of a simulation system which consists of a set of 3 hierarchical models (*micro-, meso-* and *macro-scale)* each representing different geographical scales and components of the decision process related to disease epidemiology and control.

The current model represents the *micro-scale* component. A set of four instances of this model will be run, each modelling disease dynamics within a single type of the four major vegetation types (pasture, pasture/scrub, scrubland and forest). The size of each of the areas represented by a single instance of the model will have to be decided. It is likely to be somewhere between 100 and 400 ha. Each of the four model instances will be replicated to generate distributions of values for population density and possum tuberculosis prevalence on a year by year basis. Additional instances may be required to represent the effect of implementation of control.

The *meso-scale* component of the simulation system combines the results from the *micro-scale* models and implements the spatial structure for a specific area. It uses a raster-based map of the four major vegetation types relevant for possum tuberculosis epidemiology to represent the characteristics of a specific area at the district level. The distribution of results for population size, emigration and clinical tuberculosis prevalence produced by each of the *micro-scale* model instances will be used to generate a map of possum tuberculosis risk. The risk of infection for particular species of domestic stock can be estimated by overlaying actual farm boundaries on this map. This process will be repeated on a year-by-year basis. The temporal and spatial relationships between raster cells will influence actual values used for immigration and to represent the spatial spread of tuberculosis infection within possum meta-populations.

The *macro-scale* model will introduce landform features such as rivers, lakes and mountain ranges and allow modelling tuberculosis control policies at a regional level.

CHAPTER 7

A CASE-CONTROL STUDY OF TUBERCULOSIS BREAKDOWN IN CATTLE HERDS IN THE WAIKATO REGION, NEW ZEALAND

INTRODUCTION

In 1986 it was recognised that the main objective of the tuberculosis disease control scheme had to be the containment of infection within the endemic areas. The major endemic zone in the Central North Island of New Zealand is centred around the Hauhungaroa Ranges. To achieve containment of spread for the Central North Island a management plan was set out to restrict movements of *Mycobacterium bovis* infected possums out of these areas by surrounding the endemic area with a low possum population density zone of 3-5 km width. Possum populations were to be controlled by aerial and ground poisoning operations using 1080 baits (Anon. 1986, Livingstone 1988). The buffers were supposed to act as 'dispersal sinks', hence reducing migration from the endemic area. Additionally tuberculous possums settling inside the buffer area would have a reduced probability of infecting other possums as with the low population density social interaction would be less likely to occur. During 1986 buffers were put in place on the western side of the Rangitoto Ranges (Rangitoto I), and along the western side of the Taumaranui County. In 1987 the buffer Rangitoto II and another buffer around the southern border of the endemic area of South Kaipara Head were created (Anon. 1988). In winter 1988 the low population density buffer Rangitoto III was established. The buffer zones were maintained and some were extended during the following years. Additionally areas inside or outside the buffer which are considered important possum habitat were now targeted to improve its effectiveness. This includes the reduction of possum population density for up to 15 km along all catchments draining the endemic area. During the financial years 1989/90 and 1990/91 a sum of about 1.3 Million New Zealand dollars each was budgeted by the Ministry of Agriculture and Fisheries to be spent on buffer related possum control (Livingstone 1990). The results of the study suggested that dispersal frequency and distance were independent of population density. It was hypothesized that a buffer zone with its excess of food and den sites might in fact improve survival of both uninfected and infected individuals. Efford (1991) reviewed available information on dispersal behaviour in possums. He concluded that there are indications that young male dispersers do not settle at the first vacant site they find. He adds that this may be different for female dispersers who would then have a relatively large potential for setting up new disease foci. In summary, there is still not enough information available to verify the effectiveness of the low population density buffer concept.

While the buffers were being put in place between 1987 and 1989, a number of breakdowns of cattle herds to tuberculosis infection occurred on the opposite side of the buffer zone from the endemic area. In most cases detailed epidemiological investigations did not reveal any obvious sources of infection. In 1988 two tuberculous possums were found 1 km apart in the non-endemic area. In 1989 a feral pig with tuberculous lesions was found inside the non-endemic area. These incidents were followed up by localised possum control. The Chief Veterinary Officer's report for 1990 (MacDiarmid 1991) stated that no further infected cattle herds had been identified since completion of the buffer zone in 1989. At that

time it was concluded that the buffer appeared to have contained the spread of tuberculous possums.

In 1989/90 a case-control study of cattle herds with and without tuberculosis infection was conducted in cooperation between the Ministry of Agriculture and Fisheries (Quality Management) and Massey University. The objective was to identify risk factors which are associated with the establishment of infection in herds (breakdowns).

MATERIALS AND METHODS

Study Design

The case-control study has been defined by Breslow and Day (1980) as an investigation into the extent to which subjects selected because they have a specific disease (the cases) and comparable subjects who do not have the disease (the controls) have been exposed to the disease's possible risk factors in order to evaluate the hypothesis that one or more of these is a cause of the disease. In contrast to survey research, in a case-control study, neither cases nor controls need to be representative of any population. The authors add that while usually one case and one control group is included, a second control group may be indicated in situations where one desirable control group has a specific deficiency which can be overcome by another control group. In the present study for each case a random and a matched control were selected. The use of a matched control was considered necessary, as it is difficult to control for confounding based on geographical factors during the analysis.

The study covered 285 farms - 95 cases (C), 95 random (RC) and 95 matched (MC) controls. A case was defined as a herd (previously free of infection) which was placed under movement control in the period 1986-89, due to the identification of tuberculosis-infected cattle through routine surveillance and testing procedures. A random control was a farm of any enterprise type chosen at random from the same county. A category matched management control was a farm of the same enterprise type as the case and located in its immediate vicinity. The farms are located in the Waikato region of the central North Island, New Zealand (see figures 98a,b,c).

Figure 98a: Map of farm locations

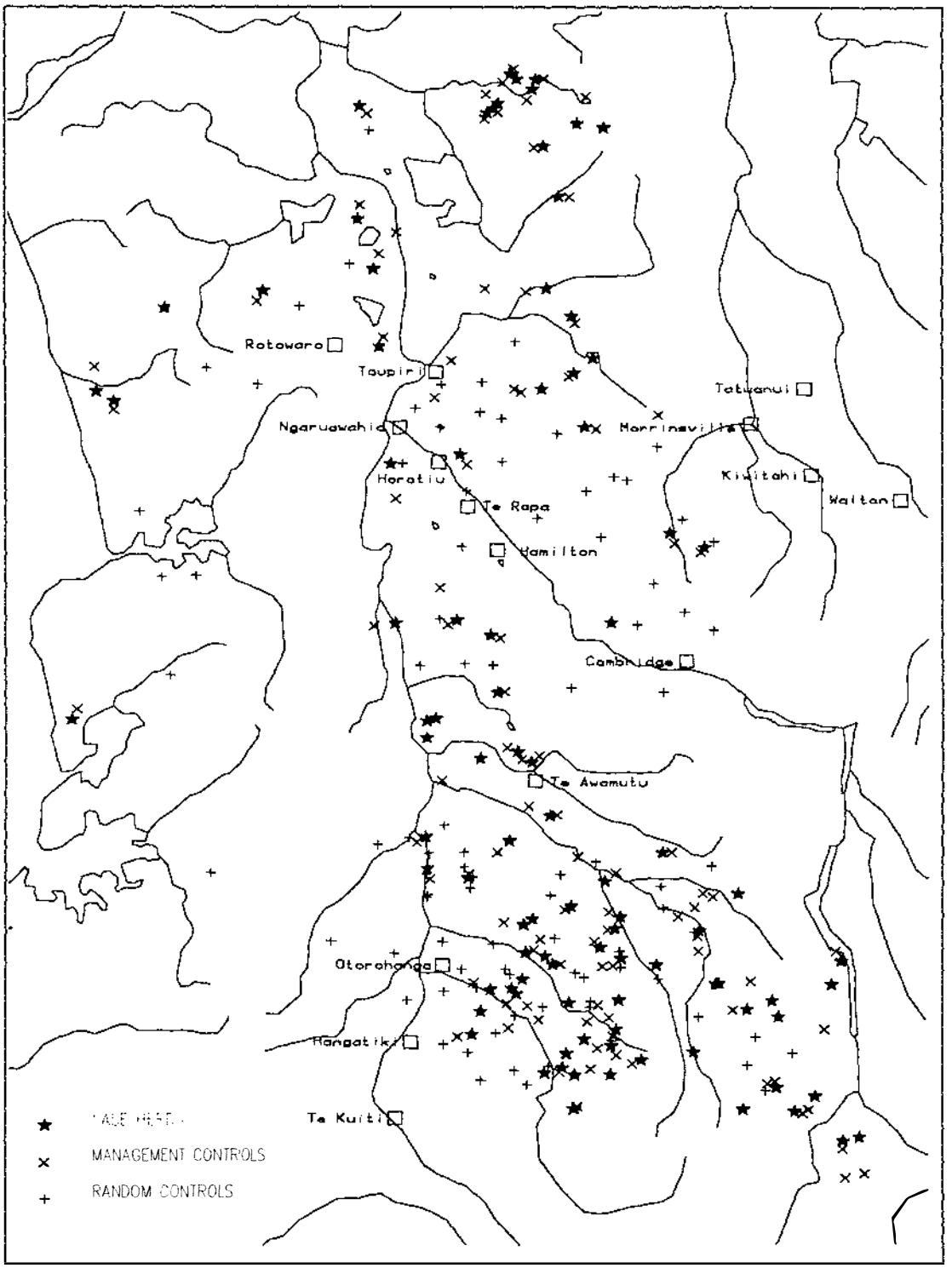


Figure 98b: Vegetation map of study area with farm locations

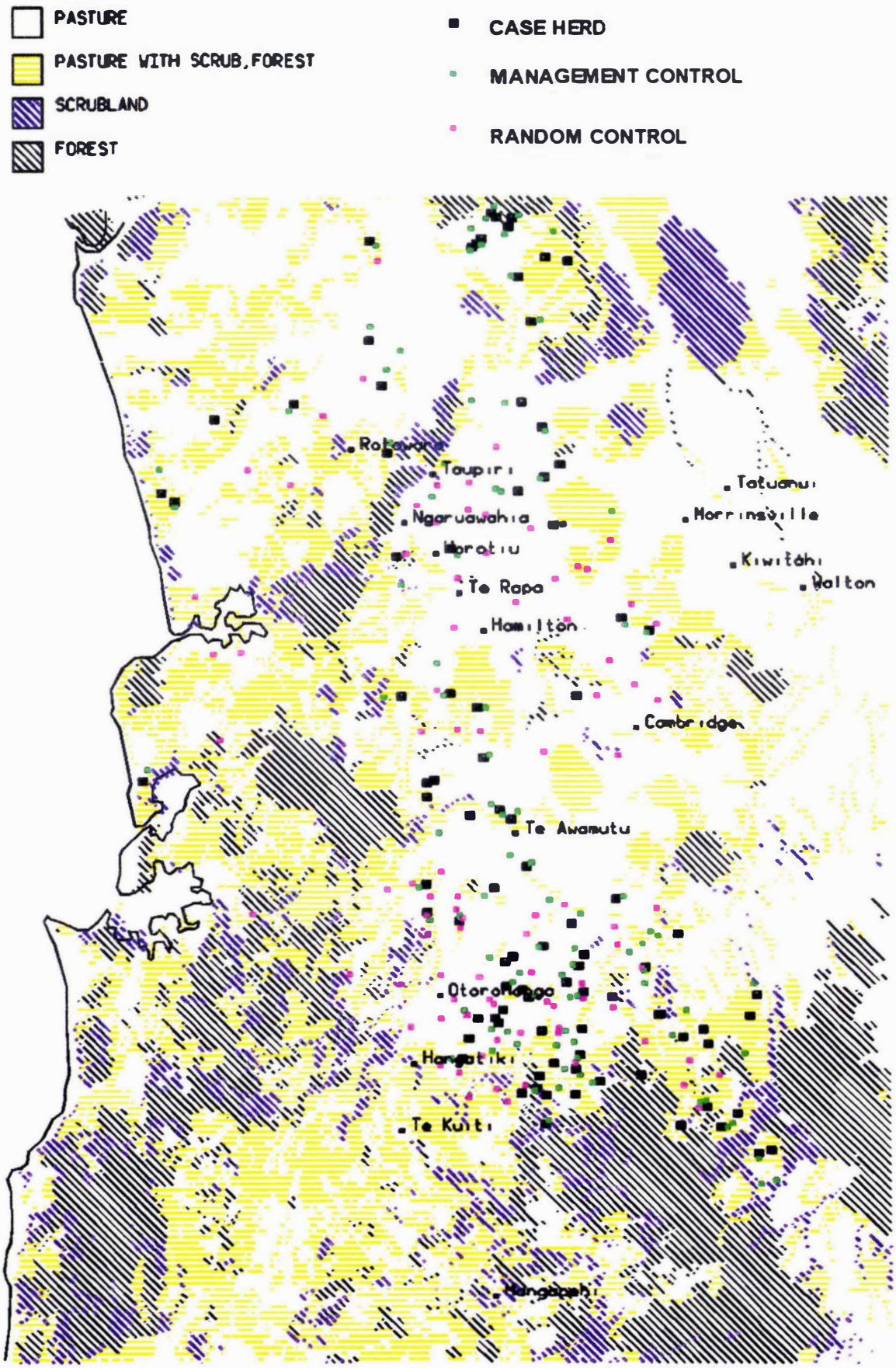
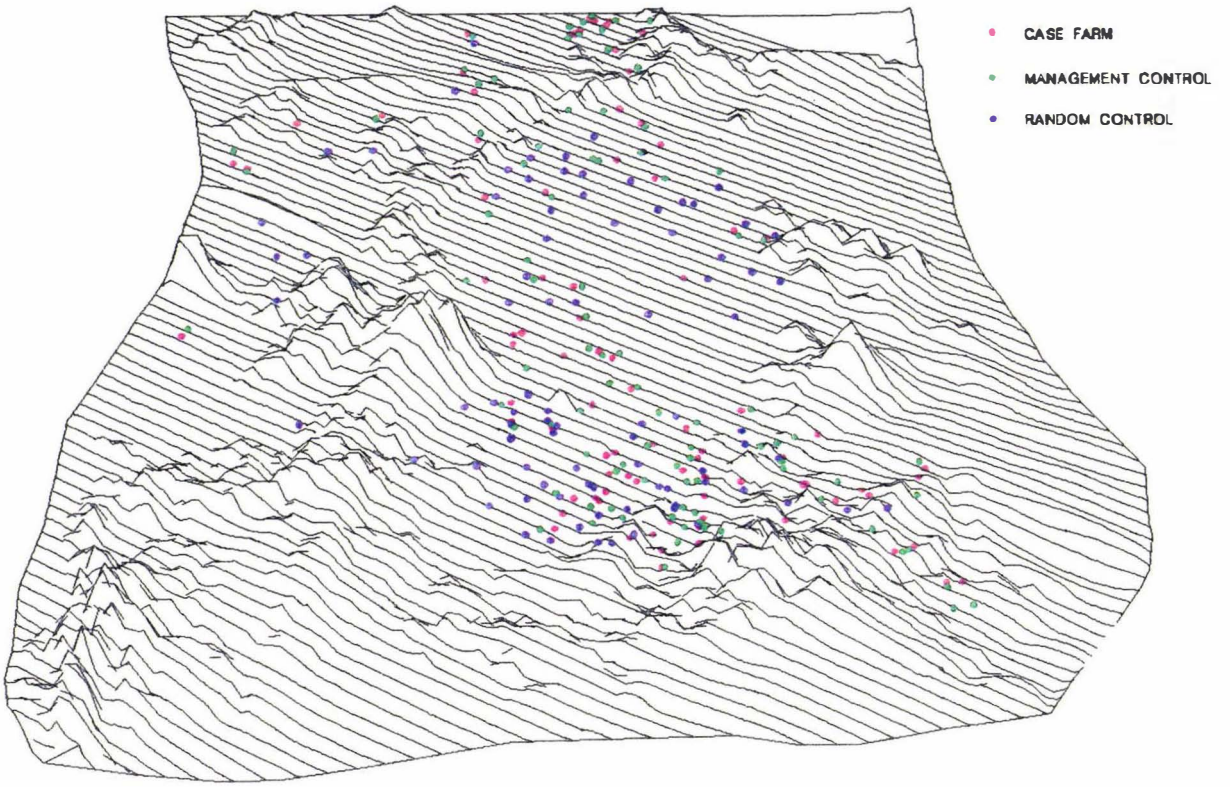


Figure 98c: Digital terrain model of study area with farm locations



Data Collection

Each herd or farm represents an ecological system consisting of a network of multiple biological, economical and sociological factors. The objective of the data collection was to describe this system with as much detail as necessary to allow a quantitative analysis with respect to the overall objective of the study while minimizing potential sources of error (Susser 1973).

A questionnaire was developed which comprised 134 items, of which 118 were to be answered by the person in charge of cattle management on the farm. The questionnaire included farm specific data, general information on the interviewee, general stock information, stock management information, and tuberculosis data (see appendix V). A section which required the interviewee to describe his assessment of himself with regard to farm management style was adapted from work by Seabrook and Higgins (Seabrook and Higgins 1988). The questionnaire was designed in close cooperation with Mr. Roy Sproule, a MAFQual veterinary officer working in the study area. Mr. Sproule also selected random and matched controls for each case herd. The questionnaire was tested on 20 farms to identify potential sources for misinterpretation of the questions and to further refine the questions. Interviews were conducted by field personnel of the Ministry of Agriculture and Fisheries between 1/12/1988 and 30/5/1990. Completed questionnaires were checked for errors by Mr. Roy Sproule. At Massey University the information was then coded and entered into the

computer data base management system PANACEA (PAN Livestock Services, University of Reading, Reading, England). As described by Rothman (1986) data editing checks were conducted to screen out errors from data entry and coding.

The locations of the farms which were included in the study were marked on 1:50000 map sheets of the Infomap 260 Topomap series (Department of Survey and Land Information, Wellington, New Zealand) and then digitized as point features using the GIS software PC-ARC/INFO version 3.4D (Environmental Systems Research Institute, Redlands, California, U.S.A.) for storage as a digital map coverage on a personal computer. Digital information based on map sheet 4 of the Infomap 262 Terrainmap series including roads, rivers, townships and contour lines was obtained from the Department of Survey and Land Information, Wellington, New Zealand. Vegetation data from the New Zealand Land Resource Inventory data base in digital form covering the study area was supplied by Soil Conservation Centre, DSIR Land Resources, Palmerston North, New Zealand.

Data Analysis

The objective of the study is to describe causal associations between potential risk factors and the occurrence of tuberculosis breakdowns of cattle herds. Lilienfeld and Lilienfeld (1980) define a causal relationship as existing "whenever evidence indicates the factors form part of the complex of circumstances that increases the probability of the occurrence of disease and that a diminution of one or more of these factors decreases the frequency of disease". It should be emphasized that causal inference is a subjective process and that as with epidemiological research in general it is impossible to unequivocally prove the causal nature of an association (Rothman 1986). Breslow and Day (1980) consider as "the basic questions to be asked in a case-control study are the degree of association between risk for disease and the factors under study, the extent to which the observed associations may result from bias, confounding and/or chance, and the extent to which they may be described as causal."

The objective of multivariate analysis is to develop a model allowing the researcher insight into the causal structure of the problem under study. Views among researchers regarding the complexity of the final model diverge considerably. While some consider "Occam's razor" as a useful maxim which leads towards a parsimonious model (Susser 1973), others emphasize that the construction of a comprehensive multivariate statistical model including all important confounders is one of the strengths of the multivariate approach (Rothman 1986). Robins and Greenland (1986) discussed the problems involved in model selection for causal inference based on nonexperimental data. They write that a compromise has to be found between a highly-saturated model which has small bias, but may have large variance and a model with only few covariates and interactions which is more likely to be biased, but with lower variances. Holland (1988) points out that while statistics has made major contributions to issues of causal inference when it has addressed the problem of measuring the effects of causes, it has done less useful things when its methodology claimed to identify the causes of effects. Hosmer and Lemeshow (1989) write that especially when

relying on statistical algorithms for covariate selection the parameters included in a final model have to be carefully scrutinized in terms of their biological plausibility.

The outcome variable in the present study was the case-control status of a herd. Separate analyses were conducted for the combination of cases with matched and random controls. Matched data should generally be analyzed using specific methods for matched analysis. If cases and controls have been matched on a variable associated with the exposure, then analysis not taking account of matching would result in an estimate of the odds ratio biased towards unity. If otherwise matching was done based on variables not associated with the exposure, a multivariate analysis accounting for matching would increase variance of the estimated parameters and hence be unnecessary (Schlesselman 1982). Schlesselman recommends a comparison of the results of a fully matched, a stratified and a unmatched analysis. He adds that the trade-off in choosing between these three approaches is one of reducing the variance of a parameter at the risk of increasing its bias. Breslow and Day (1980) state that both pooled and matched analysis provide unbiased estimates if either the stratification variables are conditionally independent of disease status given the risk factors or they are conditionally independent of the risk factors given the disease status. In general, they recommend that matching should be accounted for in the analysis whenever it has been incorporated in the design. Rothman (1986) suggests that even if individual matching is employed in a matched case-control studies, unless there is a large number of categories relative to the number of cases, it is not necessary to use methods of individually matched analysis as long as the matching factors are being controlled for in the analysis. He adds that if the matching factor is not related to disease status and therefore is not a confounder, matching represents overmatching because the effort of matching and the loss of efficiency in the required matched analysis do not improve the validity of the study. Kleinbaum *et al* (1982) point out that category matching on factors which are either weak risk factors or no risk factors at all is more detrimental in terms of efficiency in case-control studies. Matching will increase efficiency relative to random sampling, if the matching factor is a strong risk factor, as a function of the extent to which the matching factors are differentially distributed between exposed and unexposed subjects. These authors emphasize that conditional maximum likelihood methods should be used if stratum-specific sample sizes are moderate to small, the extreme being one case and one control per stratum in a pair-matched analysis. For the analysis of the present study it was decided to use standard methods of univariate analysis, but for comparison to use both standard and matched methods in the multivariate analysis.

The information regarding the self concept of the interviewees was analyzed separately. The objective of this analysis was to describe a typical farmer's perception of himself and to find out if there were differences between cases and matched / random controls. As this is an abstract and very subjective concept it can only be approximated by measuring a number of factors. The abstract variable measured then lies hidden in the matrix of the data collected. Multidimensional scaling is a technique which can be used to describe patterns in the data and

represent it in a graphical form. The objects under study are represented by points in a spatial model and significant features of the data about these objects are revealed in the geometrical relations among the points (Shepard 1972). The interviewees had been asked to rate themselves on 22 semantic differential scales. For the analysis the adjective ratings were converted on a scale from 1 to 5, with 1 representing the left-hand adjective and 5 the right-hand adjective. This data is suited to non-metric multidimensional preference scaling (MDPREF). This technique is essentially a principal components analysis applied to a data matrix where columns correspond to the different adjectives and rows to the individuals. The final result is a biplot of the resulting preference space. The analysis procedure is outlined in SAS technical report: P-179 (SAS Institute 1988). The SAS procedure PRINQUAL was used in the analysis using the Maximum Total Variance method. The technique is based on an iterative algorithm which alternates between classical principal-components analysis and optimal scaling (Young 1981). It transforms the input variables, maximizing the total variance accounted for by the first specified components. The technique also estimates missing values in the data matrix. In the resulting biplot each individual is represented by a point and each adjective by a vector. Each individual can be projected orthogonally onto a adjective vector. The individuals which project farthest along an adjective vector in the direction it points identify themselves most with the respective adjective. Individuals clustered on the plot tend to have similar adjective patterns. Adjective vectors which point into the same direction represent similar adjective patterns. It may be possible to interpret the dimensions of the plot as describing general patterns of adjectives.

General Outline of Approach to Data Analysis

The general data analysis and modelling strategy was in part adapted from Hosmer and Lemeshow (Hosmer and Lemeshow 1989). In a first step, the system under study was briefly described in a general sense disregarding case-control status of each herd as a general overview of the husbandry system. In a second step, a univariate analysis was conducted comparing the association between case and control herds with regard to the variable measured. The interpretation of the results of this analysis is used as a basis for selection of a subset of variables to be included in the third analytical step, the multivariate analysis. Considering the complexity of the underlying system it was decided to use four different approaches for the multivariate analysis and compare the results.

The following statistical methods were used in each of the three analytical steps of the data analysis. Analysis step number 1 consisted of a *descriptive analysis* of the data, including the use of graphical methods. In step number 2, a *univariate analysis* of each variable using a univariate logistic regression model was conducted to screen the data statistically for variables which were significantly associated with the case-control status of a herd based on the score test at a significance level $p < 0.15$ (Hosmer and Lemeshow 1989). The choice of a screening criterion of 0.15 is designed to ensure that all potentially important variables are included in the next analytical step, the *multivariate analysis*. A number of variables which were not

significant in the univariate analysis, but were considered to be possibly of biological importance were also included into the next step. Factors which were statistically significant at a p-value of 0.05 were described in more detail using tables and histograms.

Step number 3 consists of four different approaches which were being used to develop a multivariate model adequately describing the data space. Method 1 used a stepwise logistic regression approach to fit a multivariate model to the data. Method 2 used a combination of ordinary least squares and maximum likelihood regression (OLS-ML) to develop a path model. Method 3 describes the data using a structural equation model (LISREL). Finally, method 4 was based on recursive partitioning yielding a binary classification tree as a representation of the data space.

Methods used in Multivariate Analysis

Stepwise Multiple Logistic Regression

Method 1 uses stepwise multivariate logistic regression. A stepwise selection procedure is based on a statistical algorithm which decides on the importance of variables. During each step the variable which produces the greatest change in the log-likelihood relative to the previous model is being included in the model until the p-value of the likelihood ratio chi-square test exceeds a predetermined level. In every step a check for backward elimination of variables in the model is made, their continued importance being determined by using the likelihood ratio test at a given significance level (Hosmer and Lemeshow 1989). Continuous variables included in the final model were checked for linearity in the logit scale and transformed appropriately. Then, possible interaction terms were considered for inclusion into the model using a stepwise approach beginning with the main effects model. Goodness-of-fit of the final model was assessed based on the -2 Log Likelihood. This involves calculation of a Pearson chi-square statistic, testing the joint significance of the explanatory variables by comparing the -2 Log Likelihood for an intercepts only model with a model including intercept and explanatory variables. Akaike's Information Criterion was used to compare different models. This model-selection criterion favours models with fewer parameters. A 'better' model is indicated by a smaller value of this statistic (Akaike 1987, Amemiya 1980). Both summary statistics represent the agreement between observed and fitted values in form of a single value. Before accepting a model, logistic regression diagnostics as described in McCullagh and Nelder (1989) and Hosmer and Lemeshow (1989) were performed. These authors recommend the use of methods based on case deletion and standardized residuals. Plots of the difference in Pearson chi-square residuals due to deletion versus the predicted probability are drawn. In these plots the size of the plotting symbol is made proportional to the size of the standardized influence measure of the particular point, and these have to be interpreted visually. Hence, large values in the difference in Pearson chi-square residuals represent covariate patterns which are poorly fitted and large values of the influence measure stand for values which have a great influence on the estimated parameters. Index plots of these

particular measures can be used to identify and further examine any suspect observations. If the model is accepted, the estimated coefficients can be used to derive odds ratios and their confidence intervals for important risk factors. The results of the stepwise regression procedure will be reported as described in Hauck and Miike (1991). As outlined in the paper this method includes the use of 2 tables in addition to the standard report about the final model. The first table lists the p-values of all variables available for selection to the stepwise algorithm for each individual step. Variables which were selected at a particular step are indicated by brackets around the p-value. Carets were used to mark transitions of p-values meaning that at this step the p-value for this variable changed notably. Careful examination and interpretation of the table allows to identify correlated sets of variables and suggest possible alternative models. The second table summarizes these findings while reporting the order of entry for the variables.

Path Analysis

Methods 2 and 3 for multivariate analysis are both used to conduct a path analysis. This method of analysis dates back to 1934 when Sewall Wright developed it to study the direct and indirect effects of variables hypothesized as causes of variables treated as effects. Pedhazur (1982) writes that path analysis is not a method for discovering causes, but a method applied to a causal model formulated by the researcher on the basis of knowledge and theoretical considerations. He adds that while the analysis is able to test if the causal model is consistent with data, this should be understood to mean that the theory withstood the test and has not been falsified (Popper 1959). Cliff (1983) emphasizes that with correlational data it is not possible to isolate the empirical system sufficiently so that the nature of the relations among the variables can be unambiguously ascertained. Saris and Stronkhorst (1984) recommend the following process for developing a causal model. In the first step the researcher has to decide on which variables he considers as potentially important. Then this set of variables has to be arranged in a way indicating causal ordering. Finally a causal hypothesis can be specified. This involves writing down the names of the variables, with the position indicating causal order, then the causal hypotheses are specified by introducing arrows between variables for which one expects direct causal effects. Essentially, based on prior beliefs of the researcher the resulting causal diagram depicts the relationships between the variables attempting to subdivide the covariation between variables into spurious, direct and indirect effects. The causal hypotheses described by the causal diagram or null hypothesis model are then tested. For the testing to be valid it is absolutely essential that all potential causes are included in the analysis. Variables which are not explained by the theory are considered exogenous variables. For these factors no causal effects are specified, the covariation between them is left unexplained. Variables which are being explained are referred to as endogenous variables. In a path diagram exogenous factors are symbolized by lines connecting the variables with arrows pointing in both directions. The diagram should be developed following the principles of biological plausibility and parsimony. Up to this point,

path analysis using standard multiple regression techniques (OLS-ML) and the structural equation modelling (LISREL) approach are very similar.

Path analysis using regression techniques

When using standard multiple regression techniques (OLS-ML) for analysing a path diagram, the following assumptions have to be met as outlined in Pedhazur (Pedhazur 1982). The relationship between variables has to be linear, additive, and causal. The model is recursive, meaning that there is no reciprocal causation. Variables are measured without error. Pedhazur adds as another requirement that the variables have to be measured on an interval scale. This last assumption does not apply when using a mixture of least-squares regression, polychotomous and logistic regression. The OLS-ML approach to path analysis is centered around the estimation of path coefficients. The latter have been defined as indicating the direct effect of a variable hypothesized as a cause of a variable taken as an effect. Using the OLS approach path coefficients are estimated by regressing each variable on all factors with arrows leading to it. Path coefficients are standardized and path regression coefficients are not transformed, the latter being more useful when comparing between different studies. It is then possible to decompose the correlations between exogenous and endogenous, or between endogenous variables into direct effects, indirect effects, unanalyzed and spurious components. Goldsmith (1977) describes the decomposition of paths of association as follows. He compares the correlation between a potential cause $\mathbf{x_1}$ and an effect $\mathbf{y}$ with the partial correlation of the same variables controlling for another variable $\mathbf{x_2}$. The difference can be tested with the z-transformation for statistical significance. If it is large, the result suggests that the path of $\mathbf{x_1}$ involves $\mathbf{x_2}$. When calculating total effects, parallel paths have to be summed and series paths are multiplied. The total effect can also be estimated from a regression model which includes cause and effect while controlling for all other causes preceding the cause in question. The indirect effect can then be calculated as the difference between total and direct effect. When the effect is measured as a binary, ordinal or nominal variable, a maximum likelihood approach (ML) has to be used to estimate the regression coefficients. Partial regression coefficients derived from models for binary outcome variables (logistic regression) can be converted into conditional odds ratios which allows a direct epidemiologic interpretation (Curtis *et al* 1985). The approach to partitioning the effects in direct and indirect components is different from the OLS technique. The magnitude of direct associations is represented by the path coefficients and indirect associations are found by multiplying path coefficients along the paths. This concept is related to calculation and specific interpretation of interaction effects (Curtis *et al* 1988a, Curtis *et al* 1988b).

In the present analysis two separate null hypothesis path diagrams were developed for the comparison of cases with random and with matched controls. These path models included the factors which had been selected in the final stepwise regression models plus other variables which were selected for consideration because they were both statistically significant in the univariate analysis and considered to be of potential biological importance.

The comparison of cases with matched controls was analyzed as an unconditional analysis. During the analysis a significance level of 0.15 for entry and removal was used in a stepwise variable selection procedure for each effect variable in the null hypothesis path model. No path coefficients were estimated. The sign of each path coefficient was indicated on the path diagrams.

Path analysis using LISREL

Structural equation modelling using the LISREL model was used as an alternative approach to path analysis. LISREL is a general model for studying a set of linear structural equations. The variables in the model can be either directly observed or latent, unobserved variables. It is based on a measurement and a structural equation model. The measurement model specifies how the latent variables are measured in terms of the observed variables and the structural equation model specifies the causal relationships between the latent variables. The general model is designed to handle models with latent variables, measurement errors and reciprocal causation. It has been used in experimental and non-experimental research for exploratory and confirmatory factor analysis models, path analysis models, econometric models for time-series data, recursive and non-recursive models for cross-sectional and longitudinal data, and covariance structures (Jöreskog 1985). Pedhazur (1982) considers structural equation models as more powerful than conventional path analysis, and he adds that they are also based on less restrictive assumptions.

In order to understand the input required for the program and to get an impression of the flexibility of the technique, it is necessary to describe the mathematical procedures in more detail. As mentioned above the LISREL model consists of two major subdivisions: the structural equation and the measurement model. The structural equation model describes the relations between exogenous and endogenous variables. These can be observed or unobserved (latent) variables. In the structural equation latent dependent or endogenous variables are designated as η (eta) and latent independent or exogenous variables as ξ (ksi). The structural equation model is: $B\eta = \Gamma\xi + \zeta$; where η (eta) represents an *m* by 1 vector of latent endogenous variables; ξ (ksi) is an *n* by 1 vector of latent exogenous variables; B (beta) is an *m* by *m* matrix of coefficients of the effects of endogenous on endogenous variables; Γ (gamma) is an *m* by *n* matrix of coefficients of the effects of exogenous variables (ξ's) on endogenous variables (η's); ζ (zeta) is an *m* by 1 vector of residuals in the equations. The measurement model describes the relations between unobserved and observed variables. It consists of two equations: $\mathbf{y} = \Lambda_y\eta + \varepsilon$ and $\mathbf{x} = \Lambda_x\xi + \delta$. In the first equation **y** is a *p* by 1 vector of measures of dependent variables; Λ (lambda) is a *p* by *m* matrix of coefficients of **y** on the unobserved dependent variables (η); ε (epsilon) is a *p* by 1 vector of errors of measurement of **y.** In the second equation **x** is a *q* by 1 vector of measures of independent variables; Λ (lambda) is a *p* by *m* matrix of coefficients of **y** on the unobserved dependent variables (ξ); and δ (delta) is a *q* by 1 vector of errors of measurement of **y**. Based on these three equations the model can be described graphically using squares to represent observed

variables and circles for unobserved variables, and in matrix form. LISREL uses eight matrices to describe the covariance matrix of the observed variables:

- Λ_y (lambda) as a matrix of coefficients relating indicators of endogenous variables to latent endogenous variables (η).
- Λ_x (lambda) as the matrix of coefficients relating indicators of exogenous variables to latent exogenous variables.
- **B** (beta) as a matrix of coefficients of the effects of latent endogenous variables on latent endogenous variables.
- Γ (gamma) as the matrix of coefficients of the effects of latent exogenous variables on latent endogenous variables.
- Φ (phi) as a variance-covariance matrix of the latent exogenous variables (ξ).
- ψ (psi) as a variance-covariance matrix of residuals (ζ).
- Θ_ε (theta) as a variance-covariance matrix of errors of measurement of y's.
- Θ_δ (theta) as a variance-covariance matrix of errors of measurement of x's.

The elements within these matrices can be fixed, constrained or free parameters. A theoretical model is represented by specifying a pattern of fixed and free elements in each of the eight parameter matrices. The matrix of observed covariances or correlations is then used to estimate values for the free parameters that best reproduce the data. In LISREL version 7.16 this estimation process can be done using seven different methods (Jöreskog and Sörbom 1989). Structural equation modelling is used to test whether a given theoretical model is consistent or inconsistent with the data. The fit of the theoretical model can be assessed using multiple criteria. LISREL 7 provides five such methods: standard errors and correlations of the parameter estimates (t-values), measures of variation accounted for, overall goodness-of-fit measures, analysis of residuals and model modification indices. The modification index is a measure of predicted decrease in χ^2 if a single fixed parameter or equality constraint is relaxed and the model is reestimated. Saris and Stronkhorst (1984) discuss the assumptions behind the LISREL approach. The effects in the model have to be linear and additive. Observations should be independent. Because of the development of new estimation techniques, the assumptions of interval measurement, multivariate normality and identical parameters for all cases have been relaxed. Using the WLS (weighted least squares) and DWLS (diagonally weighted least squares) method LISREL 7 allows estimation based on the asymptotic variance and covariance matrices of estimated sample variances, covariances and correlations. These methods are the preferred estimators when working with non-normal distributions.

In the present analysis the software PRELIS (Jöreskog and Sörbom 1988) was used to estimate a matrix of product-moment, polychoric and polyserial correlations. This program calculates product-moment correlations between interval scaled variables, polychoric correlations between ordinal variables and polyserial correlations between pairs of one interval variable scaled with an ordinal type variable. The calculations were based on pairwise deletion of cases with missing values. Assuming that missing values are randomly distributed within the dataset this method allows any further analysis to be based on a maximum number

of observations. Estimations were based on the method of maximum likelihood. PRELIS also calculates a significance value for each correlation. Variables were included into the path analysis if they were statistically significantly correlated with case-control status at a p-value of less than 0.15. It was not possible to estimate the matrix of the asymptotic variances of the estimated correlations, because the sample size was too small. The correlation matrix was then used as input for LISREL. A causal model was specified including exogenous and endogenous variables. In the present analysis no latent variables were considered for inclusion into the model. LISREL submodel 2 has to be used for analysis of causal models with only directly observed variables. It is described by the following structural equation: $y = \alpha + By + \Gamma x + \zeta$. The ζ-variables represent an aggregate of all known and unknown influences on the y values that are uncorrelated with the x values. The parameter matrices **B**, Γ and Ψ (=Cov(ζ)) are to be estimated. The matrix Φ (=Cov(**x**)) is assumed to be an unconstrained free covariance matrix. If sub model 2 is applied to a recursive system as in the present analysis, matrix **B** is sub-diagonal, Ψ is diagonal and α is omitted. In the present analysis only hypothesized direct effects of exogenous and endogenous factors on case-control status were specified. The significance of these effects based on their t-values was then tested in a preliminary LISREL run. Any effects with t-values below 2.0 were dropped from the model. In the next run the automatic model modification feature of LISREL was used to free any fixed parameters in a stepwise manner which had the largest modification index statistically significant at a p-value of 0.01. The t-values of the effects in the resulting model were then reexamined and any effect which was no longer significant was dropped from the model (MacCallum 1986). These effects were left out of the model even if on subsequent reestimation of the model parameters they had a statistically significant model-modification index. The overall fit of the model was assessed by examining the χ^2- test statistic and the adjusted goodness-of-fit index. The χ^2- test can be used to assess model fit against the alternative that all parameters are unconstrained. It is sensitive to sample size and assumes that the data comes from a multivariate normal distribution. The adjusted goodness-of-fit index can be interpreted in analogy with the correction of bias of a squared multiple correlation coefficient. It compensates for the increase in goodness-of-fit of a less restricted model obtained by estimating more free parameters (Mulaik *et al* 1989). It is considered relatively robust against departures from normality (Anderson 1987). The root mean square residual was used as another indicator of fit of the model, when comparing different models. The distribution of values of normalized residuals was visually presented using a quantile plot (Q-plot). A model with perfect fit would yield residuals forming a straight line at a 45-degree angle (Herting and Costner 1985). LISREL also calculates squared multiple correlation coefficients for each structural equation and a total coefficient of determination. The latter indicates the amount of variation in the endogenous variables jointly accounted for by the model. The final path model was presented graphically showing the direction and size of the

effects between variables in the model. Direct and total effects between factors were also estimated.

Classification Tree Analysis

The multivariate classification technique recursive partitioning was employed to develop a binary classification tree as a hierarchical-type representation of the data space (Kotz and Johnson 1989). The technique was implemented using the computer program CART version 1.1 (California Statistical Software, Inc., Lafayette, California, U.S.A.). Breiman *et al (*1984) describe as the basic purpose of a classification study to either produce an accurate classifier or to uncover the predictive structure of the problem. They list the following advantages of tree-structured classification. The technique can be applied to any data structure. The analysis results can be easily applied to new data. It makes powerful use of conditional information for handling nonhomogeneous relationships. The technique can automatically perform a stepwise variable selection and complexity reduction. It provides an estimate of misclassification probability. The results do not vary under all monotone transformations of individual ordered variables. It is robust with respect to outliers and misclassification points. The output is easy to understand and interpret with regard to the predictive structure of the data.

During the analysis the program first determines the best discriminant boundary value for each variable, which is a designated level for a categorical variable and a point in the range of numerical variables, trying to most cleanly separate the observations into similar response classes. The procedure then selects the variable which splits all the individual observations into the most accurately classified subgroups at each junction of the tree among competing splits. In the present analysis the GINI criterion was used as a measure of node impurity. At each node the procedure determines a number of surrogate splits which can be interpreted as the splits which most accurately predict the action of the selected splitting variable. The quality of this relationship is assessed using a predictive measure of association. Surrogate variables are used to perform a split if for a given observation a value is missing for the selected splitting variable. At each split a misclassification rate is calculated. The process terminates when a minimum number of cases remains at a particular node which then becomes a terminal node. This procedure yields a maximum size tree which then has to be pruned back to correct for overfitting. This pruning process selects a sequence of trees which minimise apparent error rate within subtrees of the same size. The selection of the final tree is based on minimising true error rate (cost) and complexity. Estimates of true error rate for each subtree can be calculated based on two methods. With large data sets they can be estimated by randomly dividing the observations into a learning sample and a number of test sets, which are then run down each tree. For smaller samples an alternative method called cross-validation can be used. Using this method the original learning sample is divided by random selection into a user-defined number of subsets, each of the same sample size and stratified by class. The subsets are subsequently withheld from the test sample, which is run down each tree in the pruned sequence. A standard error of the true misclassification cost is estimated. In the

present analysis the final tree was selected as the simplest tree within 1 standard error from the subtree with minimum misclassification cost. The program also ranks the variables in order of importance. This is based on summing up over all nodes the decrease in impurity produced by the best split on a particular variable at each node. These values are then converted into relative magnitudes as proportions of the maximum decrease in impurity achieved by the most important variable. As in the present analysis the emphasis was on developing a classification tree for describing herds which are most likely to break down to tuberculosis infection, the proportion of case herds in each branch and terminal node was calculated and presented together with the total number of observations at each terminal node in the graphical representations of the final tree diagrams.

During any multivariate analysis the treatment of missing values is an important consideration. Knowledge about the missing data mechanism is important to decide on the treatment of observations with missing values. Commonly it is assumed that these values are missing completely at random. But quite often missingness is related to the variables under study. Two approaches which are commonly used for analysis of data with missing values are complete-case and available-case analysis (Little and Rubin 1987). When using the complete-case approach, analysis is confined to observations where all variables are present. This technique is used by many software packages for statistical analysis. Its main advantage is that univariate statistics are comparable, because they are based on the same sample of cases. However discarding incomplete cases potentially results in a significant loss of information. During a univariate analysis the available-case method uses all complete observations where the variable of interest is present. This results in changing sample sizes from variable to variable. The estimation of covariance or correlation matrices can be done using pairwise available-case methods such as is done for the LISREL analysis in this study. It has been shown that this approach yields consistent estimates given that the data is missing completely at random. In the present analysis the complete-case approach was used for stepwise logistic regression and each of the individual regressions in the path analysis based on standard regression techniques. As described above classification tree analysis uses a different approach in the treatment of missing values.

Table 46 lists descriptions of codes for variables which have been used in the analysis. The direction of risk factor effects is indicated by (+) for increase and (-) for decrease, a (+) meaning that as the factor at the tail of the arrow increases, the factor at the head of the arrow also increases. In the path diagrams, significance levels of regression coefficients for individual paths are indicated as * for $p < 0.15$, ** for $p < 0.05$ and *** for $p < 0.01$. The abbreviation "n.s." stands for a non-significant statistical relationship, "s" for standard deviation, "OR" for odds ratio, "LU" for labour units and LSU for livestock units.

Table 46: Codes and descriptions of variables used in the multivariate analysis

VARIABLE	LABEL
ADULTCA T	PROPORTION OF COWS AND BULLS IN TOTAL CATTLE
BEEFCATT	PROPORTION BEEF IN TOTAL CATTLE
BEEFLSU	NO. BEEF CATTLE IN LIVESTOCK UNITS
BUSHACCE	CATTLE ACCESS TO BUSH
BUYLIVES	PREFERENCE TO PURCHASE MACHINE / LIVESTOCK
BUYREPLA	BUYS REPLACEMENTS?
CATTDENS	CATTLE DENSITY ON PASTURE
CATTLLSU	TOTAL CATTLE IN LIVESTOCK UNITS
CATTPURC	TOTAL LSU CATTLE PURCHASED
CCSPREAD	SCORE KNOWLEDGE ABOUT CATTLE-CATTLE SPREAD
CHSPREAD	SCORE KNOWLEDGE ABOUT CATTLE-HUMAN SPREAD
CONTEFFE	OPINION ABOUT EFFICIENCY OF MAF DISEASE CONTROL
DAIRYLSU	TOTAL NUMBER DAIRY CATTLE IN LSU
DIFFHER	PURCHASE FROM 0, <= 3 OR >3 HERDS
EPIDEMIO	SCORE EPIDEMIOLOGICAL UNDERSTANDING
FARMSIZE	FARM SIZE IN HECTARES
FOREST	AREA FOREST IN HECTARES
FORFARM	AREA FOREST PER TOTAL FARM SIZE
GIVINGIN	SEES HIMSELF AS FORCEFUL/GIVING IN EASILY
GIVINGUP	SEES HIMSELF AS PERSEVERING/GIVING UP EASILY
HARDWORK	PREFERS NO HARD WORK/LIKES HARD WORK
HEISTCAT	HEIFERS AND STEERS PER CATTLE LSU
INTRODUC	KNOWLEDGE ABOUT PATHS FOR DISEASE INTRODUCTION
LIVEPREF	PREFERS MACHINERY / LIVESTOCK
LIVESDEN	LIVESTOCK DENSITY ON PASTURE
MAFCOMET	SCORE KNOWLEDGE ABOUT MAF CONTROL METHODS
MAINGRAZ	MAIN GRAZING ON OR OFF FARM
MAINOP	MAIN TYPE OF CATTLE OPERATION (DAIRY OR BEEF)
MODEST	SEES HIMSELF AS NOT MODEST / MODEST
NEWIDEAS	SEES HIMSELF AS TRADITIONAL / LIKES NEW IDEAS
NEXTCKM	DISTANCE TO NEXT CASE FARM IN KILOMETER
NEXTEKM	DISTANCE TO NEXT ENDEMIC TB AREA IN KILOMETER
NOTALKAT	CONSIDERS HIMSELF TALKATIVE / NOT TALKATIVE
OTHEMPL	OTHER EMPLOYMENT COMMITMENTS?
OTHERCAT	OTHER FARMERS CATTLE GRAZED?
PASTURE	AREA PASTURE IN HECTARES
PERLABCA	PERMANENT LABOUR UNITS PER TOTAL CATTLE LSU
PERLABFA	PERMANENT LABOUR UNITS PER HECTARE FARM SIZE
PERLABLI	PERMANENT LABOUR UNITS PER LIVESTOCK UNIT
PERMLABO	PERMANENT LABOUR UNITS
PERTOTLA	PERMANENT LABOUR UNITS PER TOTAL LABOUR UNIT
PURCATT	TOTAL PURCHASE CATTLE LSU PER CATTLE LSU PRESENT
RECORDS	DISLIKES / LIKES KEEPING RECORDS
SOCIABLE	SEES HIMSELF AS UNSOCIABLE / SOCIABLE
SPECIES	SCORE KNOWLEDGE ABOUT RESERVOIR SPECIES FOR TB
TOTALLSU	TOTAL LIVESTOCK UNITS
TOTLABCA	TOTAL LABOUR UNITS PER CATTLE UNIT
TOTLABFA	TOTAL LABOUR UNITS PER HECTARE FARM SIZE
TOTLABLI	TOTAL LABOUR UNITS PER LIVESTOCK UNIT
TOTLABOU	TOTAL LABOUR UNITS
WEACAT	WEANERS PER CATTLE LSU
WEATOTPU	WEANERS PER TOTAL CATTLE PURCHASE LSU
WEAYEACA	LSU WEANERS,YEARLINGS PER CATTLE LSU
WHSTOTPU	LSU WEANERS,HEIFERS,STEERS / PURCHASE LSU

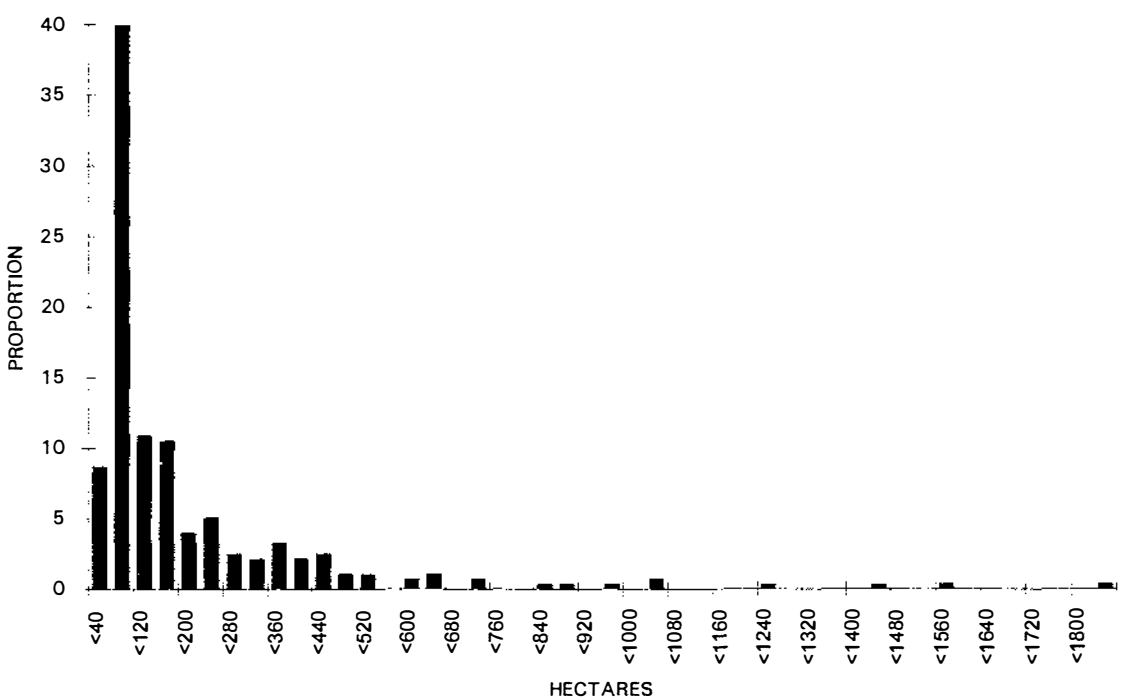
RESULTS

Descriptive Analysis

General Management and Farm Characteristics

The majority of case farms was located in "non-endemic" (21%) and "surveillance" (53%) tuberculosis control areas, as classified according to the MAF disease management plan for the Waikato region. Eighty-four percent of study herds were mainly cattle operations and 62% were involved mainly into dairy production. Twenty-one percent were mainly dry-stock fattening operations. The average farm size was 152.6 hectares (s = 161; see figure 99). About 50% of farms were smaller than 80 hectares. Interviewees were on average 35 years old (s = 11.4). 70% of interviewees owned the farm and 19% were share milkers. Twenty percent had a second job. Sixty-two percent considered themselves to be the main decision makers with regard to cattle management. Sixteen percent began their current job without farming background. Forty-nine percent had a formal education lower than school certificate and 69% did not have any farming-specific qualification.

Figure 99: Farm size distribution of properties included in study



Farmer's Interest in Disease Control and Knowledge about the Epidemiology of TB

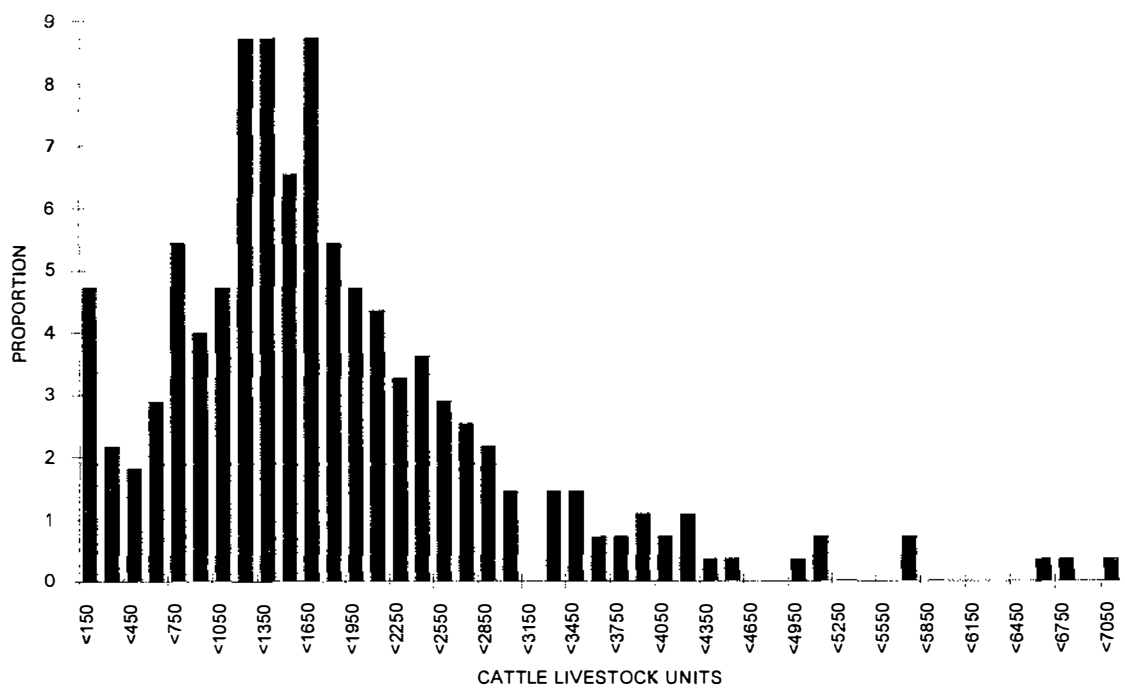
Of a total of 288 farmers 96% did not consider it as desirable to have endemic cattle tuberculosis infection in New Zealand and 98% were not prepared to tolerate the disease in their herd. Ninety-three percent of interviewees had some knowledge about the disease control program implemented by MAF. But 36% did not believe in its effectiveness or had no

opinion. Eighty-four percent of farmers thought that they had an idea why the official disease control program did not achieve eradication. Fifteen percent did not know or did not think that they themselves could make a contribution towards controlling the disease successfully. Forty-eight percent of interviewees either did not believe or did not know that there was a risk of infection from cattle with bovine tuberculosis for themselves. Eleven percent of farmers did not know about potential ways of introduction of TB infection into their herd. Thirty percent had no idea of possible mechanisms for disease spread within a herd. Ninety-six percent considered feral animals an important source of infection. Ninety-one percent thought that their cattle probably had contact with feral possums. Thirty-eight percent of interviewees were aware of TB infected herds in the neighbourhood. Fifty-seven percent were involved in some form of possum population control on their property.

Herd Characteristics

On average, cattle herds comprised 1721 livestock units (s = 1165; see figure 100). The average cattle density was 21.2 livestock units per hectare of pasture (s = 9.7). Cattle comprised on average a proportion of 0.8 of total livestock (s = 0.30) kept on farms in the study area. Sixty-eight percent of farmers kept dairy cattle, 62% had beef cattle, 10% deer, 49% had sheep and 24% had goats. Twenty-four percent of farmers had only dairy cattle, 3.5% only beef and 29% had both on their farm. Other farmers kept various combinations of animal species.

Figure 100: Cattle herd size distribution of properties included in study in livestock units



Purchase Patterns of Farmers in Study Area

Seventy-seven percent of farmers had bought stock. Forty-five percent of purchasing farmers bought from more than three different herds. On average they had bought in 30% of their current herd size during the last 2 years prior to the study. Thirty-eight percent stated that their main reason for acquiring stock was for trading purposes. Twenty-five percent bought a breeding bull and only 6% needed replacement cattle. Thirty-nine percent of farmers purchased stock from private sources, 27% bought from a sale yard and 24% bought through a stock agent. Twenty-six percent of interviewees did not consider the risk of introduction of diseases when they purchased cattle. Sixty-seven percent of farmers considered purchase from "safe" herds the most important measure for preventing disease introduction. Nine percent of farmers thought that it was important to check MAF's records about the disease status of a source herd in order to reduce the risk of disease introduction.

Stock Management

A total of 126 farmers (44%) grazed their cattle occasionally on a "run-off" or another farm. Forty-five percent reported that they used a "run-off". Eighty-seven of 288 interviewees reported that their cattle had access to bush or forest. Of these 49% stated that bush/forest was part of the paddock. Fourteen percent needed bush/forest grazing for additional food supply. Forty-six percent of farmers had to purchase replacements and 29% considered purchases their most important source of replacements. A total of 123 farmers (43.2%) used computers for animal recording and 15.8% did not use any recording system at all. Twenty-three percent did not have their cattle individually identified. Sixty-four farmers (22.5%) considered bloat and 13% internal parasites as their most important animal health problem. Only 8% of case farmers thought that tuberculosis infection was their most important animal health problem. Forty-five percent of interviewees only rarely needed to consult with a veterinarian.

Univariate Analysis

Univariate analysis of the questionnaire data reduced the number of potential risk factors to 15 and 20 for matched and random controls respectively (see tables 47a and 47b). Factors which were not statistically significant included for example farming experience, the interviewee's relationship to the property, the water source for livestock, the amount of contact with neighbouring farms and the possibility of bush access.

Table 47a: Some results of univariate analysis for random controls using logistic regression

PARAMETER	BETA COEFFICIENT	SCORETEST	DF	P-VALUE
ADULTCAT	0.3065	0.6513	1	0.420
BEEFCATT	0.5844	4.205	1	0.040
BEEFLSU	0.0004	9.916	1	0.002
BUSHACCE='yes'	0.3111	1.089	1	0.297
BUYLIVES	0.0519	1.089	1	0.297
BUYREPLA	-0.1333	0.0667	1	0.796
CATTDENS	0.0090	1.302	1	0.254
CATTLLSU	0.0002	4.919	1	0.027
CATTPURC	0.0005	9.205	1	0.002
CCSPREAD	0.1134	1.417	1	0.234
CHSPREAD	0.1615	2.099	1	0.147
CONTEFFE='yes'	-0.0952	0.2381	1	0.626
DAIRYLSU	0.0000	0.0752	1	0.784
DIFFHER='0'		7.073	2	0.029
DIFFHER='<=3'	-0.1176	0.1765	1	0.674
DIFFHER='>3'	0.6897	6.897	1	0.009
EPIDEMIO	0.0141	0.7835	1	0.376
FARMSIZE	0.0011	4.593	1	0.032
FOREST	0.0149	1.482	1	0.224
FORFARM	5.305	1.009	1	0.315
GIVINGIN	0.0804	1.286	1	0.257
GIVINGUP	0.0243	0.0729	1	0.787
HARDWORK	0.0568	1.989	1	0.158
HEISTCAT	1.173	6.063	1	0.014
INTRODUC	0.1213	2.123	1	0.145
LIVEPREF	0.0503	1.282	1	0.258
LIVESDEN	0.0079	1.203	1	0.273
MAFCOMET	0.0966	2.318	1	0.128
MAINGRAZ='off'	0.5455	0.8182	1	0.366
MODEST	0.0307	0.4298	1	0.512
NEWIDEAS	0.0164	0.1232	1	0.726
NEXTCKM	-0.0678	2.6449	1	0.104
NEXTEKM	-0.0268	4.85	1	0.028
NOTALKAT	0.0668	1.269	1	0.260
OTHEMPL='yes'	-0.4242	1.485	1	0.223
OTHERCAT='yes'	0.2500	0.5000	1	0.480
PASTURE	0.0013	3.898	1	0.048
PERLABCA	-85.71	1.286	1	0.257
PERLABFA	2.899	0.1594	1	0.690
PERLABLI	0.0000	0.0000	1	10.000
PERMLABO	0.2011	4.102	1	0.043
PERTOTLA	0.1786	0.8484	1	0.357
PURCATT	0.2981	1.326	1	0.249
RECORDS	0.0124	0.0745	1	0.785
SOCIABLE	0.0210	0.2737	1	0.601
SPECIES	0.0606	1.030	1	0.310
TOTALLSU	0.0001	3.815	1	0.051
TOTLABCA	-40.00	0.4000	1	0.527
TOTLABFA	2.491	0.1744	1	0.676
TOTLABLI	40.00	0.2000	1	0.655
TOTLABOU	0.1512	3.863	1	0.049
WEATOTPU	1.373	3.343	1	0.067
WEAYEACA	-1.598	4.834	1	0.028
WHSTOTPU	0.7632	4.400	1	0.036

Table 47b: Some results of univariate analysis for matched controls using logistic regression

PARAMETER	BETA COEFFICIENT	SCORE TEST	DF	P-VALUE
ADULTCAT	1.154	3.088	1	0.079
BEEFCATT	0.8830	1.246	1	0.264
BEEFLSU	0.0005	7.603	1	0.006
BUSHACCE='yes'	-0.3158	0.4737	1	0.491
BUYLIVES	-0.1081	0.4324	1	0.511
BUYREPLA	0.4000	0.4000	1	0.527
CATTDENS	-0.0097	0.1597	1	0.689
CATTLLSU	0.0003	3.198	1	0.074
CATTPURC	0.0004	4.586	1	0.032
CCSPREAD	0.2745	1.922	1	0.166
CHSPREAD	0.4167	4.167	1	0.041
CONTEFFE='yes'	-0.6207	2.793	1	0.095
DAIRYLSU	-0.0002	0.8474	1	0.357
DIFFHER='0'		7.679	2	0.022
DIFFHER='<=3'	-0.7586	4.172	1	0.041
DIFFHER='>3'	1.130	7.348	1	0.007
EPIDEMIO	0.0403	0.9468	1	0.331
FARMSIZE	0.0025	9.207	1	0.002
FOREST	0.0239	2.755	1	0.097
FORFARM	3.890	0.4603	1	0.497
GIVINGIN	0.1277	0.3830	1	0.536
GIVINGUP	-0.2963	2.370	1	0.124
HARDWORK	0.3467	2.253	1	0.133
HEISTCAT	1.743	7.217	1	0.007
INTRODUC	0.1500	0.4500	1	0.502
LIVEPREF	0.040	0.0808	1	0.776
LIVESDEN	-0.0106	0.1688	1	0.681
MAFCOMET	0.4688	7.031	1	0.008
MAINGRAZ='off'	-0.2857	0.1429	1	0.705
MODEST	-0.2951	2.656	1	0.103
NEWIDEAS	0.0000	0.0000	1	10.000
NEXTCKM	0.4828	16.85	1	0.0001
NEXTEKM	-0.0005	0.0025	1	0.96
NOTALKAT	0.2182	1.309	1	0.253
OTHEMPL='yes'	-0.1538	0.0769	1	0.782
OTHERCAT='yes'	0.5000	1.000	1	0.317
PASTURE	0.0033	8.034	1	0.005
PERLABCA	-66.67	0.6667	1	0.414
PERLABFA	18.18	1.000	1	0.317
PERMLABO	0.6411	8.398	1	0.004
PERTOTLA	0.7837	1.242	1	0.265
PURCATT	0.4228	0.9957	1	0.318
RECORDS	-0.0566	0.1698	1	0.680
SOCIABLE	-0.0153	0.0076	1	0.930
SPECIES	0.3185	3.981	1	0.046
TOTALLSU	0.0002	4.379	1	0.036
TOTLABCA	-66.67	0.6667	1	0.414
TOTLABFA	-0.0000	0.0000	1	0.000
TOTLABOU	0.3937	4.173	1	0.041
WEATOTPU	0.9885	1.310	1	0.252
WEAYEACA	-2.793	4.049	1	0.044
WHSTOTPU	1.500	7.837	1	0.005

The following figures 101a - q describe the results for some of the variables which were found to be statistically significant in the univariate analysis.

Figure 101a: Beef component per cattle livestock unit stratified by case-control status

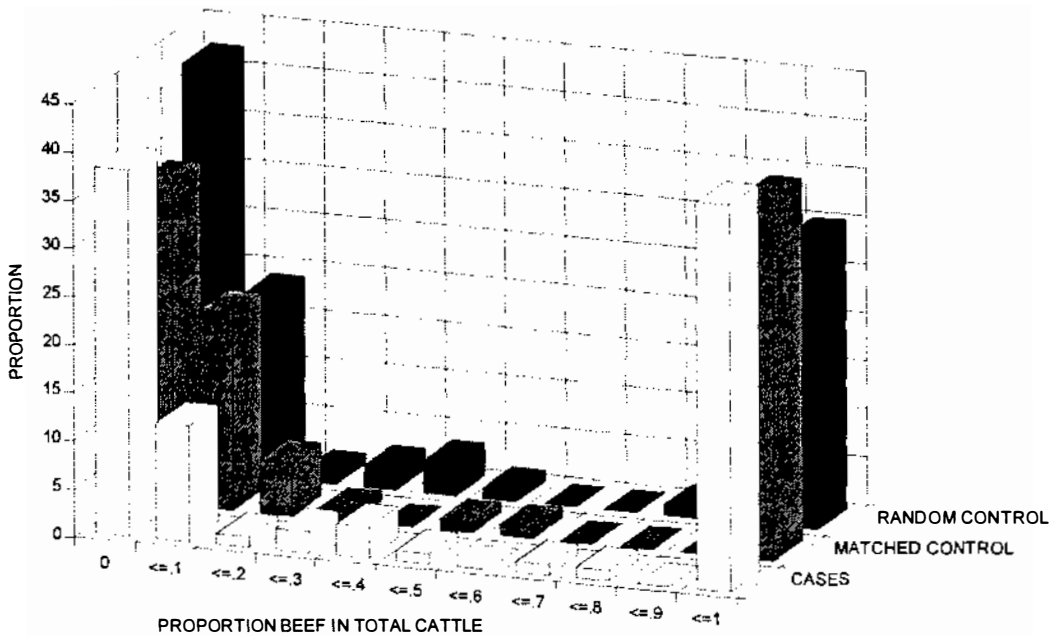


Figure 101b: Total beef cattle in livestock units stratified by case-control status

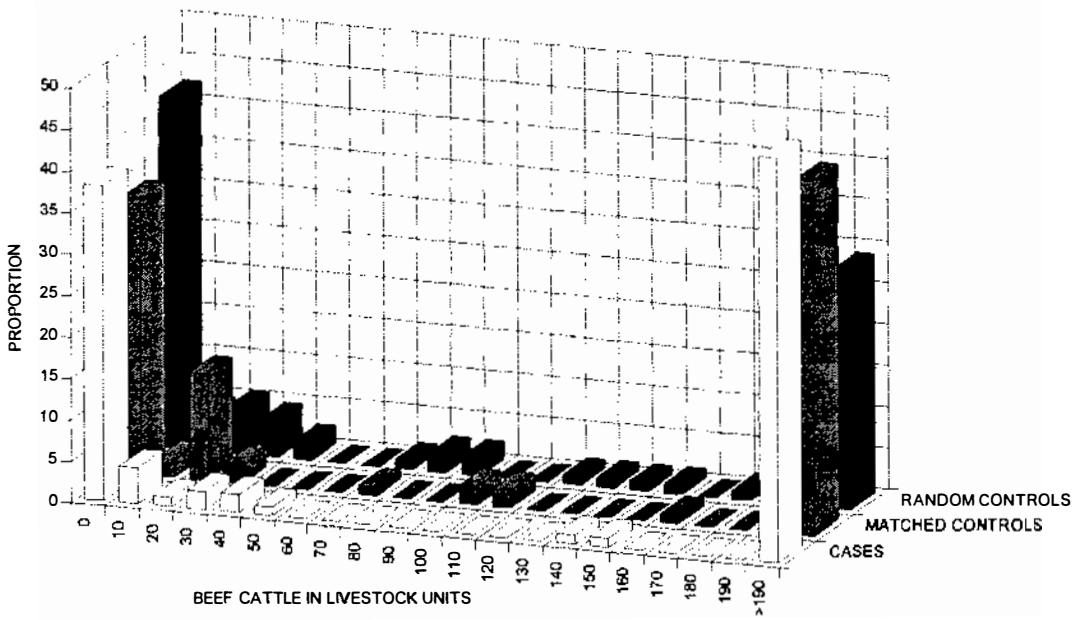


Figure 101c: Total cattle in livestock units stratified by case-control status

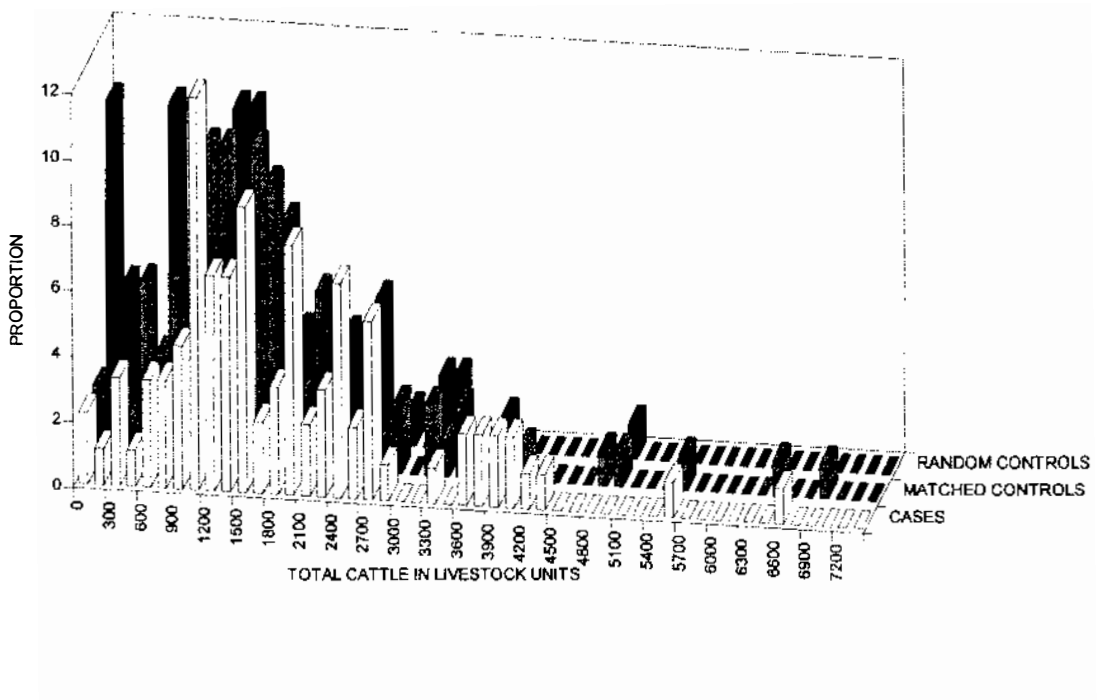


Figure 101d: Total cattle livestock units purchased stratified by case-control status

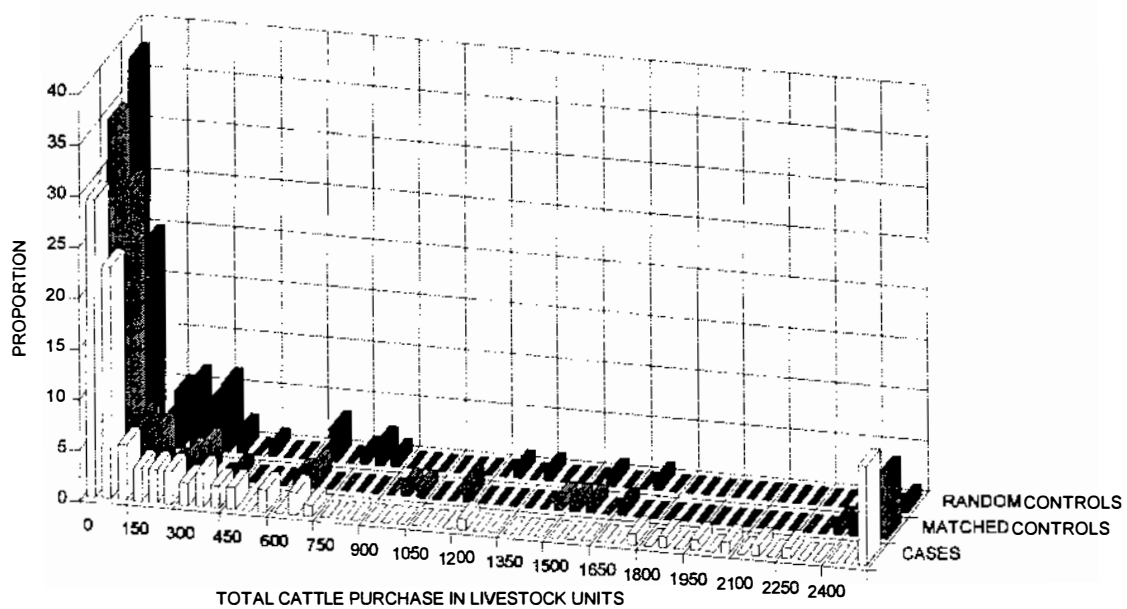


Figure 101e: Proportion of heifers/steers per cattle livestock unit stratified by case-control status

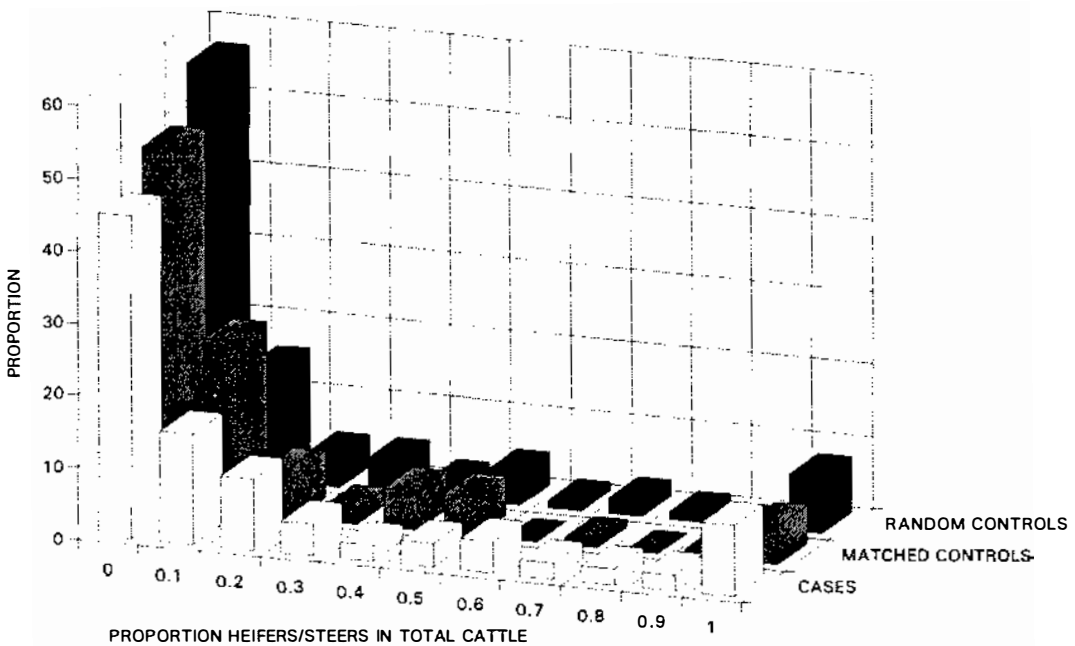


Figure 101f: Distance to next case farm stratified by case-control status

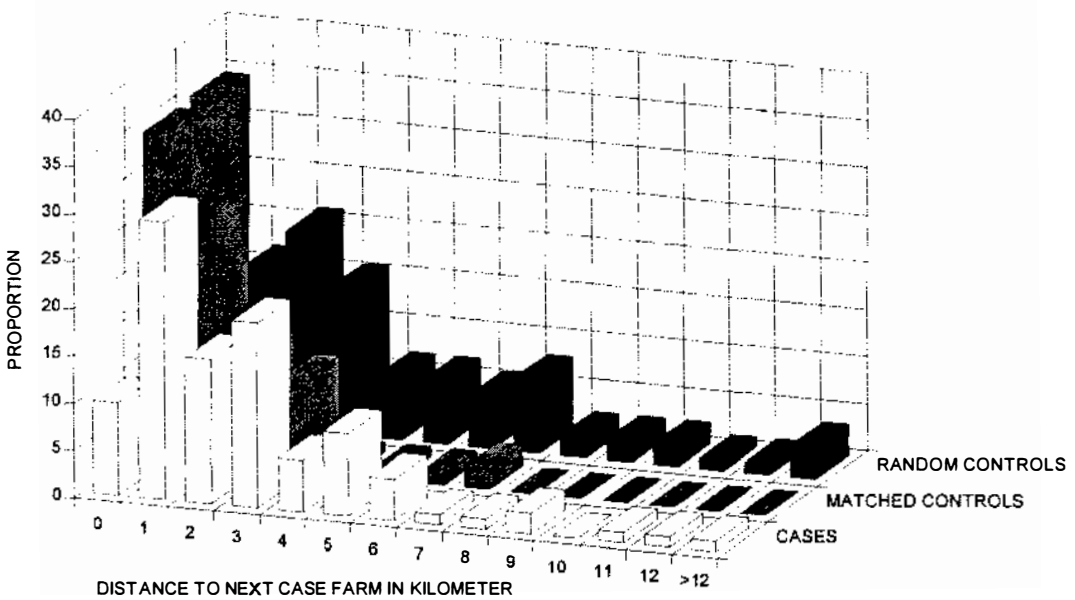


Figure 101g: Distance to next endemic TB area stratified by case-control status

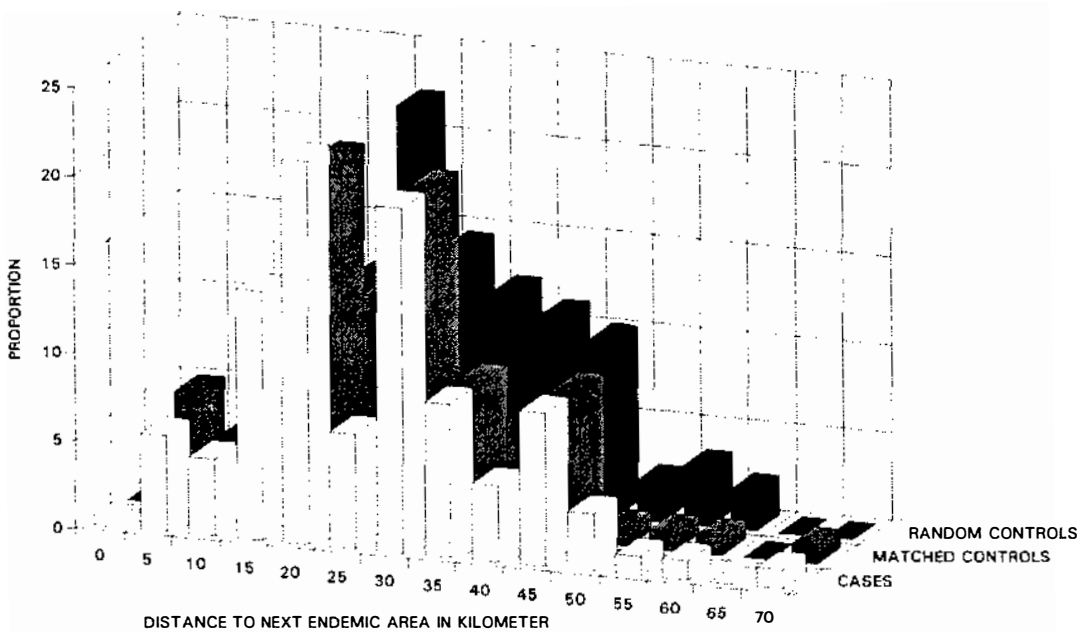


Figure 101h: Total area pasture stratified by case-control status

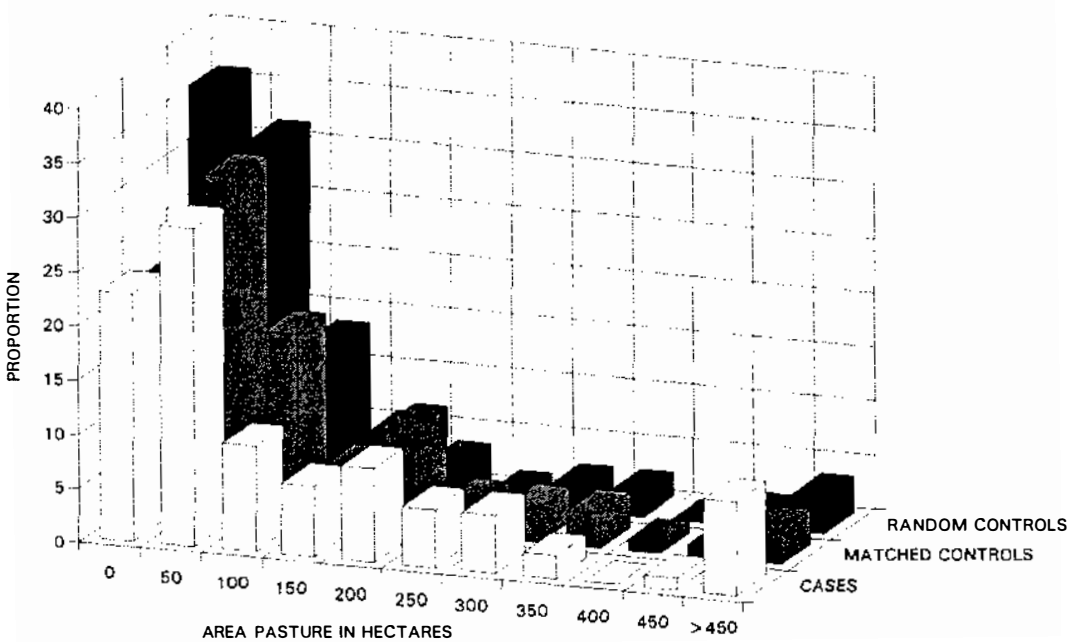


Figure 101i: Permanent labour units stratified by case-control status

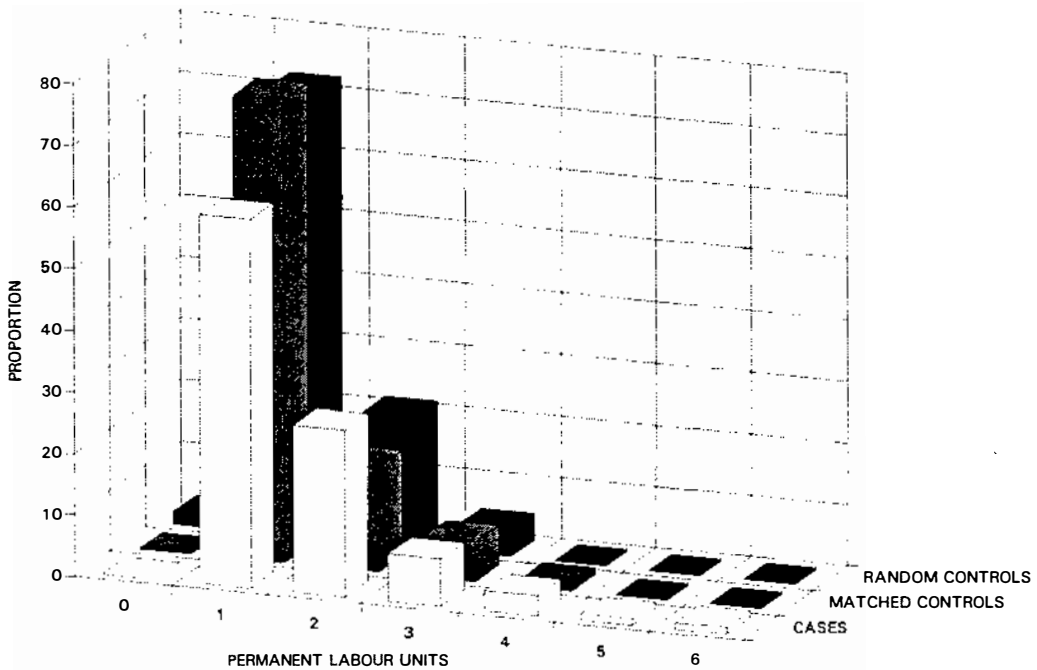


Figure 101j: Total labour units stratified by case-control status

Figure 101k: Total livestock units stratified by case-control status

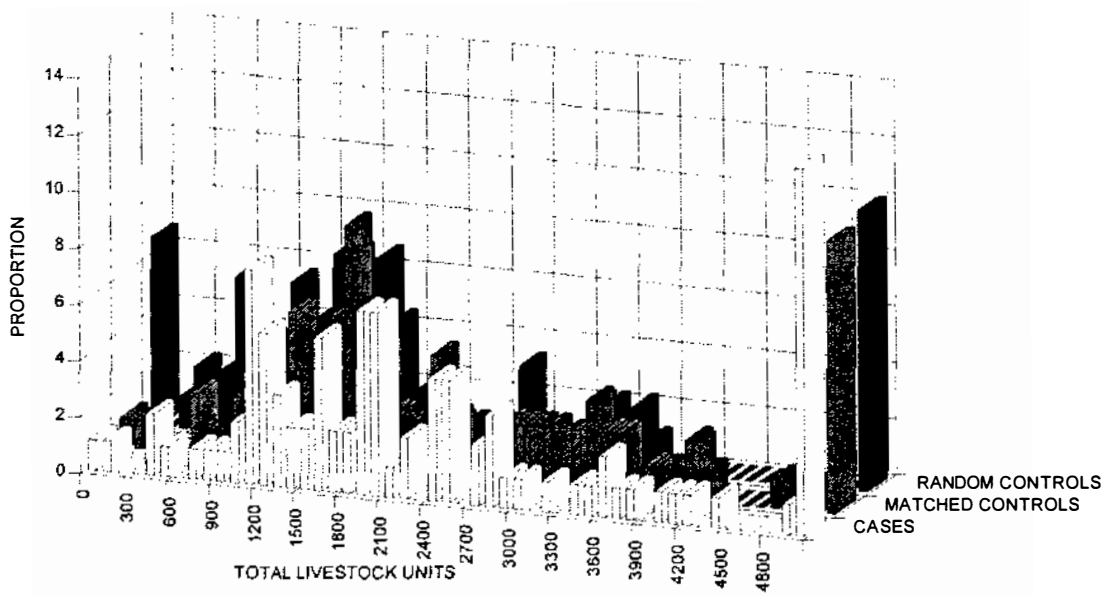


Figure 101l: Proportion of weaners/yearlings per cattle livestock unit stratified by case-control status

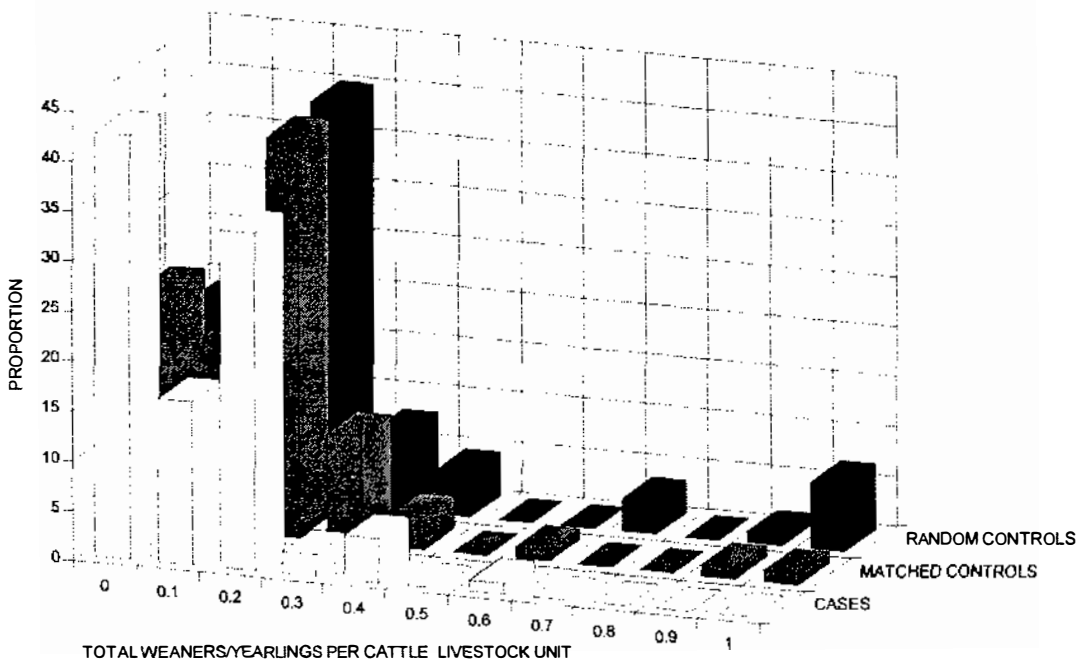


Figure 101m: Proportion of weaners/heifers/steers per cattle livestock unit stratified by case-control status

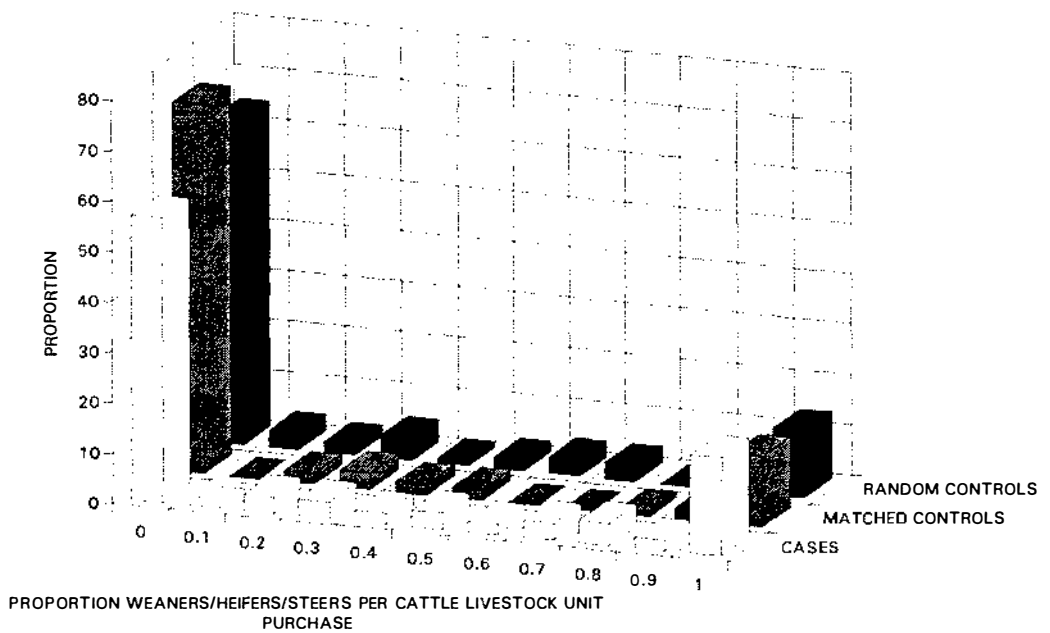


Figure 101n: Scores for knowledge about possible mechanisms of tuberculosis transmission between cattle and humans stratified by case-control status

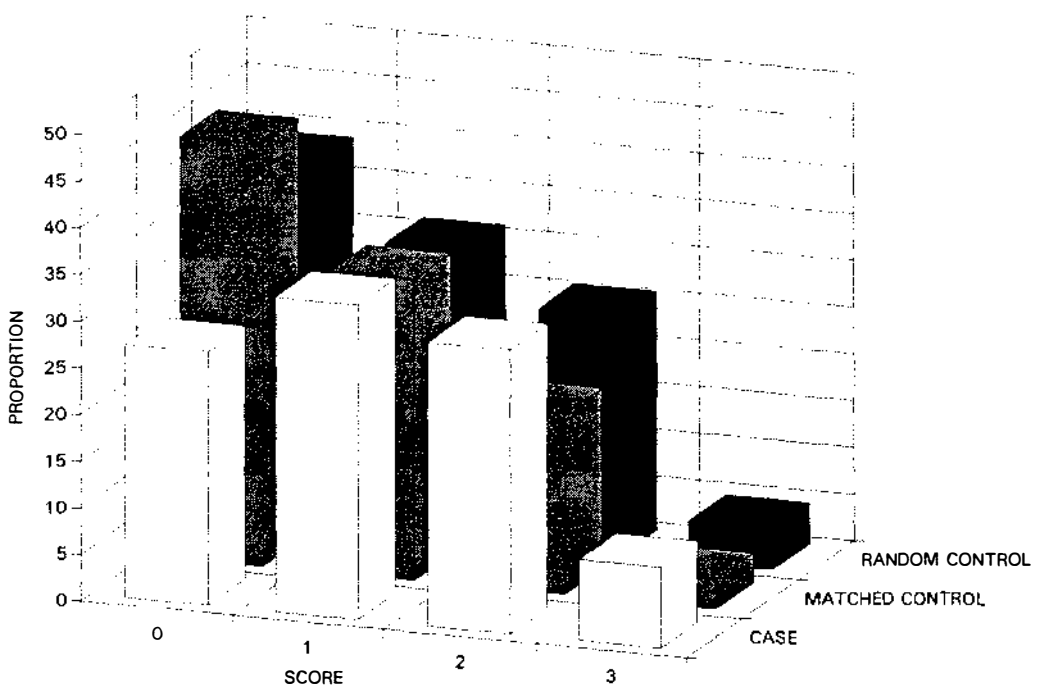


Figure 101o: Purchase of replacements and number of different sources stratified by case-control status

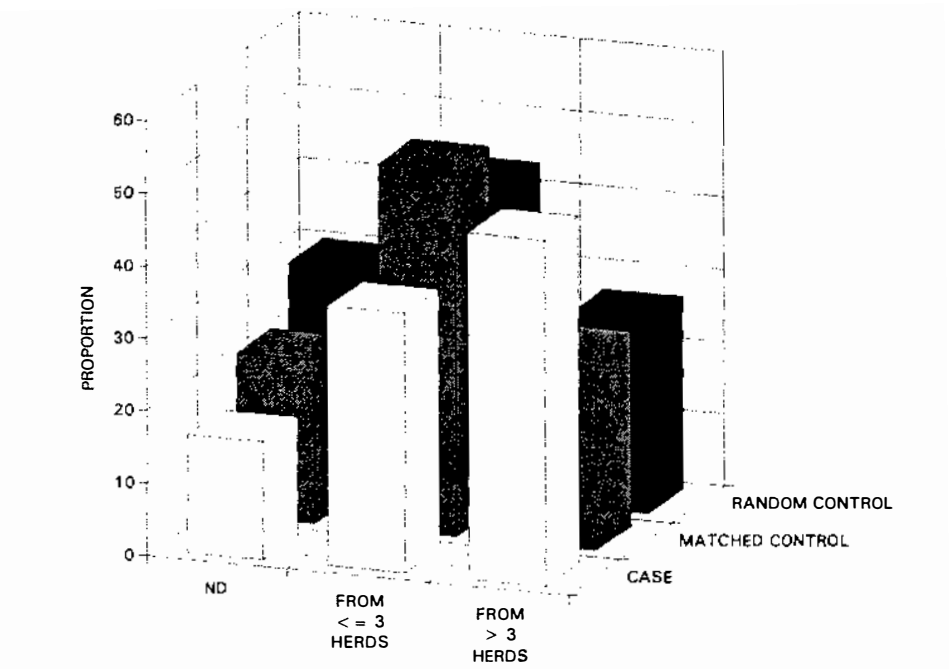


Figure 101p: Scores for knowledge about MAF TB control methods stratified by case-control status

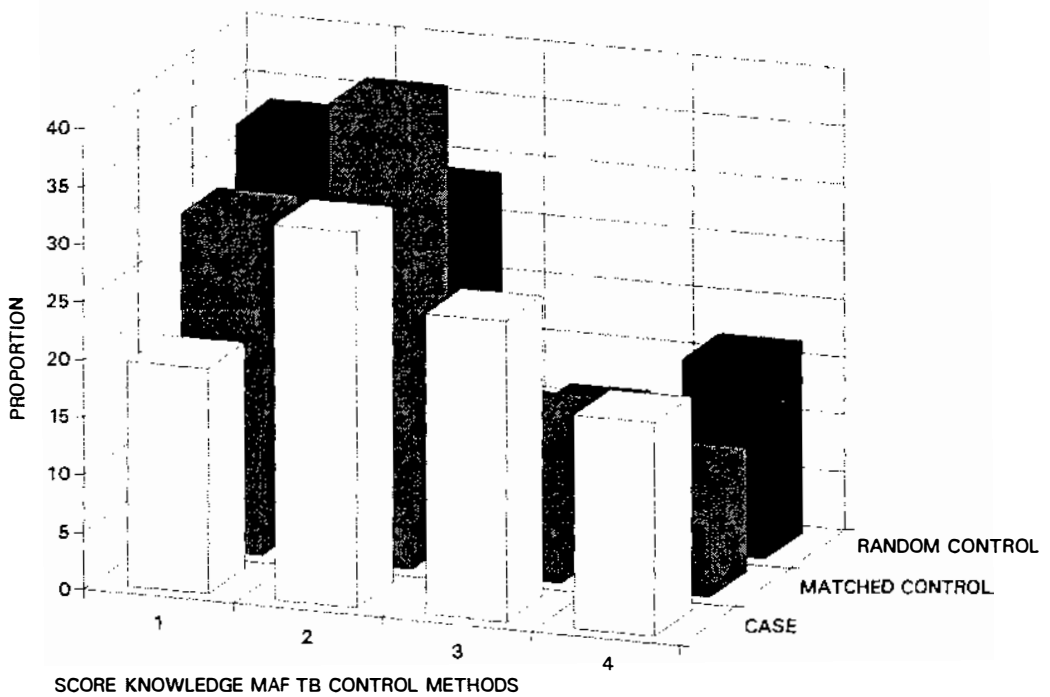
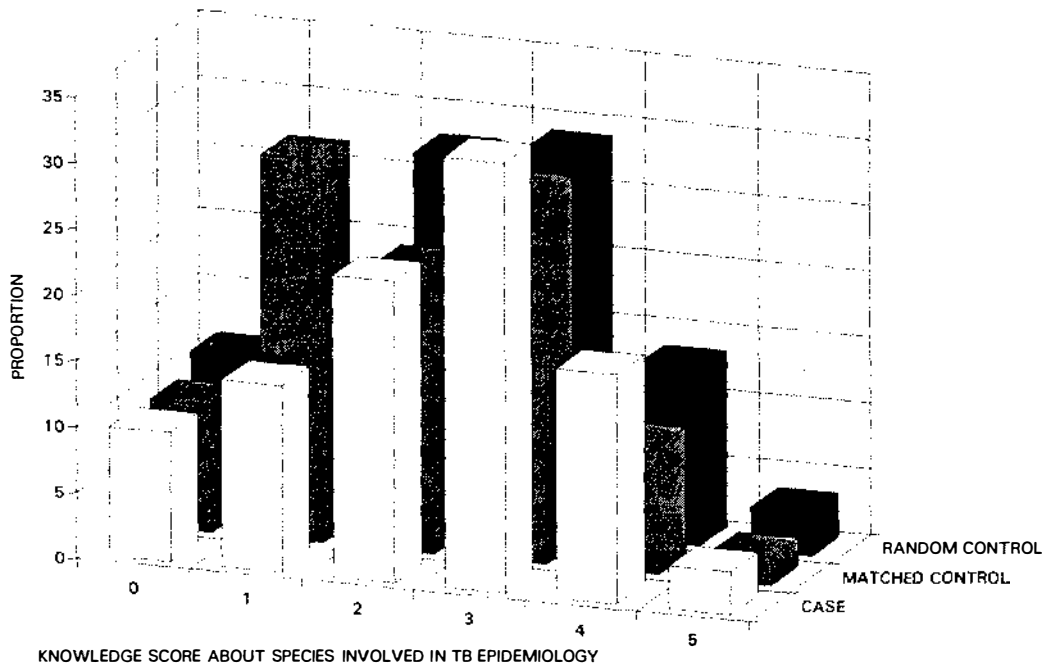
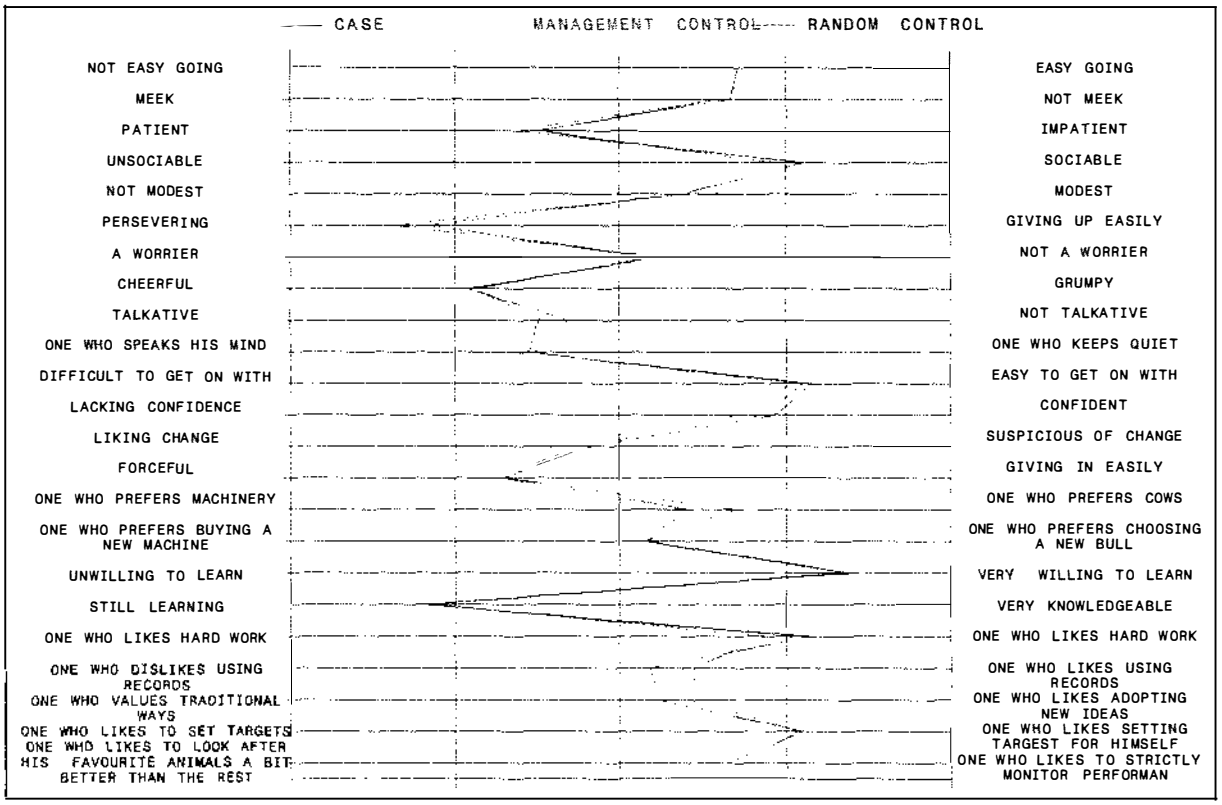


Figure 101q: Scores for knowledge about species involved in epidemiology of tuberculosis stratified by case-control status



Analysis of the interviewee's self concept using arithmetic means suggests that case farmers were less sociable, less talkative, more persevering, more livestock orientated, less likely to use herd records and were more traditional (see figure 102). These differences were not statistically significant, when using the Kruskal-Wallis test.

Figure 102: Personality trait means for interviewees by case-control status

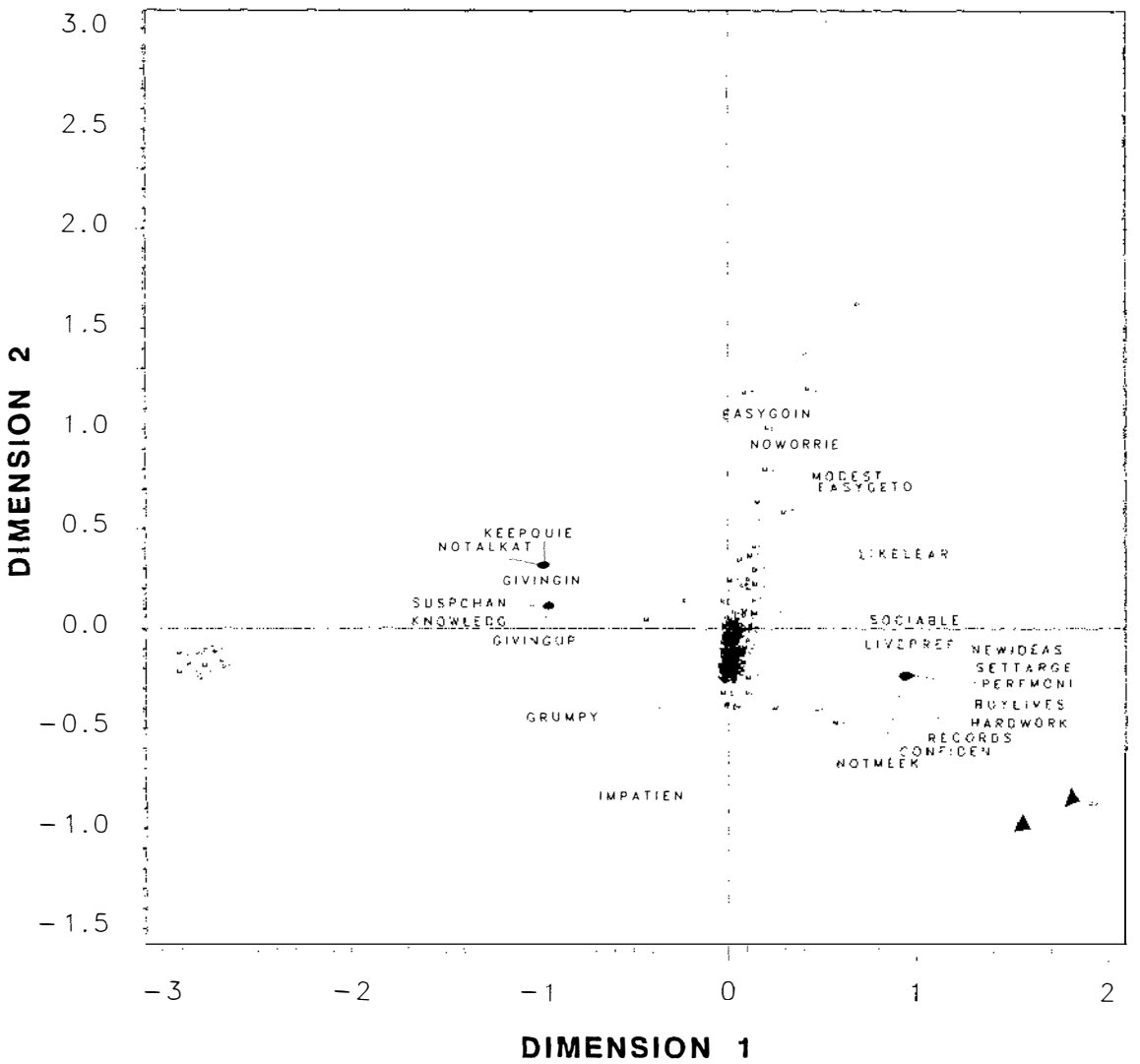


Multivariate Analysis

Multidimensional Preference Mapping

Ordinary principal components analysis of the information about farmer's self concept resulted in the first 2 components explaining 3.35 and 2.07 percent of variance respectively. After optimal scaling the monotonically transformed first 2 principal components explained 18.18% and 4.34% respectively. The biplot shows that most individuals are clustered around the origin. The directions of the adjective vectors indicate that dimension no. 1 separates conservative, quiet from innovative, communicative farmers. Dimension no.2 distinguishes the more relaxed, social from the less social farmer personality. A cluster of farmers including cases and controls consider themselves very conservative and unsocial (see figure 103).

Figure 103: Biplot of multidimensional preference mapping of study farms within the preference space describing their self concept



Stepwise Logistic Regression Models

A summary of the results of the stepwise regression process comparing cases with random controls is described in tables 48a and b.

Table 48a: Stepwise logistic regression analysis for cases compared with random controls

	P- VALUES prior to step							
PARAMETER	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
ADULTCAT	0.2685	0.9773	^ 0.2505	0.2488	0.4205	0.4747	0.6301	
BEEFLSU	0.0004	0.0063	^ 0.0801	^ 0.2170	0.2439	0.2461	0.3538	
BUSHACCE	0.1533	0.4210	0.7012	0.7022	0.8227	0.9189	0.9541	
BUYREPLA	0.9811	0.9780	0.6944	^ 0.8574	0.7992	0.7269	0.6210	
CATTDENS	0.5628	0.5581	0.2295	0.2368	0.3085	0.2772	0.4287	
CATTLLSU	0.0009	^ 0.0062	^ 0.0327	^ 0.2861	0.3739	0.3432	0.7185	
CATTPURC	0.0079	^ 0.0660	^ 0.4573	0.4340	0.4154	0.4098	0.4898	
CCSPREAD	0.3336	0.2363	0.2435	0.2588	0.7336	0.9584	0.9394	
CHSPREAD	0.0939	0.0590	0.0416	^ 0.0900	^ 0.2926	0.3222	0.3635	
CONTEFFE	0.2954	0.2206	0.3129	0.7103	0.7889	0.9177	0.7833	
DAIRYLSU	0.7720	0.9992	0.6264	0.8647	0.7829	0.8342	0.5766	
DIFFHER	0.0012	[0.0033]	0.0039	0.0052	0.0087	^ 0.0137	0.0087	0.0144
EPIDEMIO	0.3738	0.3796	0.3087	0.5813	0.4875	0.4643	0.3637	
FARMSIZE	0.0053	^ 0.0221	^ 0.0953	^ 0.3512	0.3263	0.3454	0.5046	
FOREST	0.1845	0.0698	0.1376	0.2443	0.3206	0.5570	0.6946	
FORFARM	0.2699	0.1264	0.1973	0.3215	0.4231	0.6694	0.7677	
HEISTCAT	0.1745	0.9853	0.2545	0.2536	0.4296	0.4845	0.6415	
LIVESDEN	0.5354	0.7689	0.5616	0.5962	0.8170	0.7851	0.9480	
MAFCOMET	0.0083	0.0086	0.0156	[0.0343]	0.0362	0.0384	0.0189	0.0102
MAINGRAZ	0.2397	0.4695	0.6422	0.4458	0.4484	0.3868	0.4392	
NEXTCKM	0.1151	0.1658	0.1341	^ 0.0545	^ 0.1041	0.4220	0.3782	
NEXTEKM	0.0351	0.0408	0.0677	0.0348	[0.0373]	0.0399	0.0454	0.0510
OTHEMPL	0.0454	^ 0.0764	0.0841	^ 0.1554	^ 0.0615	[0.0706]	0.0735	0.0355
OTHERCAT	0.8911	0.4342	0.8650	0.7719	0.8171	0.8780	0.8175	
PASTURE	0.0044	^ 0.0240	^ 0.1210	0.4805	0.4359	0.4797	0.6850	
PERLABCA	0.0180	^ 0.0702	0.1003	0.1329	0.2579	0.2519	0.4569	
PERLABFA	0.0541	^ 0.2679	0.7089	0.6778	0.8843	0.9039	0.9384	
PERLABLI	0.9901	0.8793	0.5881	0.5034	0.3181	0.3589	0.1347	
PERMLABO	0.0015	0.0084	0.0086	^ 0.2687	0.2622	0.3198	0.3686	
PERTOTLA	0.9200	0.9771	0.9381	0.1972	0.1731	0.1780	0.2692	
PURCATT	0.9993	0.3632	^ 0.0315	^ 0.0991	^ 0.1505	0.2128	0.3381	
SPECIES	0.2765	0.4744	0.5331	0.5084	0.7749	0.9502	0.8240	
TOTALLSU	0.0119	^ 0.0668	^ 0.2641	0.8520	0.9992	0.9587	0.5932	
TOTLABCA	0.0263	^ 0.1679	0.2232	0.2752	0.4596	0.4802	0.7646	
TOTLABFA	0.0561	^ 0.3109	0.8141	0.4955	0.6301	0.9039	0.9543	
TOTLABLI	0.7257	0.6238	0.3879	0.3371	0.2195	0.2459	[0.0785]	0.2730
TOTLABOU	0.0016	0.0054	[0.0071]	0.0094	^ 0.0182	0.0119	0.0214	0.0226
WEATOTPU	0.1839	0.2856	0.7585	0.9884	0.8963	0.8668	0.8409	
WEAYEACA	[0.0001]	0.0012	0.0014	0.0032	0.0028	0.0028	0.0044	0.0042
WHSTOTPU	0.1163	0.2409	0.9588	0.9764	0.9343	0.8530	0.9711	

[ ] variable selected at that step **^ transition**

Table 48b: Summary of the results of stepwise logistic regression analysis for cases compared with random controls

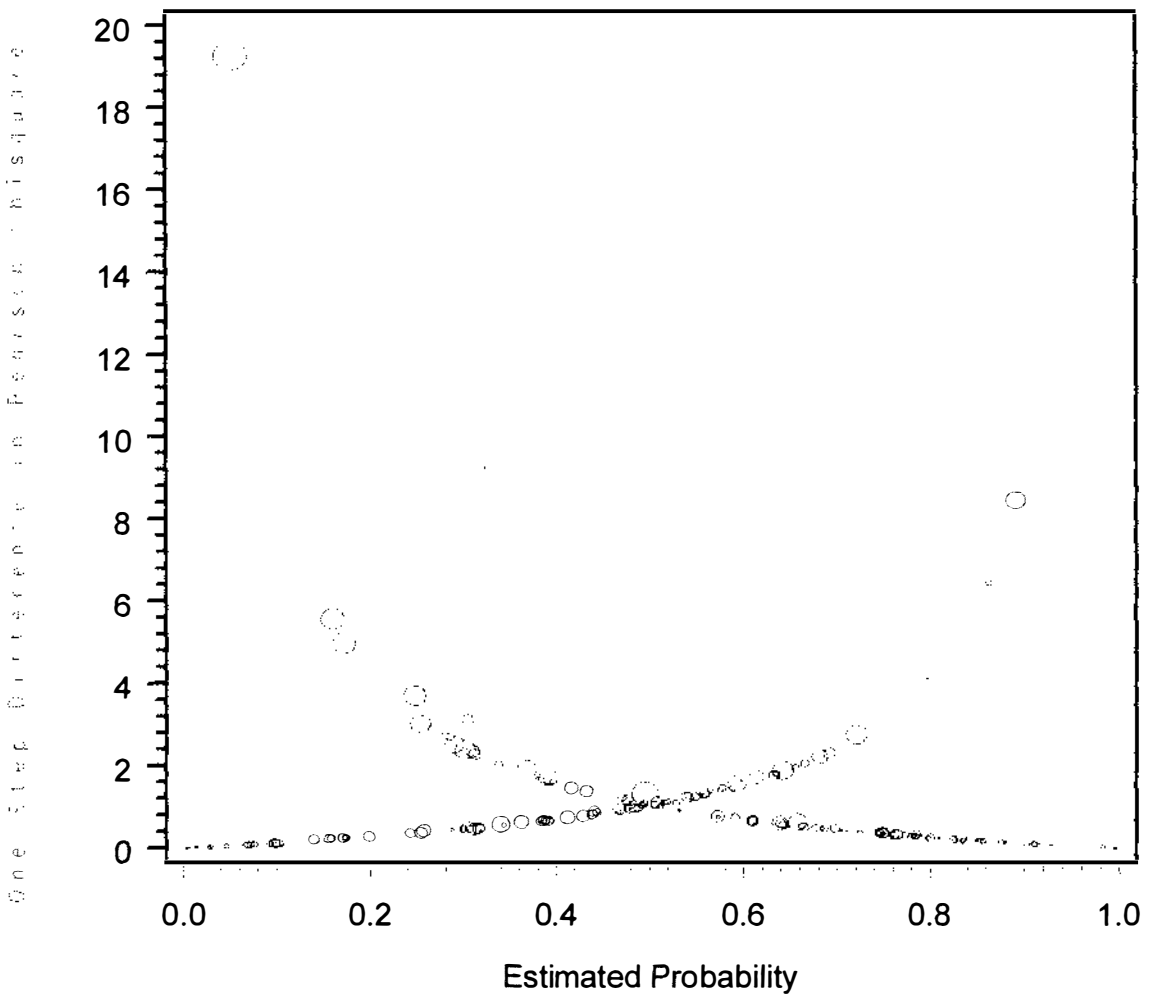
STEP	PARAMETER ADDED	P-VALUE	CLOSE ALTERNATIVES
1	WEAYEACA	0.0001	?BEEFLSU (0.0004 -> 0.0063), ?CATTLLSU (0.0009 -> 0.0062), CATTPURC (0.0079 -> 0.0660), FARMSIZE (0.0053 -> 0.0221), ?OTHEMPL (0.0454 -> 0.0764), PASTURE (0.0044 -> 0.0240), PERLABCA (0.0180 -> 0.0702), PERLABFA (0.0541 -> 0.2679) ?OTHEMPL (0.0454 -> 0.0764), TOTALLSU (0.0119 -> 0.0668), TOTLABCA (0.0263 -> 0.1679)
2	DIFFHER	0.0033	BEEFLSU (0.0063 -> 0.0801), CATTLLSU (0.0062 -> 0.0327), CATTPURC (0.0660 -> 0.4573) FARMSIZE (0.0221 - > 0.0953), PASTURE (0.0210 -> 0.1210), TOTALLSU (0.0668 -> 0.2641)
3	TOTLABOU	0.0071	BEEFLSU (0.0801 -> 0.2170), CATTLLSU (0.0327 -> 0.2861), ?CHSPREAD (0.0416 -> 0.0900), FARMSIZE (0.09513 -> 0.3512), ?OTHEMPL (0.0841 -> 0.1554), PERMLABO (0.0086 -> 0.2687), ?PURCATT (0.0315 -> 0.0991)
4	MAFCOMET	0.0343	CHSPREAD (0.0900 -> 0.2926), ?NEXTCKM (0.0545 -> 0.1041), ?PURCATT (0.0991 -> 0.1505)
5	NEXTEKM	0.0373	
6	OTHEMPL	0.0706	
7	TOTLABLI	0.0785	REMOVED IN NEXT STEP

Examination of table 48a reveals that the variables BEEFLSU, CATTLSU and FARMSIZE are confounded with the variables selected during the first 3 steps of the analysis. The final logistic regression model resulting from the stepwise selection process includes the parameters WEAYEACA, DIFFHER, TOTLABOU, MAFCOMET, NEXTEKM and OTHEMPL. The model was examined for the presence of first-order interaction terms. There was a statistically significant interaction between TOTLABOU and MAFCOMET and between TOTLABOU and OTHEMPL. Detailed information about the coefficients and goodness of fit estimates are presented in table 49. Figure 104 shows a diagnostic plot for the final model plotting the difference in chisquare versus predicted probability with the size of the plotted symbol proportional to the product of 1.5 and the standardized influence measure. There are six covariate patterns with very large values for the difference in chi-square. About 8 observations have a moderate size difference in chi-square and large influence.

Table 49: Final logistic regression model comparing cases and random controls

VARIABLE	ß- COEFFICIENT	S.E.	P - VALUE
WEAYEACA	-4.9334	1.6670	0.0031
DIFFHER	0.5143	0.2414	0.0331
TOTLABOU	1.4539	0.5019	0.0038
MAFCOMET	1.5324	0.4337	0.0004
NEXTEKM	-0.0230	0.0139	0.0973
OTHEMPL	-3.5109	1.2795	0.0061
TOTLABOU * MAFCOMET	-0.5866	0.2026	0.0038
TOTLABOU * OTHEMPL	1.4595	0.6959	0.0360
AIC INTERCEPT ONLY	254.284	AIC INTERCEPT + COVARIATES	210.715
-2 LOG LIKELIHOOD CHI^2	59.6 with 8 df (p=0.0001)	SAMPLE SIZE	89 CASES + 88 CONTROLS
SENSITIVITY	70.7%	SPECIFICITY	62.2%%

Figure 104: Diagnostic plot of difference chi-square versus predicted probability with plot symbol proportional to standardized influence measure for final logistic regression model comparing cases and random controls



A summary of the results of the stepwise regression process comparing cases with matched controls using the unconditional logistic regression approach is described in tables 50a and b. Tables 50c and d report the results for the analysis using conditional logistic regression. Examining the summary table 50a it is interesting to note that in the unconditional analysis four variables (CATTLLSU, CATTPURC, PURCATT, WEACAT) which during the univariate analysis had not been statistically significant at a p-value of 0.10 were included into the final model. The same was the case in the conditional analysis for the variables DAIRYLSU, PURCATT AND WEACAT.

Table 50a: Stepwise logistic regression analysis for cases compared with matched controls using the unconditional approach

	P-VALUE PRIOR TO STEP									
PARAMETERS	1	2	3	4	5	6	7	8	9	10
ADULTCAT	0.3525	0.9820	0.9324	^ 0.092	^ 0.356	^ 0.090	^ 0.129	^ 0.069	^ 0.212	0.2226
BEEFLSU	0.1459	0.4833	0.769	0.3064	0.9017	0.8202	0.7946	0.4143	0.5454	0.7728
BUSHACCE	0.6007	0.3710	0.5543	0.3148	0.7756	0.6329	0.617	0.7094	0.7197	0.7457
BUYREPLA	0.9266	0.9724	0.9561	0.744	0.9221	0.9595	0.8973	0.9721	0.8138	0.9371
CATTDENS	0.6588	0.9300	0.9385	0.3355	0.7634	0.2335	0.3057	0.7758	0.9555	0.9123
CATTLLSU	0.6088	0.8344	0.9296	0.9378	0.6003	0.324	[0.033]	0.038	0.0391	0.0102
CATTPURC	0.6053	0.3929	0.4118	0.7049	0.0366	^ 0.541	0.5997	^ 0.053	[0.066]	0.064
CCSPREAD	0.0284	0.0070	^ 0.086	0.0982	^ 0.185	0.1574	0.1349	0.1172	0.1956	0.2182
CHSPREAD	0.005	0.0130	^ 0.160	0.2136	0.218	0.1816	0.2154	0.2589	0.2773	0.2798
CONTEFFE	0.7554	0.9947	0.456	0.3368	0.1856	0.181	0.1073	0.1745	0.2278	0.2251
DAIRYLSU	0.3088	0.6151	0.829	0.3434	0.6739	0.2077	^ 0.062	0.4143	0.5454	0.7728
DIFFHER	0.009	0.0054	0.0169	[0.002]	0.002	0.0001	0.0001	0.0001	0.0003	0.0002
EPIDEMIO	0.0586	0.0298	^ 0.460	0.2858	0.4099	0.2947	0.3878	0.6118	0.6288	0.7466
FARMSIZE	0.1453	0.3590	0.3354	0.1619	0.519	0.3615	0.9335	0.5806	0.6565	0.4132
FOREST	0.261	0.3081	0.4641	0.4198	0.6063	0.5973	0.8744	0.7863	0.7331	0.6874
FORFARM	0.65	0.6429	0.9279	0.8956	0.9348	0.894	0.6806	0.4042	0.3326	0.2616
HEISTCAT	0.0409	^ 0.353	0.2976	0.1103	0.5788	0.151	0.2389	0.1343	0.3748	0.4114
INTRODUC	0.2735	^ 0.066	0.4325	0.5092	0.9132	0.8764	0.9192	0.9144	0.7939	0.6619
LIVESDEN	0.518	0.8950	0.6742	0.3079	0.5379	0.1142	0.1504	0.4397	0.4893	0.4988
MAFCOMET	0.005	[0.001]	0.002	0.0016	0.0066	0.0152	0.0218	0.0287	0.0261	0.0225
MAINGRAZ	0.72	0.8746	0.9378	0.7302	0.9517	0.5408	0.3638	0.3238	0.2912	0.3095
MAINOP	0.3118	0.7846	0.9939	0.2736	0.998	0.4172	0.4133	0.525	0.6902	0.6784
NEXTCKM	[0.000]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
NEXTEKM	0.9827	0.5385	0.5609	0.3041	0.5106	0.2233	0.2623	0.3198	0.3055	0.2387
OTHEMPL	0.7401	0.4976	0.9826	0.8609	0.9576	0.9065	0.9192	0.6187	0.5472	0.4307
OTHERCAT	0.6062	0.2944	0.7509	0.8548	0.9536	0.7705	0.8187	0.93	0.9809	0.9378
PASTURE	0.308	0.6324	0.5842	0.3177	0.813	0.603	0.6408	0.8969	0.9569	0.6454
PERLABCA	0.9623	0.8858	0.8803	0.7638	0.8387	0.9702	0.8879	0.4193	0.3002	0.4321
PERLABFA	0.3708	0.2250	0.2355	0.6184	^ 0.072	0.0898	^ 0.242	0.5262	0.5191	0.6546
PERLABLI	0.2564	0.5774	0.5645	0.6177	0.4924	0.5573	0.5237	0.6424	0.6182	0.6553
PERMLABO	0.0201	0.0124	0.0148	0.0211	0.0262	[0.029]	0.034	0.0077	0.0053	0.0025
PERTOTLA	0.1115	0.1757	0.2516	0.2328	0.1248	0.2736	0.5232	0.5059	0.582	0.8005
PURCATT	0.6856	^ 0.016	0.0225	0.0433	[0.000]	0.007	0.0078	0.0044	0.0022	0.0022
SPECIES	0.0267	0.0193	^ 0.290	0.2061	0.4981	0.5091	0.5576	0.5467	0.6299	0.6692
TOTALLSU	0.2642	0.4729	0.7126	0.2713	0.7285	0.8288	0.5145	0.4919	0.6064	0.4072
TOTLABCA	0.9589	0.9468	0.9611	0.8243	0.8425	0.8955	0.8712	0.3576	0.3357	0.4488
TOTLABFA	0.7608	0.4153	0.4324	0.9946	0.272	0.3433	0.4105	0.7172	0.6082	0.6654
TOTLABLI	0.2753	0.5854	0.5824	0.6371	0.5268	0.5719	0.5719	0.7369	0.6922	0.7317
TOTLABOU	0.5363	0.3514	0.2381	0.3447	0.6236	0.4143	0.2776	0.222	0.2883	0.4448
WEACAT	0.1089	^ 0.007	[0.011]	0.043	0.0192	0.0302	0.0321	0.0263	0.0238	0.0156
WEATOTPU	0.2749	0.2456	0.5258	0.6136	0.7723	0.865	0.9839	0.8691	0.1598	0.1672
WEAYEACA	0.0113	0.0412	0.0489	0.8368	0.3171	0.5867	0.3965	0.452	0.3946	0.3363
WHSTOTPU	0.0301	0.0704	^ 0.102	^ 0.025	0.2469	0.062	0.041	[0.043]	0.045	0.0719

[ ] variable selected at that step **^ transition**

Table 50b: Summary of the results of unconditional stepwise logistic regression analysis for cases compared with matched controls

STEP	PARAMETER ADDED	P-VALUE	CLOSE ALTERNATIVES
1	NEXTCKM	0.0001	HEISTCAT (0.0409 -> 0.3529)
2	MAFCOMET	0.0011	CCSPREAD (0.0070 -> 0.0862), CHSPREAD (0.0130 -> 0.1597), EPIDEMIO (0.0298 -> 0.4601), SPECIES (0.0193 -> 0.2898), ?WHSTOTPU (0.0704 -> 0.1024)
3	WEACAT	0.0105	WEAYEACA (0.0489 -> 0.8368)
4	DIFFHER	0.0017	ADULTCAT (0.0916 -> 0.3558), CCSPREAD (0.0982 -> 0.1847)
5	PURCATT	0.0001	CATTPURC (0.0366 -> 0.5405)
6	PERMLABO	0.0290	PERLABFA (0.0898 -> 02419)
7	CATTLLSU	0.0328	
8	WHSTOTPU	0.0427	ADULTCAT (0.0687 -> 0.2115)
9	CATTPURC	0.0661	

Table 50c: Stepwise logistic regression analysis for cases compared with matched controls using the conditional approach

	P-VALUE PRIOR TO STEP							
PARAMETER	1	2	3	4	5	6	7	8
ADULTCAT	0.5997	0.6813	0.9301	0.4643	0.418	0.9679	0.6588	0.6588
BEEFLSU	0.1482	^ 0.0419	0.0313	^ 0.2731	0.4893	0.6604	0.7547	0.7547
BUSHACCE	0.8527	0.495	0.4105	0.4856	0.5386	0.2482	0.1594	0.1594
BUYREPLA	1	0.5079	0.4759	0.8651	0.6693	0.8539	0.9598	0.9598
CATTDENS	0.9655	0.9288	0.4936	0.6881	0.4671	0.3853	0.5623	0.5623
CATTLLSU	0.4812	0.2795	0.334	0.5086	0.2797	0.2753	0.7547	0.7547
CATTPURC	0.7382	0.2953	0.178	0.2569	0.4351	0.5491	0.9815	0.9815
CCSPREAD	0.0474	0.0345	0.0349	0.0129	[0.0109]	0.0185	0.0312	0.0544
CHSPREAD	0.0066	0.0427	0.0324	0.0526	0.0811	^ 0.3967	0.7193	0.7193
CONTEFFE	0.3994	0.3485	0.5539	0.9448	0.7943	0.5546	0.3995	0.3995
DAIRYLSU	0.4359	0.4124	0.2536	^ 0.0351	0.0261	[0.0417]	0.045	0.0578
DIFFHER	0.0282	0.0302	0.0047	[0.0024]	0.0058	0.0065	0.0051	0.0048
EPIDEMIO	0.092	^ 0.222	0.4464	0.364	0.4442	0.6941	0.6628	0.6628
FARMSIZE	0.1501	^ 0.0917	0.0761	^ 0.4824	0.3797	0.5569	0.6451	0.6451
FOREST	0.2981	0.427	0.3384	0.8801	0.9078	0.7129	0.7057	0.7057
FORFARM	0.7151	0.8523	0.7982	0.4367	0.5236	0.8822	0.8096	0.8096
HEISTCAT	0.0806	^ 0.3091	0.2341	0.8996	0.9116	0.5511	0.9241	0.9241
INTRODUC	0.3946	0.1334	0.1178	0.1396	0.2234	0.9547	0.876	0.876
LIVESDEN	0.6739	0.9638	0.6009	0.4042	0.1649	^ 0.0932	0.1193	0.1193
MAFCOMET	0.0058	0.0042	0.0092	0.0093	0.0316	0.1493	0.2037	0.2037
MAINGRAZ	0.4795	0.7047	0.6958	0.3682	0.6118	0.8871	0.8255	0.8255
MAINOP	0.2482	0.1105	^ 0.0712	0.0665	^ 0.1136	0.303	0.4896	0.4896
NEXTCKM	[0.0001]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0007
NEXTEKM	0.7914	0.8909	0.7719	0.9081	0.8344	0.7239	0.3001	0.3001
OTHEMPL	0.6547	0.3045	0.2915	0.1554	0.3553	0.5801	0.9749	0.9749
OTHERCAT	0.6831	0.5327	0.6758	0.5278	0.6045	0.6688	0.6891	0.6891
PASTURE	0.3471	0.1376	0.1052	0.6378	0.5152	0.7529	0.8484	0.8484
PERLABCA	1	0.4134	0.5743	0.1431	0.4196	0.7011	0.382	0.382
PERLABFA	0.127	0.0925	^ 0.2348	0.5644	0.5952	0.6835	0.9085	0.9085
PERLABLI	0.2715	0.6048	0.5276	0.4397	0.4029	0.392	0.5876	0.5876
PERMLABO	0.0116	0.0044	[0.0025]	0.0057	0.0048	0.0035	0.0018	0.0096
PERTOTLA	0.051	^ 0.2544	0.4026	0.9933	0.5955	0.6259	0.5898	0.5898
PURCATT	0.4672	[0.0008]	0.0159	0.0123	0.0074	0.0088	0.012	0.0105
SPECIES	0.0522	^ 0.1671	0.3198	0.327	0.9017	0.7205	0.7598	0.7598
TOTALLSU	0.2934	0.1997	0.1891	0.6959	0.4687	0.3203	0.3182	0.3182
TOTLABCA	1	0.6291	0.8572	0.3943	0.7387	0.9855	0.5987	0.5987
TOTLABFA	0.6051	0.3369	0.5348	0.6212	0.4778	0.5454	0.7896	0.7896
TOTLABLI	0.2885	0.6144	0.4988	0.4237	0.392	0.3903	0.5871	0.5871
TOTLABOU	0.6246	^ 0.0803	^ 0.0297	^ 0.841	0.4927	0.5182	0.4997	0.4997
WEACAT	0.109	^ 0.0081	0.0279	^ 0.0959	^ 0.0351	0.0589	[0.0826]	0.2647
WEATOTPU	0.5023	0.1304	0.1272	0.3068	0.6671	0.8761	0.9809	0.9809
WEAYEACA	0.0241	0.016	0.0276	^ 0.1308	0.1206	0.3239	0.3956	0.3956
WHSTOTPU	0.0612	^ 0.2695	^ 0.0643	^ 0.1131	0.2809	0.1816	0.1309	0.1309

[ ] variable selected at that step **^ transition**

Table 50d: Summary of the results of conditional stepwise logistic regression analysis for cases compared with matched controls

STEP	PARAMETER ADDED	P-VALUE	CLOSE ALTERNATIVES
1	NEXTCKM	0.0001	EPIDEMIO (0.092 -> 0.222), HEISTCAT (0.0806 -> 0.3091), PERTOTLA (0.051 -> 0.2544), WHSTOTPU (0.0612 -> 0.2695)
2	PURCATT	0.0008	PERLABFA (0.0925 -> 0.2348)
3	PERMLABO	0.0025	BEEFLSU (0.0313 -> 0.2731), FARMSIZE (0.0761 -> 0.4824), TOTLABOU (0.0297 -> 0.841), WEACAT (0.0279 -> 0.0959), WEAYEACA (0.0276 -> 0.1308), ?WHSTOTPU (0.0643 -> 0.1131)
4	DIFFHER	0.0024	?CHSPREAD (0.0526 -> 0.0811), ?MAINOP (0.0665 -> 0.1136)
5	CCSPREAD	0.0109	CHSPREAD (0.0811 -> 0.3967), MAFCOMET (0.0316 -> 0.1493)
6	DAIRYLSU	0.0417	
7	WEACAT	0.0826	REMOVED IN NEXT STEP

Table 51: Comparison of coefficients of logistic regression models for cases and matched controls using the unconditional and the conditional approach

	UNCONDITIONAL ANALYSIS			CONDITIONAL ANALYSIS		
VARIABLE	BETA	S.E.	P - VALUE	BETA	S.E.	P-VALUE
CATTLLSU	-0.0006	0.0002	0.0102			
CATTPURC	0.0007	0.0004	0.0641			
CCSPREAD				0.8436	0.3916	0.0312
DAIRYLSU				-0.0009	0.0004	0.0450
DIFFHER	1.3784	0.3697	0.0002	1.5703	0.5613	0.0051
MAFCOMET	0.3587	0.1528	0.0189			
NEXTCKM	0.7885	0.1504	0.0001	1.5857	0.4230	0.0002
PERMLABO	0.9506	0.3150	0.0025	2.1831	0.6979	0.0018
PURCATT	-2.8984	0.9457	0.0022	-2.5212	0.9814	0.0102
WEACAT	-0.0074	0.0031	0.0156			
WHSTOTPU	1.1271	0.6263	0.0719			
AIC INTERCEPT ONLY	254.218	AIC INTERCEPT + COVARIATES	181.969			
-2 LOG LIKELIHOOD CHI^2	90.25 with 9 df (p=0.0001)			77.09 with 6 df (p=0.0001)		
SENSITIVITY	71.9%	SPECIFICITY	76.3%%			
SAMPLE SIZE	89 CASES + 93 CONTROLS			89 MATCHED PAIRS		

A comparison of the estimates for the coefficients of variables included into the final logistic regression models for the matched controls show that while less variables were included into the model based on the conditional analysis approach the estimated coefficients for variables included into both models were generally more conservative in the unconditional analysis. It has to be taken into consideration that due to missing values in the unconditional analysis 89 cases and 93 controls were used while in the conditional analysis 89 complete risk sets were included. Table 52 shows the coefficients of the final conditional logistic regression model including one statistically significant first-order interaction term.

Table 52: Final conditional logistic regression model comparing cases and matched controls

VARIABLE	ß-COEFFICIENT	S.E.	P - VALUE
CCSPREAD	0.7882	0.3562	0.0269
DAIRYLSU	-0.0007	0.0004	0.1082
DIFFHER	3.6812	1.4245	0.0098
NEXTCKM	2.9582	1.0844	0.0064
PERMLABO	1.9284	0.6579	0.0034
PURCATT	-1.9917	0.9082	0.0283
DIFFHER * NEXTCKM	-0.9704	0.5513	0.0784
-2 LOG LIKELIHOOD CHI^2	81.60 with 7 df (p=0.0001)	SAMPLE SIZE	92 matched pairs

Path Analysis using Standard Regression Procedures

For path analysis potential risk factors were grouped into parameters describing physical farm characteristics, operational characteristics, herd characteristics, purchase behaviour, self concept, problem understanding and other factors.

Figures 105a and 105b show the null hypothesis and the final path diagram for comparison of cases with random controls. The final path model includes factors representing herd characteristics, cattle purchase behaviour, knowledge about the disease problem and proximity to the next TB endemic area. Farms which were located closer to the TB endemic areas were at increased risk of breakdown. Farmers who bought cattle from more than 3 different herds were more likely to have TB reactors. If farms had more beef cattle, they were also more likely to buy more cattle and to purchase from more than 3 different herds. Larger herds had a smaller proportion of weaners and yearlings. Herds which had a smaller proportion of weaners and yearlings were less likely to break down to tuberculosis infection. Case farmers appeared to be better informed about the disease problem. They tended to have

more employees and were less likely to have another job at the same time. Larger herds and larger farms had more employees. Table 53 lists the logistic and least-squares regression results for direct effects in the final path model for random controls. The factors with direct effects on TB breakdown status were identical with the final model based on the stepwise regression approach (see 51).

Table 53: Results of regression analyses for final path model comparing cases with random controls

MODEL		PATH COEFFICIENTS			MODEL FIT			
DEPENDENT	INDEPENDENT	ß-COEFF.	S.E.	P-VALUE	SAMPLE SIZE	-2 LOG L OR R^2	D.F.	P-VALUE
CATTPURC	BEEFLSU	0.4565	0.0559	0.0001	180	0.27		0.0001
DIFFHER	CATTPURC	0.0051	0.0011	0.0001				
	BEEFLSU	0.0007	0.0002	0.0015	182	89.8	2	0.0001
MAFCOMET	CHSPREAD	0.4502	0.0875	0.0001				
	OTHEMPL	0.4506	0.1949	0.0219	174	0.15		0.0001
PERMLABO	PASTURE	0.0014	0.0003	0.0001	182	0.11		0.0001
TOTLABOU	PERMLABO	0.7010	0.0670	0.0001				
	PASTURE	0.0008	0.0003	0.0043				
	CATTLLSU	0.0001	0.0000	0.0089	182	0.55		0.0001
WEAYEACA	CATTLLSU	-0.00004	0.00001	0.003	182	0.05		0.003

Figure 105a: Null hypothesis path diagram for comparison of cases with random controls

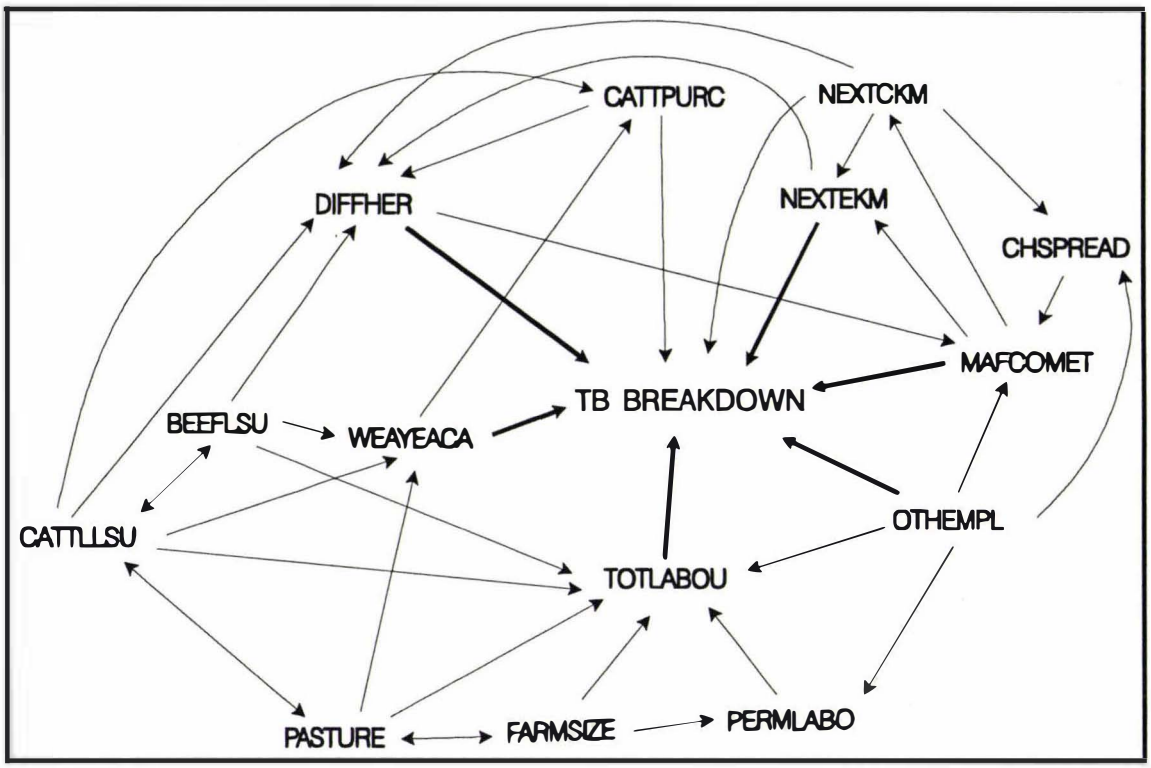
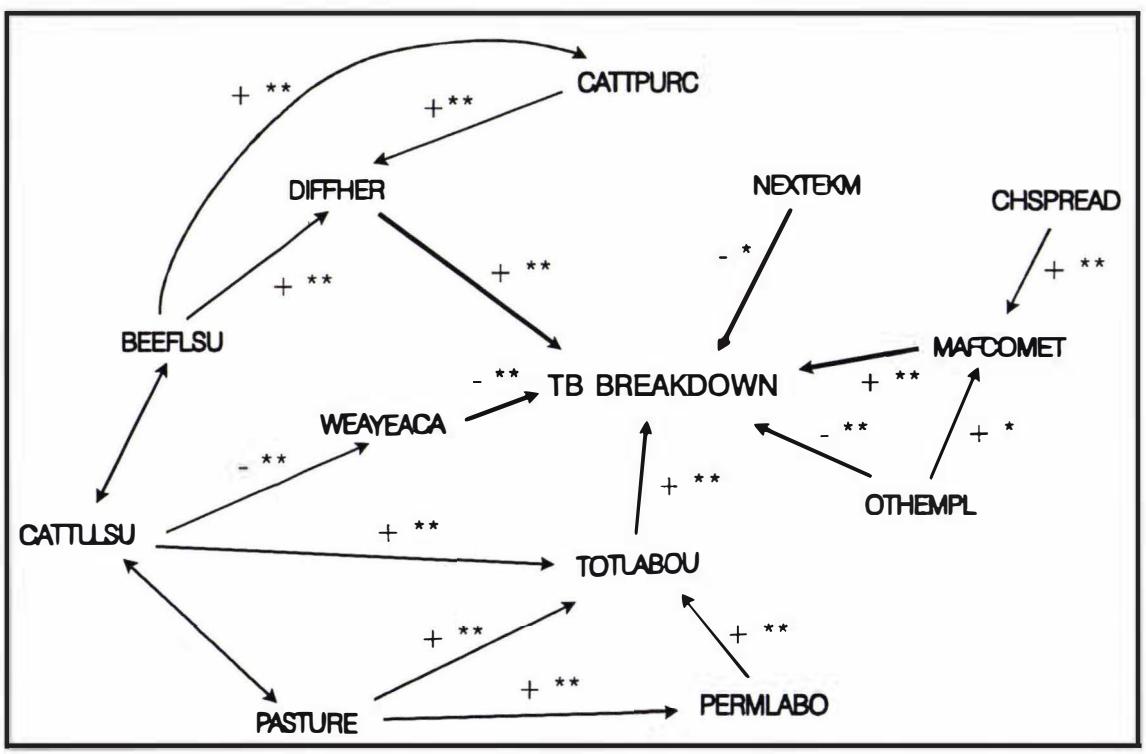


Figure 105b: Final path diagram for comparison of cases with random controls



Figures 106a and 106b show the null hypothesis and the final path diagram for comparison of cases with matched controls. The final path diagram includes factors describing herd characteristics, information about cattle purchase behaviour, knowledge about the disease problem and proximity of the next case farm. The latter factor indicates that case herds are not spatially clustered. Better knowledge about the disease problem was associated with an increased risk of breakdown. Farmers who bought more cattle including a larger proportion of weaners, heifers and steers and who bought from more than 3 different herds were at a higher risk of having reactor cattle to the tuberculin test. The proportion of weaners, heifers and steers in purchased cattle was higher, if the herd had a stronger component of heifers and steers. Herds with more dairy cattle appeared to buy less cattle, replaced a smaller proportion of their herd, had more weaners and yearlings and were more labour intensive. Case herds tended to be smaller than controls and had a smaller proportion of weaners in the herd. Case herds had more permanent labour employed. Table 54 lists the logistic and least-squares regression results for direct effects in the final path model for matched controls. The factors with direct effects on TB breakdown status were identical with those in the final model based on the stepwise regression approach (see table 51).

Table 54: Results of regression analyses for final path model comparing cases with matched controls

MODEL		PATH COEFFICIENTS			MODEL FIT			
DEPENDENT	INDEPENDENT	ß-COEFF.	S.E.	P-VALUE	SAMPLE SIZE	-2 LOG L OR R^2	D.F.	P-VALUE
ADULTCAT	CATTLLSU	0.0001	0.00001	0.0001				
	DAIRYLSU	-0.0001	0.00001	0.00001	189	0.3783	2	0.00001
CATTPURC	WHSTOTPU	956.66	206.03	0.0001				
	CATTLLSU	0.4497	0.0706	0.0001				
	DAIRYLSU	-0.3942	0.0766	0.0001	189	0.3599	3	0.0001
DIFFHER	NEXTCKM	-0.1928	0.0696	0.0056				
	CATTPURC	0.0038	0.0008	0.0001				
	WHSTOTPU	1.0503	0.4860	0.0307	187	96.246	3	0.0001
HEISTCAT	DAIRYLSU	-0.0002	0.00001	0.0001				
	CATTLLSU	0.0001	0.00001	0.0001	189	0.4732	2	0.0001
MAFCOMET	EPIDEMIO	0.0464	0.0192	0.0168				
	SPECIES	0.1693	0.0684	0.0142				
	CCSPREAD	0.1369	0.0829	0.1007				
	CHSPREAD	0.1408	0.0877	0.1099	187	0.2633	4	0.0001
PERMLABO	FARMSIZE	0.0014	0.0002	0.0001				
	DAIRYLSU	0.0002	0.00005	0.0001	189	0.23	2	0.0001
PURCATT	WHSTOTPU	0.5355	0.1310	0.0001				
	DAIRYLSU	-0.0001	0.00004	0.0036	189	0.1644	2	0.0001
WEACAT	DAIRYLSU	-0.0648	0.0134	0.0001				
	CATTLLSU	0.0595	0.0129	0.0001	189	0.1400	2	0.0001
WEAYEACA	ADULTCAT	0.2228	0.0363	0.0001				
	DAIRYLSU	0.0001	0.00001	0.0001				
	CATTLLSU	-0.00004	0.00001	0.0001	189	0.1948	3	0.0001
WHSTOTPU	HEISTCAT	0.8000	0.0773	0.0001				
	WEAYEACA	0.2603	0.1778	0.1450	189	0.3657	2	0.0001

Figure 106a: Null hypothesis path diagram for comparison of cases with matched controls

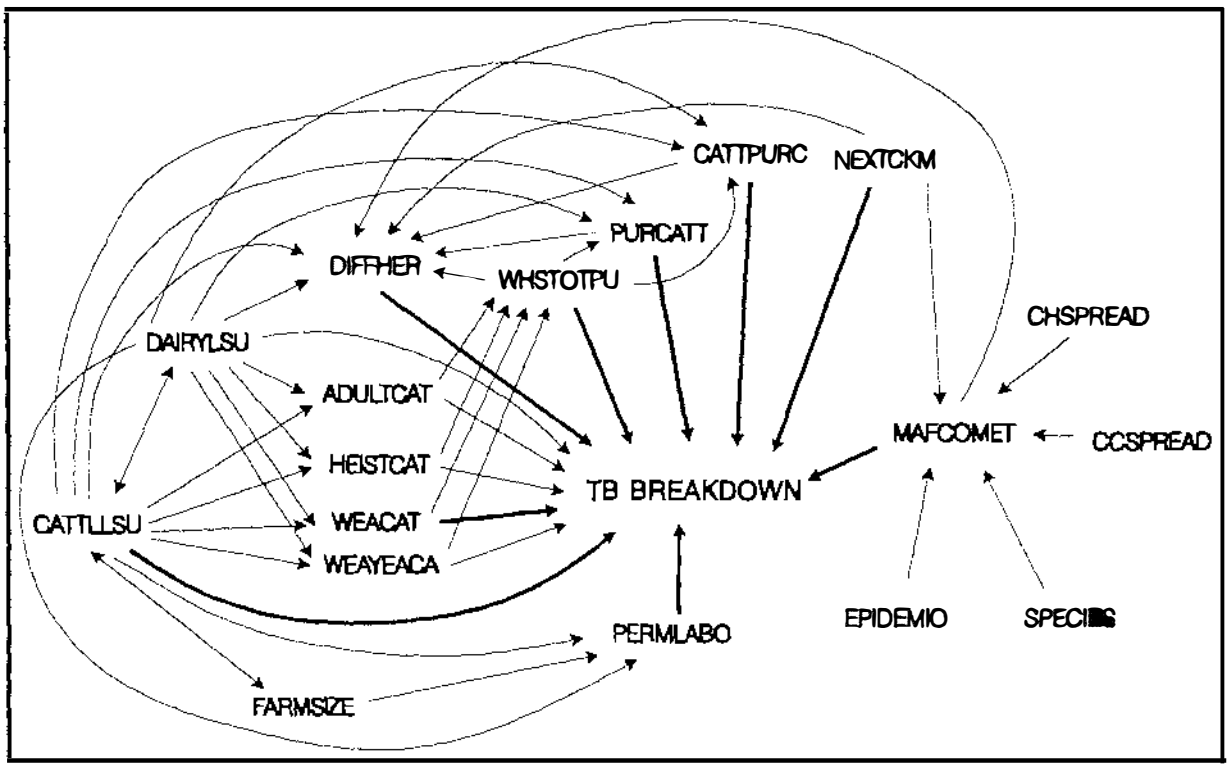
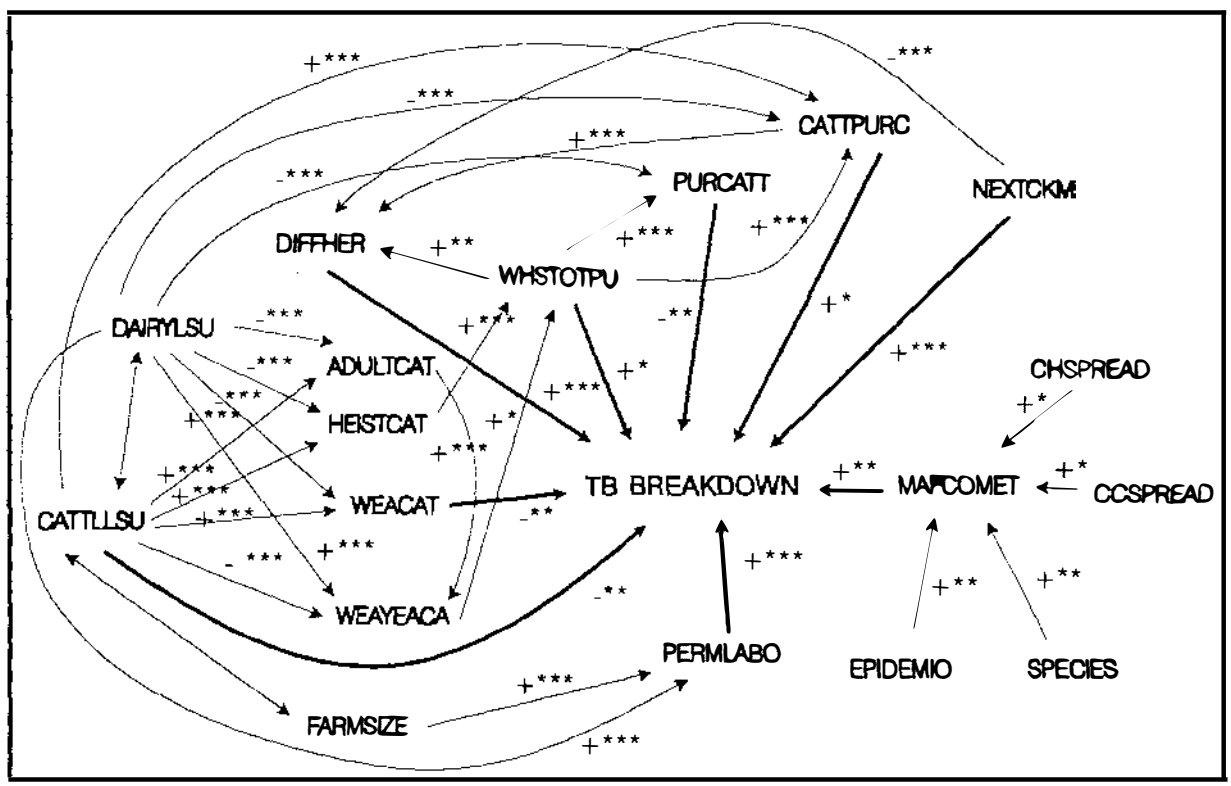


Figure 106b: Final path diagram for comparison of cases with matched controls



Path Analysis using LISREL

Separate path models were developed comparing cases with matched and with random controls. In total, 18 factors were included in the final model comparing cases with matched controls and 25 factors when comparing cases with random controls. The model for matched controls explained 47% of variation in the outcome variable case-control status, and the model for random controls explained 64%. The following table 55a summarises the results of the analysis. The χ^2-test of model fit suggests that both models would have to be rejected. Mulaik *et al* (1989) report that typically the χ^2 statistic implies for most researchers' models that they must reject it. But on inspection of the residuals these are small in an absolute sense, suggesting that the model is not as inadequate as the χ^2 statistic suggests. The adjusted goodness-of-fit index and the root mean square residual indicate a reasonable fit of the model to the sample correlation matrix. The multiple coefficient of determination shows that the structural equations in the model for comparison of cases with matched controls explain 68% of variation in the endogenous variables and for comparison of cases with random controls 92%. The path model for random controls explained 64% and the model for matched controls 47% of variation in the endogenous variable case-control status. Figure 107a displays the Q-plot of the normalized residuals for both models. The Q-plot for matched controls indicates that there are some large positive residuals for this model. The normalized residuals for the model comparing cases with random controls shows a few larger positive and negative residuals. Figures 107b and c show the diagrams of the final path models.

The final model for matched controls implies that if a case herd was in close proximity to another case farm the risk of breakdown was reduced. Purchase behaviour was represented by the number of different herds where cattle were bought from. If the enterprise was mainly a beef operation and if it was a large herd, farmers were more likely to source their animals from more than 3 different herds. An increasing proportion of weaners and yearlings in the herd was associated with a reduction in the risk of tuberculosis breakdown. Cattle operations with more employees were at increased risk of breakdown. Larger herd size had 2 indirect and one direct effect on TB infection status of a herd. Larger herds were more likely to introduce cattle from more than 3 herds and had more employees, both resulting in an increased risk of breakdown. The direct effect of herd size was a reduction in the risk of TB breakdown. Farmers who had TB reactors were better informed about the disease problem. In addition they tended to be more conservative.

The final path diagram for random controls emphasizes the importance of factors related to herd management and herd characteristics. Factors related to purchase behaviour appear to be only of minor importance and show no direct effect on TB breakdown status. Knowledge about the disease problem and factors related to the farmer's self concept are included in the model as direct effects on disease status. Herds in close proximity to areas declared endemic for tuberculosis were more likely to break down with infection. If farmers were grazing their stock mainly "on farm", they were less likely to have TB reactors. This factor represented

indirect effects from a number of other factors. Farmers who sourced cattle from more than 3 herds or who considered themselves as preferring hard work were more likely to graze their animals "off farm". If a herd was close to a case herd or if the proportion of weaners and yearlings was higher, farmers were more likely to graze mainly "on farm". The size of the beef herd had a number of indirect and a direct effect on TB breakdown status of the herd. Herds with a larger beef component were more likely to have TB reactors. Larger beef herds bought more cattle and from more different sources. They required less labour units, were more likely to have bush access and mostly grazed "on farm". Larger cattle herds bought more cattle livestock units, were less likely to have bush access, were managed by more employees and the manager was typically working on the farm full-time. Herds which had more employees were more likely to have tuberculin test reactor animals. Farmers with a TB problem in their cattle herd were better informed about the disease problem than farmers without TB reactors. Persons managing case herds were more conservative, less sociable, but more persevering.

Table 55b lists the magnitude of the total and direct effects on case-control status of variables in both path models. The model for random controls indicates that the variable NEXTCKM has the strongest total effect on case-control status. The variables DIFFHER, LIVEPREF and PERMLABO have total effects on case-control status which are moderate in magnitude. For random controls the most important factor is TOTALLSU followed by MAINGRAZ, PASTURE, PERMLABO and BEEFLSU in that order.

Table 55a: Goodness of fit of the final path models

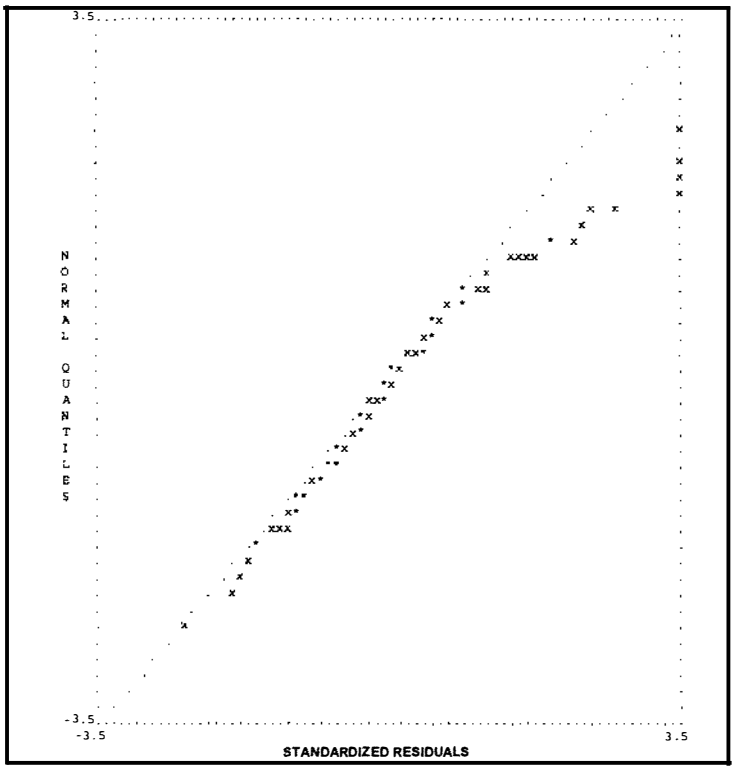
	Matched Controls	Random Controls
χ^2 test	246.65 (106 d.f.)	286.36 (180 d.f.)
Adjusted Goodness-of-Fit	0.801	0.809
Root Mean Square Residual	0.083	0.065
Multiple Coefficient of Determination	0.679	0.923
Squared Multiple Correlation for Case-Control status	0.466	0.642
No. of Factors	19	26
No. of Observations	185	182

Table 55b: Total and direct effects on case-control status in the final path models

	Matched Controls			Random Controls		
Variable	Total Effect	Direct Effect	T-value	Total Effect	Direct Effect	T-value
BEEFLSU				0.432	0.653	7.590
BUSHACCE				0.004	0.196	3.337
CATTLLSU	-0.047	-0.226	-3.554	0.356	0	
CATTPURC				0.078	0.078	
CHSPREAD	0.210	0.210	3.288	0.044	0	
DIFFHER	0.252	0.252	4.309	0.196	0	
EPIDEMIO	0.127	-0.101	-1.417			
FARMSIZE	-0.056	0		-0.278	0	
GIVINGUP	-0.099	-0.099	-1.739	-0.141	-0.141	-2.573
HARDWORK				0.058	-0.140	-2.344
INTRODUC				0.059	0	
LIVEPREF	0.251	0.251	4.395	0.296	0.245	4.601
MAFCOMET	0.194	0.194	3.039	0.167	0.208	4.023
MAINGRAZ	-0.013	0		0.560	0.560	10.070
MAINOP	0.177	0				
NEWIDEAS				-0.028	0	
NEXTCKM	0.427	0.427	7.328	-0.079	0	
NEXTEKM				-0.139	-0.139	-2.671
OTHEMPL				-0.160	0	
PASTURE				0.456	0	
PERMLABO	0.259	0.259	4.272	0.453	0.318	4.401
RECORDS				-0.024	0	
SOCIABLE				-0.222	-0.222	-4.128
SPECIES	0.057	0				
TOTALLSU				-0.614	-0.557	-6.461
TOTLABOU				0.217	0.217	3.012
WEAYEACA	-0.179	-0.179	-3.149	-0.143	0	

Figure 107a: Q-plots of normalized residuals for final path models

Cases - Matched Controls



Cases - Random Controls

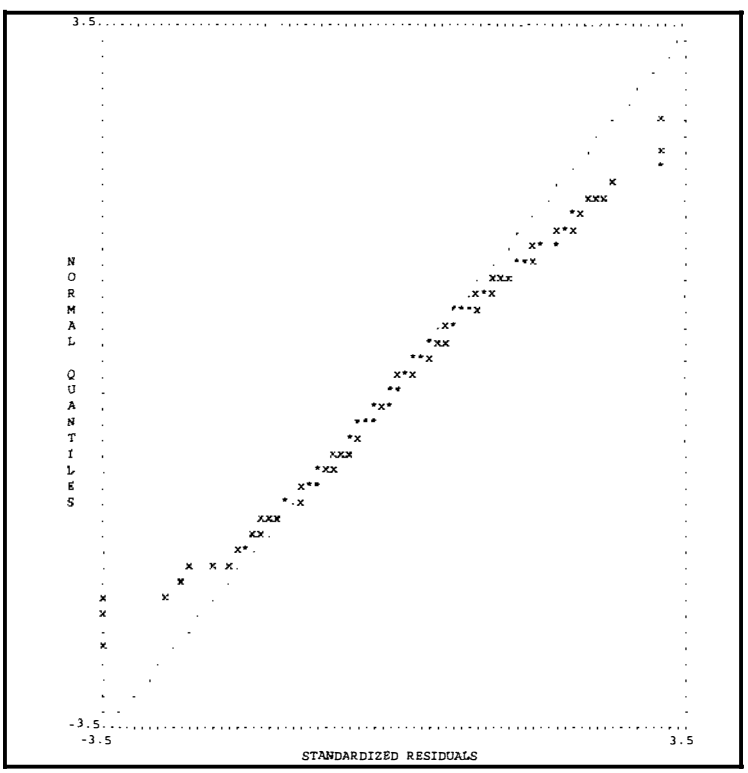


Figure 107b: Path diagram for final path model comparing cases and matched controls

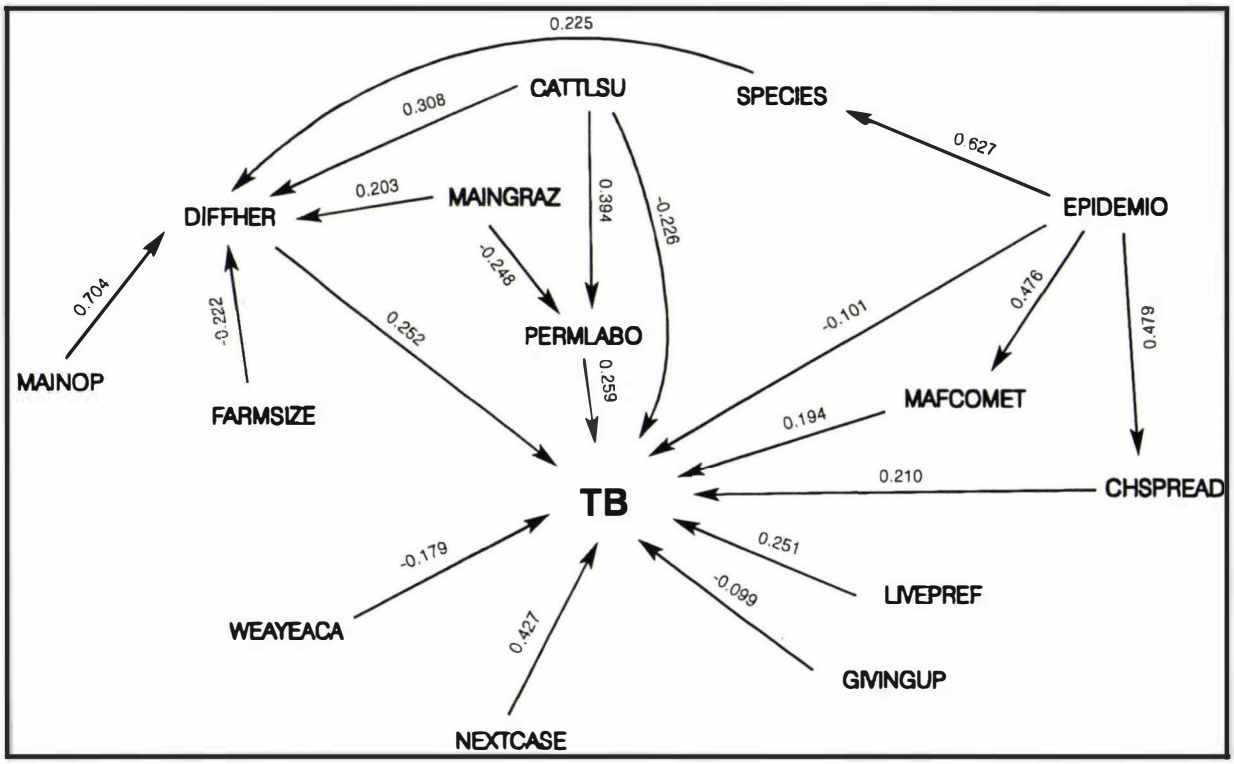
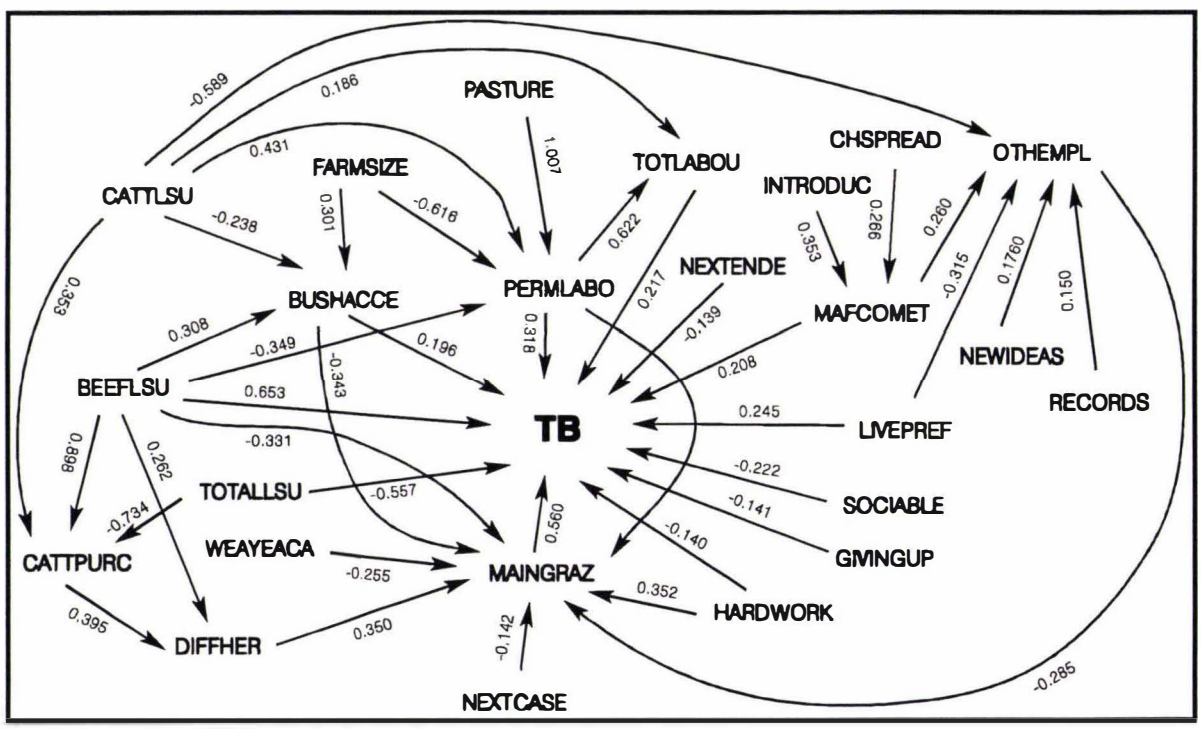


Figure 107c: Path diagram for final path model comparing cases and random controls



Classification Tree Analysis

Classification tree analysis was based on the variables which were statistically significant in the univariate analysis. Separate analyses were conducted for comparison of cases with matched and cases with random controls. A summary of information about the final trees is listed in table 56a. The classification tree for matched controls overall was able to classify herds as case herds with a probability of 0.66 and the tree for random controls with a probability of 0.47. The probability of correctly classifying a herd as a control was 0.69 for matched controls and 0.67 for random controls.

Table 56b ranks the variables included in the analysis according to their order of importance. The most important classifier for comparison of cases with matched controls was NEXTEKM with BEEFLSU, CATTLLSU and WEAYEACA above 75% importance. The most important classifier for random controls was PASTURE with CATTLLSU, WEAYEACA and FARMSIZE above 75% importance. The final classification trees are presented in figures 108a and b.

The classification tree diagram for comparison of cases with matched controls indicates that 68% of 99 herds at a distance of more than 1.7 km from the closest endemic area had TB reactor cattle. If the herds were closer or equal to 1.7 km from the endemic areas, 29% of 86 herds had broken down to TB infection. Of 28 herds within 1.7 km from the endemic areas who bought cattle from more than 3 different herds, 57% showed reactors during TB testing. These reactor herds were likely to have more than 13 livestock units of dairy cattle. If herds were located at a distance of more than 1.7km from the endemic areas, 85% of 33 farms with more than 0.25 labour units had TB reactor cattle. The figure of 1.7 km was chosen by the classification process as the distance giving the best split between cases and controls.

Comparison of cases with random controls showed that 76% of 42 herds with more than 865 livestock units of beef cattle were case herds. Of 30 farms with beef herds with less or equal than 865 LSU and where the manager was not satisfied with the current tuberculosis disease control program of the New Zealand Ministry of Agriculture and Fisheries, 70% had TB reactor cattle. Of 81 herds that had less than 865 LSU beef cattle, who were satisfied with the effectiveness of the TB disease control program and who had less than 1830 LSU dairy cattle, 27% had TB reactor cattle.

Table 56a: Summary of information about the final classification trees

	MATCHED CONTROLS	RANDOM CONTROLS
CROSS-VALIDATED RELATIVE COST	0.65 +/-0.069	0.87 +/-0.072
RESUBSTITUTION RELATIVE COST	0.29	0.42
COMPLEXITY	0.0108	0.0109
NO. TERMINAL NODES	9	10
SENSITIVITY (BASED ON CROSS-VALIDATION)	0.66	0.47
SPECIFICITY (BASED ON CROSS-VALIDATION)	0.69	0.67
SAMPLE SIZE	185	182

Table 56b: Variable rankings according to relative importance

VARIABLE	RELATIVE IMPORTANCE (%)	
	MATCHED CONTROLS	RANDOM CONTROLS
beeflsu	81	65
bushacce	3	10
buylives	38	25
buyrepla	2	6
cattdens	65	55
cattllsu	80	91
cattpurc	46	52
ccspread	45	1
chspread	30	18
conteffe	28	56
dairylsu	71	74
diffher	56	39
epidemio	62	4
farmsize	52	78
forest	42	41
givingin	41	32
givingup	42	28
hardwork	7	53
introduc	49	13
livepref	48	33
livesden	54	57
mafcomet	63	38
maingraz	11	3
mainop	18	23
modest	25	14
newideas	52	9
nextckm	63	25
nextekm	100	33
notalkat	17	32
othempl	12	2
othercat	10	31
pasture	69	100
permlabo	47	47
records	6	9
sociable	44	27
species	26	37
totallsu	57	69
totlabou	41	44
weayeaca	79	84

Figure 108a: Classification tree for comparison of cases and matched controls

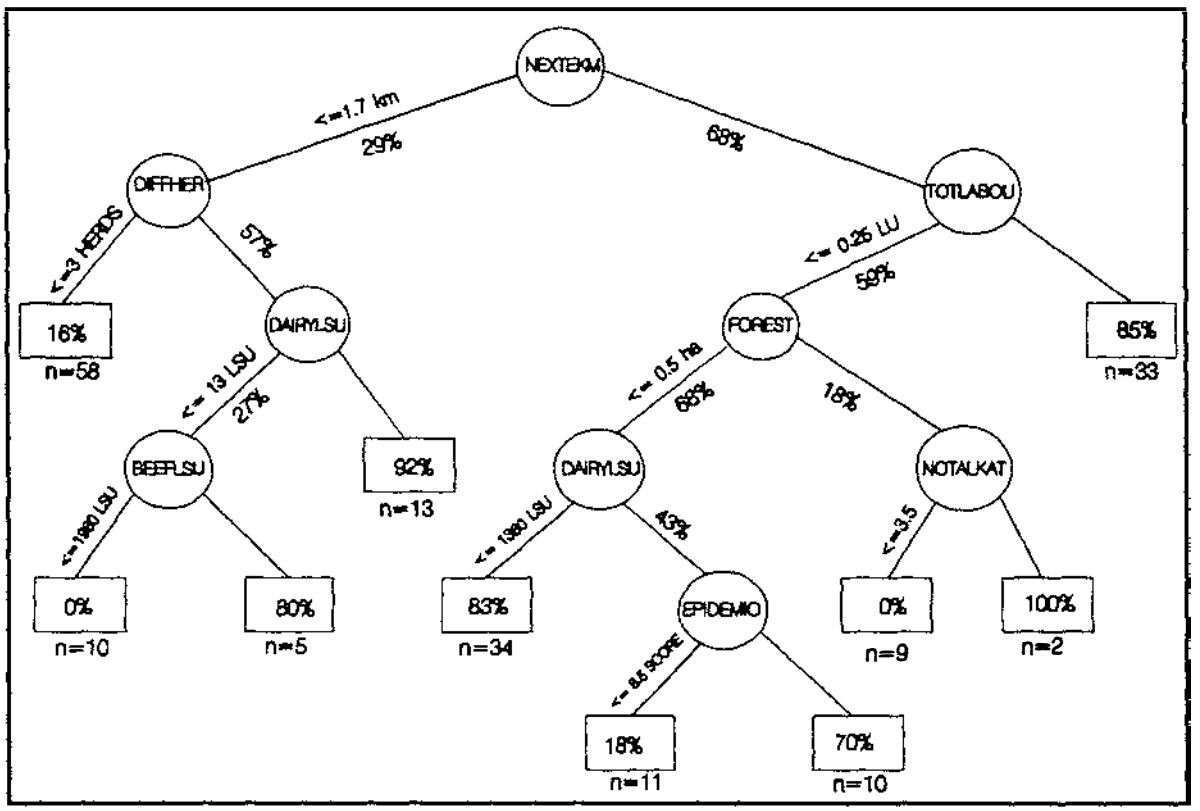
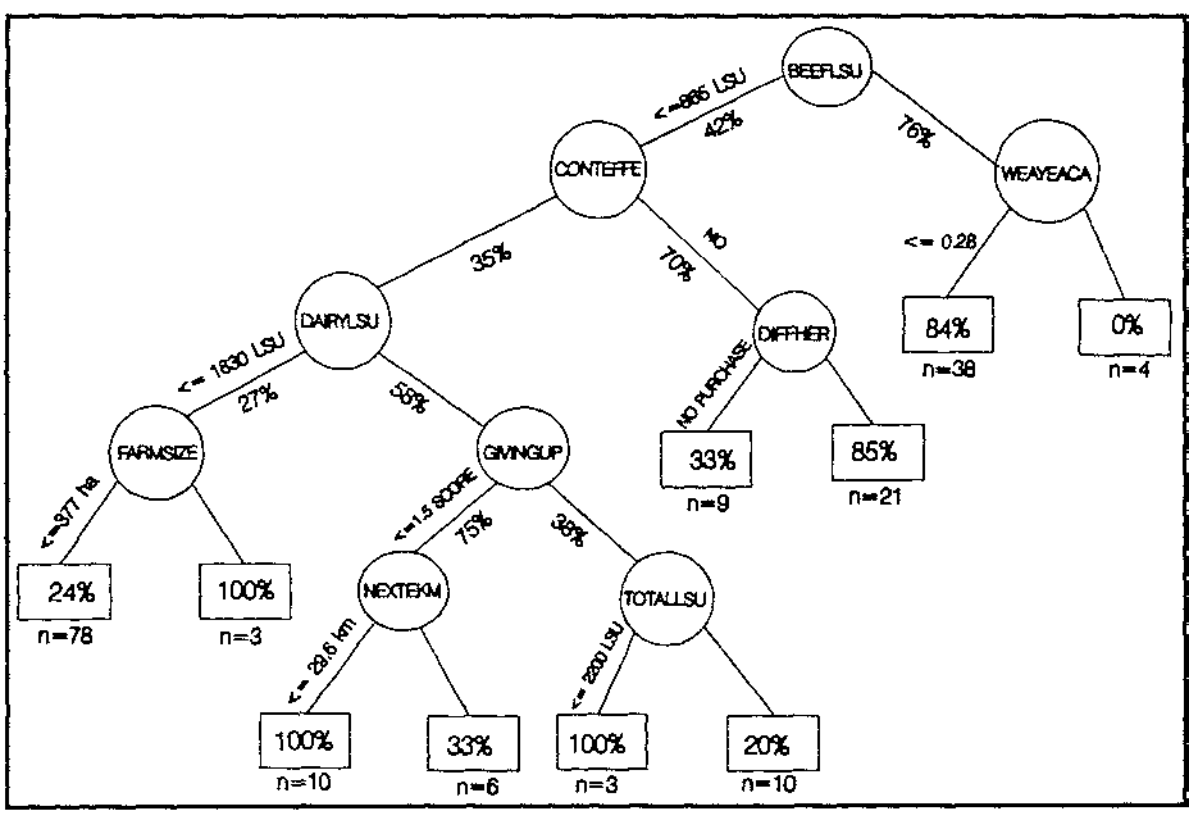


Figure 108b: Classification tree for comparison of cases and random controls



DISCUSSION

This study was intended to identify factors which were different between tuberculosis breakdown farms (cases) and two groups of control farms which did not break down with TB. These risk factors are either contributory causal factors or are in some way associated with unmeasured causal factors so that they represent them in the analyses. Just as important as finding factors which were linked to TB breakdown was to find factors which did not come out of the analysis as important, even though on first principles they may have been considered of significance.

In order to make the most valid comparisons, two different control groups were selected. The random control group was designed primarily to determine whether breakdown herds were atypical of the total population of farms in the study area - for example, whether the farms which owned beef cattle were more or less likely to break down than other herds. It was assumed that enterprise type could be an important confounding factor. Therefore it was decided to also select a sample of controls which were matched to individual cases based on type of cattle operation (dairy, beef). The matched control was designed to allow identification of factors which might explain why (say) a dairy farm in a particular locality broke down while a nearby dairy farm apparently exposed to the same local risks did not. Both comparisons have specific analytical advantages and particular dangers of misinterpretation which must be borne in mind.

The multivariate analyses were conducted using four different approaches. The LISREL approach to path analysis demonstrated an advantage as it allows to describe a complex system of relationships using one single model. It also estimates direct and indirect effects of factors included into the model. No latent variables were included into the LISREL model. An inclusion of such variables might improve the goodness-of-fit of the model, because for example the parameters describing self-concept and knowledge would be suitable estimators of latent variables. LISREL also has the advantage that its calculations are based on a correlation or covariance matrix, which in the presence of missing values allows to maximise the number of observations contributing to the analysis. The classification tree analysis provided an alternative view of the data. But it did not contribute significantly to an improved understanding of the biological system under study. It may have its uses as the basis for assigning risk scores to individual herds given certain characteristics. Goldman *et al* (1982) pointed out that classification tree analysis allows interactions to be described easily. A logistic regression model assigns the same weight for each case unless complex interaction terms are included. Breiman and Friedman (1988) emphasize that when decision boundaries in classification problems are highly nonlinear, recursive partitioning has the potential to achieve substantially higher accuracy than classical approaches. Gilpin *et al* (1983) compared the two approaches. They came to the conclusion that logistic regression was superior when used to construct predictive schemes which were then applied to new populations. Hadorn *et al* (1992) compared the performance of ordinary-least squares regression, logistic regression,

Cox regression and classification tree analysis for mortality prediction models. They found that the logistic regression model was marginally superior compared with the other approaches and that the regression tree showed the poorest performance. But they pointed out that recursive partitioning models had the advantage of being able to produce results which are similar to practical clinical guidelines. For both the LISREL and the classification tree analysis it is advised to use a larger sample size than was available in the present study.

The interpretation of the results from the present study was structured according to the following clusters of factors describing important aspects of the problem under study. They comprised parameters related to purchasing patterns, variables describing characteristics of the cattle herd, factors related to general farm characteristics, knowledge about both the disease control program and the epidemiology of tuberculosis infection. The project was originally designed to provide guidance on the relative importance of tuberculous possums and livestock movements as factors responsible for the continuing series of breakdowns in the area, the answer to this question being derived from which of these two explanations fitted better to the results of the analysis, rather than emerging directly from the analysis itself.

Likelihood of Involvement of Infection from Wildlife Reservoir Species

As the infection status of local possum populations was unknown, proximity to areas declared endemic with tuberculosis infection and geographical features (type of vegetation, grazing management, etc.) was used to estimate likelihood of contact with possums and TB infection in possums for a particular herd. At the time when the study was designed, no tuberculous possum had been found in the study area. The study arose from concerns about the high and unexplained rate of breakdowns in the area, given that intense efforts over a period to find tuberculous possums in the area to the north of the Rangitoto buffer had been uniformly unsuccessful. In the early stages of the study, infected possums began to be found in the study area and clearly had been there at low prevalence for some time before discovery.

The variables which did not appear to be of importance for discriminating cases from control herds included the following factors: grazing in bush/forest, type of vegetation and exposure to potential wildlife reservoir species (based on assessment by interviewee). These factors were considered to be surrogate indicators of the likelihood of contact with local possum population. Proximity to the closest endemic area had to be seen as an indicator both of the presence of TB infection in local possum populations and (to a lesser exent) an increased likelihood of introduction through purchase of infected cattle from the endemic area. It did not appear that herds which were located closer to the endemic areas and were more likely to have the opportunity of contact with possums were at an increased risk of infection. Therefore it seems unlikely that contact with possums alone was responsible for the breakdown pattern seen in the study area.

Farmer's Self Concept

The study also included an investigation of the view farmers had of themselves as farm managers, using a technique developed in the United Kingdom. It is widely acknowledged (though rarely documented scientifically) that farmers have disparate probabilities of having various animal diseases on their farms, according to the way in which they manage their farms, which in turn reflects in part their different personalities. Practising veterinarians take this into account all the time in the advice they give to farmers on disease control. Yet official disease control programs typically treat all farms as identical from a management point of view, and concentrate on the herd of animals rather than the herd and its manager. This study therefore endeavoured to determine whether the view which case farmers had of themselves (their self-concept) differed from the self-concept held by the two groups of control farmers. It is interesting that based on the univariate analysis the two control groups came out as virtually identical on this evaluation, but the case group had different scores on a number of the items assessed. Case farmers were more livestock-oriented than controls in this study, which may represent a greater tendency to trade in stock. They were also more towards the traditional end of the spectrum in attitudes to farming practices. They also saw themselves as less talkative than controls did. However differences between the groups were not large enough to be statistically significant in a direct comparison, given the subjective nature of the personal evaluation, the relatively small sample sizes which could be obtained, and the 5 point scaling system used. Studies of dairy farmers in the United Kingdom (Seabrook and Higgins 1988, Higgins and Seabrook 1986) found livestock orientation, efficiency and progressiveness differed among farmers and were strongly linked to acceptance of innovations in the dairy industry. In the present study multidimensional scaling was used to map the preference space describing the self-concept of farmers. It did not indicate any significant differences between case and control farmers. But the results of this analysis provided some interesting information about attitudes of farmers in general. The majority of farmers was clustered around the origin, which means that they did not identify themselves with any of the extremes in the preference space. They tended slightly towards seeing themselves as impatient. There was a group of individuals who thought they were conservative and less interested in social contact. A number of farmers perceived themselves as having a rather relaxed attitude in general. Only a few farmers identified themselves strongly with being innovative, progressive and efficient. An understanding of cattle farmers' self-concept may be important in a situation where cooperation on the part of the farmer is required to ensure success with a disease control program. It is easier to gain support from a person who is innovative, interested in taking on new ideas and efficient in management. On the other hand it would probably be difficult to convince someone to cooperate enthusiastically who thinks that he "knows it all" and prefers to keep "things as they are". Herdsmen who have a relaxed attitude may be less inclined to take the problem seriously. These results describe the personalities of cattle farmers in the study area. They suggest that it may be necessary to target information campaigns accurately to influence the attitudes of farmers, that herds which have no

experience of TB need a different approach from those which have dealt with the disease, and that incentives may be a useful way to gain their cooperation.

A small number of interviewees refused to fill in the section on self-concept in the questionnaire because they did not see its relevance to disease control and did not want to provide such information on themselves. To maximise the number of observations, variables describing self-concept were excluded from the analyses which required complete observations such as stepwise logistic regression and path analysis using standard regression techniques. In the final LISREL path model and the final classification tree, variables describing self-concept were included in the comparison with both matched and random controls. The result of the LISREL analysis suggests that case farmers were more likely to see themselves as preferring to work with livestock rather than machinery than were matched and random controls. This could be an indication that these persons are more interested in livestock trading which would increase the risk of introducing infected animals. They also thought that they were more persevering than both samples of controls. This characteristic could indicate that they are more likely to insist on "doing things their way". Therefore they may be less willing to change their cattle management practices if advised to do so in order to reduce the risk of TB infection. Case farmers considered themselves less sociable than the average random control. They were also less innovative (less inclined to take on new ideas) and less motivated by efficiency (less interested in maintaining a animal recording system) than the average cattle farmer. They were more likely to enjoy hard work than random controls. These four characteristics which distinguish farmers with cattle TB reactors from typical farmers in the area suggest that case farmers are personalities which may be less receptive to recommendations by MAF. They may be less likely to take action in order to prevent the disease problem or may not consider it to be their problem. The variable NOTALKAT was included in the classification tree discriminating cases and matched controls, but did not appear to have a strong discriminating power. Overall, it would appear that there is a cluster of characteristics which differs moderately between case and control farmers, and which would appear likely to represent one of the risk factors for herd breakdown. This group are probably not adequately influenced by MAF promotional activites for the TB control program, and efforts should be made to target efforts directly at this risk-prone group, using novel methods of extension. Gathering views from such people in an unstructured discussion may well help define how to work with other people like them.

Farmers' Views about Disease Control and Knowledge about Tuberculosis Infection

Information about factors reflecting on farmers' interest in the disease problem and epidemiological understanding was collected. Most farmers were able to identify themselves with the objective of disease eradication. But a third of all farmers in the sample did not have confidence in the current disease control program. Most farmers believed that they could make their own contribution to achieving control of the disease. The knowledge of farmers

about the epidemiology of the disease was limited, but most farmers were aware of the risk of infection represented by feral animal reservoirs. It appeared that they considered infected possums the single most important factor for introduction of tuberculosis into a cattle herd and did not give comparable weight to stock purchase policy.

Farmers were better informed about the disease and the control program if they had experienced a breakdown to tuberculosis infection in their cattle herd. This result points out that many farmers acquired knowledge about the disease only after they had a breakdown. The poorer knowledge of control farmers suggests that they did not seek to prevent a disease outbreak by taking specific action other than possibly possum control. It is likely that for reasons other than TB control they did not follow practices which were positive risk factors. Case farmers considered it more likely that their cattle had the possibility of contact with feral animals such as possums, deer or goats. This probably reflects the emphasis which MAF has publicly given to tuberculous feral animals and especially infected possums as major reservoirs of tuberculosis infection.

The multivariate analysis showed that factors describing farmer's understanding of the epidemiology of *Mycobacterium bovis* infection and their attitude towards the disease control program were highly collinear, suggesting that they were very closely related. Case farmers knew more about the disease control program than control farmers, which indirectly also represented better understanding about the epidemiology of the disease.

The relatively low level of understanding of TB and its control shown by control farmers, despite the efforts to inform the farming community on TB control, indicate that a more precisely targeted extension effort is required on the subject. Tuberculin testing in the absence of an adequately persuasive public information campaign clearly has major limitations, since high-risk farmers may continue to follow risky practices and put themselves in a danger of an outbreak.

General Management and Farm Characteristics

Most farms generated the main part of their income from cattle and the majority of those were involved in dairy production. Almost 50% of interviewees had a formal education below school certificate and only about a third had any farm-specific qualification. Most interviewees were the owners of the farm and herd.

In terms of area of pasture and total area, case farms were larger than both control groups. There was no difference with regard to total areas covered by gorse or bush/scrub, but case farms had a larger area of forest. They also had more permanent employees.

In the multivariate analyses the number of labour units consistently was an important risk factor across the control groups and different analytical approaches. Case farms had more employees than did the controls. Farm size and area of pasture usually had indirect effects through herd size or number of labour units. Area pasture was the overall most important variable in the classification tree analysis comparing case herds with random controls.

Patterns of Stock Purchase

A number of variables in the questionnaire described the methods and patterns which were used by farmers in relation to purchase of stock. These factors are likely to be influenced by the type of cattle operation and probably by distance from the areas which are the major sources for replacement and finishing cattle. For this part of the country, these source areas are mostly endemic with tuberculosis infection.

Most farmers stated that they had introduced cattle, which they mainly aquired from private sources. About two thirds of them were aware that this did expose their herd to the risk of introducing tuberculosis. But only 8.8% would consider checking the disease status of the source herd with MAF. In order to prevent disease introduction 10% did not purchase any stock. A third of farmers stated that they bought cattle mainly for trading purposes and 25% said that they mainly bought breeding bulls. Only 6% bought mainly replacements for their breeding stock. These results indicate that most farmers are involved in cattle trading while being aware of the risk of tuberculosis introduction. But they are unlikely to involve MAF to make sure that they are purchasing from low risk herds.

When comparing case farmers with controls, the results of the analysis suggest that case farmers were more likely than controls to buy stock from more than 3 different herds. There was no difference between farmers who did not buy at all and those who bought from a maximum of 3 different herds. On average case farmers had also bought more cattle. The importance of these factors was confirmed by the results of the multivariate analysis. In the comparisons between cases and random controls the single most important factor with regard to purchase patterns was the number of different herds farmers had bought their animals from. A comparison between cases and matched controls was likely to produce more reliable results, because purchase patterns are probably associated with type of operation. This comparison with matched controls using standard regression path analysis showed that case farmers bought more cattle and that their herds contained a higher proportion of beef cattle than did control farms. But case farmers replaced a smaller proportion of their herd in the year prior to interview than did the matched controls. The LISREL analysis confirmed the importance of the number of different herds farmers had purchased from as a risk factor, but did not include any of the factors related to purchase patterns. According to the classification tree for matched controls, herds within 1.7 km from an endemic area who purchase from a maximum of 3 different herds are at a low risk of TB breakdown.

These results indicate that purchase pattern was a major factor discriminating case farmers from both matched and random controls. It is likely that major sources for non-breeding cattle were adjacent TB endemic areas such as the King Country, thus exposing the herd to a substantial risk of infection. Hence, the total scale of purchases, the number of sources and probably the extent to which these animals are non-breeding stock (presumably including a large proportion of animals bought for fattening and then resold before long) are important factors associated with breakdown. Farmers may well not consider these animals as

representing a risk to their breeding herd because they are grazed for a relatively short time. These determinants are well-known risk factors in principle, but what is significant in this study is that they had greater explanatory value than possum factors, suggesting that an important issue in the spate of breakdowns in this area was failure to follow desirable purchase practices, more than exposure to tuberculous possums. This can be linked back to the relatively poor knowledge about TB spread found in those farmers who have not yet suffered a breakdown, and who hence may be at risk if they adopt these risky practices in the future, unaware of the risk attached to them.

Herd Characteristics

Many farms kept various combinations of animal species on their properties. 35% had only cattle. About 75% of herds had more than a thousand livestock units of cattle.

The univariate analysis showed that case farmers had larger herds and a larger proportion of total livestock units as beef cattle. Case herds also had a larger proportion of heifers and steers and a smaller proportion of weaners and yearlings in their herd than did both groups of controls.

As a result of the stepwise regression and path analyses it became apparent that case herds were more likely to have more beef cattle while at the same time keeping less weaners and yearlings. The importance of these variables becomes most evident when interpreting the results of the LISREL path analysis. Matching on type of cattle operation effectively resulted in controlling for differences in herd composition. But the proportion of weaners and yearlings as part of the total cattle herd remained an important risk factor. In the classification tree analysis the variables size of the cattle herd, size of the beef herd and the proportion of weaners and yearlings in the total cattle herd all had a relative importance above 60% for discrimination between cases and both groups of controls. The classification tree for random controls shows that 84% of 38 herds which had a large beef herd and a smaller proportion of weaners and yearlings in this herd had broken down to tuberculosis infection.

These results fit in with the conclusions based on interpretation of the data on purchase patterns. They show that case farmers had a larger beef herd than did random controls, and had a lower proportion of weaners and yearlings to total cattle owned than did either group of controls. This suggests that the farms most at risk of breakdown are those who for one reason or another rely on purchasing replacements and finishing stock to keep their stock numbers at the level they desire for farm operation.

Stock Management

Almost fifty percent of farmers grazed their herd on occasion at locations other than the main farm. A "run-off" was used for grazing by 45% of farmers. A third of interviewees stated that their herd had access to bush or forest, which was almost always on the main farm. 48% of farmers grazing cattle in bush or forest stated as their main reason that it was part of the same paddock needed for grazing.

During the univariate analysis none of the factors describing stock management patterns was statistically significantly associated with case-control status. In the multivariate analysis the LISREL path model for comparison of cases and random controls included location of main grazing area as one of the most important total effects. Case farmers were more likely to graze their cattle "off farm". Grazing cattle "off farm" absorbed the indirect effects exerted by 8 other risk factors.

It is notable that the use of a "run-off" did not come out of the analysis as a major risk factor for tuberculosis breakdown. The assumption had been made previously that the use of "run-offs" would increase the opportunity for contact with cattle from other herds. It was also thought that they were more likely to be located in less developed areas and therefore increase the likelihood of contact with feral animals. Surprisingly, the possibility of access to bush or forest also did not appear to be a factor associated with case-control status. Therefore, the results suggest that in most of the study area cattle having more extensive contact with feral possums were not more likely to get infected with *Mycobacterium bovis* from possums than matched and random controls. The evidence therefore favours the hypothesis that infection was being introduced into the area primarily through livestock purchases, and at the time infection in possums was still very limited in geographical distribution and in prevalence, so was not yet a significant influence on the occurrence of breakdowns. This answers the question for which the study was orignally designed.

Synthesis

The results of this exploratory case-control study suggest that tuberculous possums were not the dominant cause of breakdowns in these herds over the time period under consideration, and that the occurrence of such clusters of outbreaks does not by itself demonstrate that local possum populations are infected with *Mycobacterium bovis*. There are many reported examples of clusters occurring in both in infectious and non-infectious diseases, without a common source necessarily being responsible. In this case it would appear that farmers who had a breakdown with TB were more likely to be among those who followed purchase and herd management practices traditionally considered to put farms at risk of purchasing TB infection. Thus there are still plenty of farmers, even in high risk areas of the country such as the southern Waikato, who appear from the evidence in this study to follow practices that make TB breakdown likely. Moreover, at the time of interview control farmers were considerably less knowledgeable about TB than were case farmers who had been forced to deal with the issue, and there were clearly deficiencies in the level of understanding of TB and its spread in the TB-free members of the farming community, which suggests that this group remains at risk of introducing infection. Enhanced educational efforts and incentives aimed at encouraging modified purchase policies would therefore be a wise course to follow in reducing the probability of breakdowns in herds in this area. Bearing in mind also that introduction of infected stock to an area which has an infection-free possum population also

increases the danger of infection establishing in the possums from farm livestock, such educational efforts would seem to be an excellent investment.

The comparison of the four multivariate analysis techniques demonstrates that both path analysis techniques are suitable for describing the causal structure of a system. The approach to path analysis using standard multiple regression techniques is based on a much less complicated modelling procedure than structural equation modelling, but has the disadvantage that no overall model fit can be estimated, no latent variables can be used and a model has to be recursive. Successful use of each of the path analysis methods arises out of a combination of a thoughtful comprehension of the processes occurring in the biological system under study and effective use of the power of statistical analysis. Of the two, LISREL is generally considered the more powerful, but is more demanding both of the analyst and of the minimum size of usable sample. The other two methods, classification tree analysis and stepwise multiple regression, both rely solely on the statistical analysis. The researcher does not have to formulate a preliminary hypothetical causal model. The computer generates a causal model based on statistical algorithms. Classification tree analysis has the advantage that it summarizes the meaning of the data by producing a tree structure which it is easy to understand and which can be used directly for decision making. However conclusions derived from these latter techniques failed to produce results which were as plausible or practically useful as those arising from the path analysis approaches.

CHAPTER 8

GENERAL DISCUSSION - TOWARDS A STRATEGIC APPROACH TO WILDLIFE DISEASE CONTROL

INTRODUCTION

Individual parts of this overall study have been discussed at the end of each chapter, and this final chapter will deal primarily with putting the work in context in relation to epidemiological understanding of wildlife diseases, and on evaluating the effectiveness of the research strategy which has been pursued in order to understand tuberculosis in possums, and to identify improved control methods.

WILDLIFE RESERVOIRS OF DISEASE

The importance of wild animals as reservoirs for diseases in domestic livestock was first recognized in the latter half of the 19th century. This relationship was particularly evident in Africa where outbreaks of rinderpest, African swine fever and foot and mouth disease in the domestic animal population were blamed on transmission from wild animals (Fowler 1985). Wildlife reservoirs of disease can complicate the control of diseases in domestic animals to such an extent that it may seem impossible to achieve the goal of eradication or control of the disease. Methods which have proven their effectiveness in the control of diseases in domestic animals such as test-and-slaughter policies cannot be applied in most situations to wildlife populations. Effective control requires an understanding of the epidemiology of a disease, including its infection dynamics within domestic as well as wildlife populations. Studies of the epidemiology of diseases in wildlife species are quite challenging because individual animals are more difficult to follow up, wildlife populations are more subject to variation in environmental factors (vegetation, topography and climate) than domestic populations and most often the veterinary and ecological knowledge about the species involved is quite limited. Schnurrenberger *et al* (1987) stress that special thought must be given to the interrelationship of wild and domestic populations if they coexist in an area subjected to a disease control or eradication effort. One of the major questions which will have to be answered in such cases is whether or not any of the susceptible wildlife species is likely to be a maintenance or reservoir host of infection - in which case control efforts must take this fully into account- or whether the wildlife are merely spillover or dead-end hosts - in which case control efforts can focus almost entirely on the domestic animal population..

Schwabe *et al* (1977) define a reservoir host to be any species in which an infectious agent multiplies or develops and upon which it depends as a species for survival in nature. Other major veterinary epidemiology texts by Thrusfield (1986) and by Martin *et al* (1987) use similar definitions. Fowler (1985) lists a number of characteristics which are found in a reservoir host. First, the reservoir host must be able to maintain the disease in the absence of infection in other species. Second, the host must be able to shed the organism to allow infection of other species. Third, transfer of the organism from the wild host to a domestic animal must be demonstrated. This is usually quite difficult to demonstrate under field conditions. Fourth, reservoir hosts need not show overt signs of disease. Fifth, the reservoir host is usually not seriously affected by the organism. A reservoir host has to be distinguished from a spillover host (infected incidentally but infection is not self-sustaining in the species

without regular transfer from other species) and also from a dead-end host (infected incidentally, but cannot transfer infection to any other animals). If a species is a true spillover host for a particular organism, it would not be necessary to control the disease in the spillover host, because it would disappear or become epidemiologically insignificant once the disease has been controlled in the species which is a reservoir host. A spillover host provides an indicator for the presence of a particular disease in an area, but should not be the main target of a disease control effort.

Rinderpest, rabies and African swine fever are three animal diseases which represent a spectrum with regard to the degree of involvement of reservoirs of infection in wild animal species in producing disease in domestic animals. It was therefore considered appropriate to compare these three diseases with tuberculosis, because of the insights which this might offer.

Rinderpest

Rinderpest is an ancient plague which regularly devastated the cattle and buffalo populations of Asia and Europe and occasionally wrought havoc in North Africa. Currently the disease is endemic in most African countries north of the equator, in the Middle East, Pakistan and in India. It causes substantial losses in these countries unless vaccination is practised. Rinderpest is caused by a Morbillivirus and probably affects all cloven-hoofed animals (Scott 1990).

In Africa there has been a lot of discussion about the importance of wild animal reservoir species in the epidemiology of rinderpest. It was for example suggested that large concentrations of wild animals, as in the Serengeti region of East Africa, could act as "long-term reservoirs" of the rinderpest virus, in the absence of the disease among cattle. This was based on finding specific antibodies against the virus in these wild species. Clearly this cannot be considered sufficient evidence for a species to be considered a reservoir host (Pastoret *et al* 1988). As a consequence of the possibility that wild animals could be a reservoir for rinderpest virus some authorities of national parks and game reserves minimise or delay reports of mortality in rinderpest-susceptible species in order to avoid subsequent investigations (Plowright 1988). Plowright (1982) used the disappearance of rinderpest from the Serengeti region in Tanzania to suggest that game animals are not acting as reservoir hosts for the virus. He concluded that in the Serengeti region, at the periphery of which cattle-game contacts are frequent, a feed-back mechanism of cattle-to-game and game-to-cattle transmission had been necessary to maintain the disease continuously during the decades up to 1962-63, after which it disappeared. By that time vaccination campaigns had been under way in East Africa during the previous ten years. Plowright writes that it is likely that after 1963 local outbreaks have still occurred, but tended to die out rapidly in the presence of high densities of susceptible animals in relatively small areas. From the evidence in the literature it appears that in East Africa game animals are acting as spillover hosts for the rinderpest virus and the reservoir host is likely to be the domestic cattle population.

Rabies

Rabies is a viral infection of mammals which is transmitted in the saliva of rabid animals and usually manifested by a fatal encephalomyelitis. It is caused by a Lyssavirus and it occurs world-wide, except in Antarctica, Australasia and a number of islands and small countries. In addition to maintenance through dog infection (which was the primary problem but has been controlled in many countries by vaccination), rabies has been found to circulate in wild species - mainly in foxes, skunks, raccoons, mongooses and bats. Domestic animals as well as humans are at risk of contracting the disease dues to exposure to rabid wild animals.

In large parts of Asia, Africa and Latin America the rabies virus still circulates principally within the dog population - accounting for 95% or more of all diagnosed rabies cases. In contrast, in Western Europe and North America dog populations do no longer constitute a major reservoir of rabies infection. This has been achieved through stray dog control and widespread vaccination. Yet, the disease still has not been eradicated, due to the presence of smaller but nonetheless significant reservoirs of infection in wildlife species. It is widely accepted that notably foxes, mongooses, skunks and raccoons represent major maintenance hosts for the rabies virus. This problem has proven to be a much greater challenge than control of the disease in domestic animal populations. In the past, efforts have been made to control or eradicate rabies in wildlife reservoirs using techniques such as population reduction and more recently vaccination against infection with the rabies virus. It is now widely accepted that a thorough understanding of the epidemiology of rabies infection is required in order to make progress towards the ultimate goal of rabies eradication.

MacDonald (1980) describes the epidemiology of rabies in Europe as relatively simple because the fox is the single most important maintenance host in wildlife. The general view is that in Western Europe rabies can be eradicated, once the disease is controlled in foxes. Kaplan *et al* (1986) warn that this may not be the case as there are other biting animals such as the raccoon dog which could replace the fox as a maintenance host of infection.

Fox Rabies

The epidemiology of fox rabies has been extensively studied. It has been concluded that the social behaviour of foxes and the social structure of fox populations is of crucial importance for a bite-transmitted disease such as rabies. Social behaviour follows an annual cycle determined by reproduction and mortality. Adult foxes seasonally share a home range as male-female pairs with their young of the year. They occupy home ranges which in some areas are exclusive and in others overlap. Juvenile foxes may leave the family territory at the age of about six months and disperse over quite a large area. Young female foxes may stay in or near the home territory, whereas most males emigrate. Rabies incidence and spread in Europe reaches its minimum during May or June, immediately after the whelping season, when the population has reached its maximum. Then incidence increases steadily over the second half of the year until it reaches a maximum in February or March of the following year, and diminishes thereafter. Considering the relative brevity of the rabies incubation

period (around 1 month), it is relatively easy to identify social behaviour which may be associated with this seasonal incidence pattern. During the mating season wandering increases and contacts multiply, thus favouring transmission of the virus during fights among males and later, biting of females. The increase in incidence during the second half of the year is attributed to the dispersion of young foxes. During this time of the year the fox population is artificially elevated by newly independent young who are trying to find a territory. The majority of rabies cases during the second half of the year involves young male foxes which are more precocious and disperse over greater distances than young females (Toma and Andral 1977). During the clinical stages of the disease rabid foxes can show three different types of behaviour. If they develop the less common furious form (11% of artificially infected foxes in captivity), they wander around and attack any object encountered. They might show increasing paralysis and become very docile. MacDonald and Voigt (1985) concluded that given the available information it appears that rabid foxes in general behave like healthy ones (i.e. have the same movement patterns and frequency of social encounters) yet become more aggressive.

In Europe, cattle almost exclusively contract rabies from foxes. Cattle are exposed to infection between April and November when they are kept on pasture. The incidence of bovine rabies parallels the pattern of fox rabies incidence with a lag of 2 to 3 months. Given that they are only exposed until November, incidence diminishes as expected after December (Toma and Andral 1977).

Fox rabies in Europe shows a cyclic temporal pattern. During an epidemic, rabies reduces the fox population by about 50%, with an additional proportion destroyed by population control conducted by humans in response to the increased risk of human exposure. In the year following the epidemic, rabies incidence is very low. But the sharp reduction in fox population density improves survival and increases reproduction. In the absence of any population control over the next 2 to 3 years the population can increase to the levels it had prior to the occurrence of the epidemic and the conditions are favourable for an increase in rabies incidence (Toma and Andral 1977).

In Europe the fox rabies front advanced at the relatively slow rate of about 30 to 60km/year suggesting that spread of infection is generally of nearest neighbour type (Ball 1985). Occasionally the rabies front appears to "jump" ahead due to infected juvenile animals which dispersed in autumn into the rabies-free area and set up a local epidemic. The shape of the front is influenced by geographical obstacles such as mountain ranges and streams. Rivers running parallel to the rabies front can represent temporary barrier, but can in fact accelerate spread when running parallel to the general axis of the progression (Toma and Andral 1977).

Field experience and simulation modelling exercises suggest, that if in Western Europe a fox population has been reduced below a certain level (by rabies itself and/or fox control), rabies disappears in foxes and in all other terrestrial mammal species (except in bats;

Wandeler *et al* 1993). Despite this situation only few fox population control campaigns have been successful. This can be partly explained by the fox's resilience to persecution and its high reproductive potential in connection with high carrying capacities of rural and suburban habitats (Wandeler 1988). Denmark for example succeeded in creating an artificial barrier of low fox density in South Jutland, which protected the rest of the peninsula. But over the years the country has experienced a number of invasions by rabid foxes which were very expensive and difficult to control. MacDonald (1980) questions the successfulness of the Danish rabies control programme because at the same time the disease also disappeared in the German state of Schleswig-Holstein bordering on Denmark where no control had been conducted. Macdonald and Voigt (1985) conclude that in Europe culling of foxes had little effect on the speed at which the rabies epidemic advanced. To prove the success of a control campaign such as that for fox rabies is complicated by the cyclical occurrence of rabies. The disease can be completely absent for periods of up to 5 years. The use of vaccination shows signs of producing more promising results than population reduction. Belgium has for example reported that since a large-scale vaccination campaign was conducted in a 2200km^2 region in 1989-1990 no livestock rabies has been reported. MacDonald (1980) writes that there are only two outbreaks of rabies in Europe which can claim to have been completely eradicated by fox control, one in Dijon in 1923 and the other in Corsica in 1943.

Rabies in Other Species

In North America there are four wildlife reservoir species for rabies - namely foxes, raccoons, skunks and bats. In contrast to Western Europe population reduction has not been encouraging at all as a method for controlling rabies in wild animals (MacInnes 1988). Smith and Baer (1988) report that in most geographical areas of Canada and the U.S.A. a number of different wildlife species may be involved in the epidemiology of rabies, but most often there is a predominance of cases in a single host species. For example in 1986 75% of 3565 skunk rabies cases occurred in a large area extending from southern Alberta, Saskatchewan and Manitoba in Canada across to the central United States to the Rio Grande River. Ninety-nine percent of 1609 rabid raccoons reported occurred in the southeastern and mid-Atlantic United States. Of 1915 fox rabies cases reported in 1986, 90% occurred in southern Ontario, Quebec and northern New York. Within any of these areas the cases which occur in other animals are regarded as "spill-over" infections from the major reservoir animal(s). Monoclonal antibodies have been used to identify antigenically different variants of rabies virus. This information was then utilized to analyze the geographical occurrence of rabies in different animal species. In Texas where skunk rabies predominates a cluster of rabies in gray foxes is observed every year. Virus isolates from these foxes were easily distinguishable from the skunk isolates. It was concluded that there may be an independent cycle of infection within the fox population. Under such circumstances it would not be possible to eradicate rabies by eliminating skunk rabies. Monoclonal antibody analysis of isolates was also used to investigate the role of bats in the epidemiology of rabies in North America. It was found that none of the reaction

patterns that characterize bat rabies was represented in the patterns of viruses isolated from the major terrestrial rabies enzootics. Parker (1975) conducted a detailed study of rabies in skunks. He identified some aspects of skunk behaviour which contributed significantly to the spread of rabies virus infection within a skunk population. One factor is that skunks are using communal dens with a single dominant male in the den and the other is that young unattached males tend to wander around during the late winter months. Parker suggests that this contributes to the peak of cases in the second quarter of the year, since the breeding season is late February and March, and a subsequent incubation period of 30-60 days would coincide with the peaks observed. He also mentioned the possibility of perinatal transmission.

Carey (1985) discusses multispecies rabies in the eastern United States. He concludes that there are three manifestations of multispecies rabies in this region. First, rabies virus is maintained concurrently, but independently, in bats and in one common terrestrial carnivore such as the striped skunk, gray fox, red fox or raccoon. Second, during epidemics in terrestrial carnivores, up to 20 other species may be infected which can be considered "spill-over" hosts. Third, the major terrestrial carnivore species involved may change over time. Rabies is known to occur in epidemic cycles. It is not understood whether it reaches an endemic state at low prevalence between epidemics or if it disappears from the maintenance host population and after a period of time is reintroduced from an inapparent reservoir such as mustelids, bats or felids. Carey also suggested that fox rabies persists in areas of rugged topography with a mixture of farms and forest. Such an environment results in patchiness of fox population density and may reduce depletion of the number of susceptible foxes by producing more rapid spread of the epidemic, leaving islands of unaffected foxes.

Voigt and Earle (1983) conducted a study on the interaction between coyotes and red foxes in the Canadian province of Ontario. They found that foxes avoid raising pups in areas where coyotes traditionally travel and raise pups. Voigt and Earle suggest that foxes may have vacated certain areas due to the presence of coyotes. And this may explain why in some areas of Ontario no rabies has been reported during a 10-20 year period, whereas southern Ontario has the highest incidence of wildlife rabies in North America.

Bat rabies occurs in a number of countries of the world. Bats live at extremely high densities which provide favourable conditions for transmission through aerosol or biting. Epidemiologists have concluded that rabies in insectivorous and fructivorous bats exists as an endemic largely independent from the cycle in terrestrial animals, and that rabies in bats cannot be considered an important reservoir for infection of terrestrial wildlife populations (Smith and Baer 1988). Brass (1993) quotes a number of studies in support of this theory. In most of these studies it was found that in regions where rabies was endemic in local bat populations, no rabies infection had been detected in terrestrial species. In contrast Tinline (1988) writes that it may well be possible that bats may cause epidemics in other species. They migrate over long distances and could set up new reservoirs of infection or introduce new strains into an area. These views may not be totally inconsistent, since bat rabies may be

a separate cycle under normal circumstances, with spillover on rare occasions - which are of note when they precipitate an epidemic in a particular region.

In Latin America the epidemiological situation is different again, and dog rabies still accounts for over 50% of reported animal rabies. Vampire bats are believed to be a significant reservoir for rabies infection in some of these countries, with certain areas being particularly at risk. The importance of this source can only be indirectly estimated through the considerable incidence of bovine rabies, which is considered to be mainly caused by transmission from vampire bats.

Mongooses are a principal maintenance host of rabies infection in the Caribbean. On the island of Grenada, rabies has been continuously monitored since the late sixties. During this time a number of control campaigns of the mongoose population have been conducted, which led to temporary reductions in mongoose-transmitted rabies. A poisoning campaign in 1973 destroyed about one to two thirds of the mongoose population. Unfortunately in most areas the population recovered within 6 to 9 months. Everard and Everard (1985) conducted surveys for the presence of serum-neutralizing antibodies in the mongoose population of Grenada which led them to the conclusion that these animals actually developed immunity to rabies which in turn caused the temporary localized disappearance of rabies. They describe the occurrence of a cycle of high antibody/ low rabies and low antibody / high rabies cycle, because after the dispersal or death of the immune individuals in the population, more susceptibles are available to promote resurgence of the disease. As the immune population builds up, the cycle is repeated. At the end of a cycle up to 60% of individuals may show antibodies. Everard and Everard conclude that poisoning campaigns for the control of mongoose populations may defeat the purpose as it also removes immune animals. They recommend the use of vaccination soon after the peak of an epidemic has been reached, when the proportion of immune mongooses is at its maximum and it should be possible to eradicate rabies completely.

In Africa knowledge about the involvement of wildlife species in the epidemiology of rabies infection is limited, with the exception of South Africa. Blancou (1988) suggests that the dog may be the major reservoir of rabies infection in most of Africa as the disease disappears in countries where canine rabies has been controlled. Given the experience from other continents Blancou suggests that this may be a result of inadequate reporting in some of the African countries. The importance of the interaction between a particular type of habitat and a wild animal species can be illustrated using the example of the yellow mongoose. In the grassveld, an area from 1200 to about 1800 above sea level, rabies is considered to be endemic. In this region the yellow mongoose is the most important host of rabies and spread of infection to any other species is not common. This area is well defined in terms of vegetation and altitude. Further north, in the sub-tropical and tropical climate zones, the vegetation changes to predominantly thorn bushes and trees, the bushveld. In this region rabies occurs typically in epidemics, and the species involved are mainly feral dogs and black-

backed jackals. The limits of the area where rabies epidemics occur is less clearly defined than the grassveld where endemic disease is found. There is also a larger number of vector species involved and spread from canid hosts to for example farm animals is much more common in the bushveld than in the grassveld (Kaplan *et al* 1986). Blancou (1988) writes that between 1977 and 1988 an independent cycle of rabies had established in the kudu antelope in Namibia.

The above examples show that there are major differences in the epidemiology of rabies between geographical regions. It is interesting to note that in some areas of the U.S.A. it appears that foxes are "spill-over" hosts of infection rather than maintenance hosts, which they generally are in regions where rabies is present. It is likely that fox densities are too low in order to maintain endemic disease levels. The possibility of having several independent cycles of rabies infection in different wildlife in a single area complicates any control attempts to a great extent.

African Swine Fever

African swine fever provides one of the classic examples of a disease where wildlife was the primary reservoir but initial spillover of infection into domestic animals later produced a new epidemiological situation with different maintenance hosts. This acute contagious viral disease emerged as a disease of domestic pigs in East Africa at the beginning of this century, where it probably had previously been endemic only in wild species of the Suidae family. In 1957 the disease occurred in Portugal through importation of infected pork and it spread despite control efforts into Spain, Italy and France (Losos 1985). It is now considered to be endemic in Sardinia.

African swine fever occurs in many countries of the African continent where warthogs, bushpigs and forest hogs act as reservoir hosts of the virus. Typically the disease is only locally endemic in the wild pig population. Wild pigs do not develop clinical disease, but occasionally are the source of infection with virulent strains for domestic pigs which suffer high morbidity and mortality. The disease is transmitted between warthogs primarily by a argasid tick which live in the same burrows as the pigs. The virus can be maintained in the argasid tick for long periods in the absence of fresh infection from pigs, so that they can act as a temporary reservoir. Young warthogs, which have virus circulating in the blood, are the main source of infection for ticks. In older pigs the virus is mainly found in lymphatic tissue. In Africa the endemic infection is maintained through infection cycling between ticks and the warthog population.

Transmission from warthogs to domestic pigs was a relatively rare event, and was thought to result mainly from transmission through the tick vector. Double fencing to exclude wild Suidae and their ticks from access to domestic pigs was a very successful control method, and the disease did not occur commonly in commercial piggeries. Chronic cases of the disease and asymptomatic carriers of infection were unusual in domestic pigs.

Until 1957 it was thought that African swine fever was one of the specifically African diseases. In that year it appeared in Portugal and in 1960 in Spain where it caused the death of thousands of pigs. In both situations contaminated pork was found to be responsible for the initial outbreaks. Local argasid tick species became involved in the cycle (Liess 1988; Blood and Radostits 1989; Sanchez-Vizcaino 1992). Later it spread into Italy.

Over the next few years a new situation developed in Africa as well as in affected European countries with regard to the pathology of African swine fever. A less acute form of the disease emerged, with more frequent chronic cases and asymptomatic carriers. Within an infected herd, virus spreads rapidly via direct and indirect transmission. Asymptomatic carrier pigs and contaminated meat both became important sources of infection, and the ticks were no longer essential for maintenance of the disease. Outbreaks have occurred in a number of countries, notably in the Americas, due to consumption by pigs of pig meat scraps in garbage, mainly from airline food. Wild European pigs are susceptible to infection and are the maintenance host for African swine fever on Sardinia. In contrast, wild pigs became infected in some other European countries, but infection either disappeared spontaneously or it proved possible to control it.

BOVINE TUBERCULOSIS

Mycobacterium bovis has one of the broadest host ranges of all known pathogens (Grange and Collins 1987), with a complex epidemiological pattern which involves interaction of infection among human beings, domestic animals and wild animals. However the epidemiological patterns and the importance of various species in transmission of the disease appears to have varied over time, and among different countries. For example, bovine tuberculosis in man has declined from an important syndrome to unimportance over the last century and does not appear to have had a resurgence in parallel to that of *Mycobacterium tuberculosis* in man, bovine tuberculosis is important in badgers in the United Kingdom and the Republic of Ireland but not in other parts of Europe, and the disease is widespread in the possum in New Zealand but not in its native Australia. While the explanation for these recorded differences is in some cases well-known but in others is unclear, the differences result broadly from issues of host susceptibility, from the existence and scale of different transmission pathways, and from behavioural factors in various species which determine whether a specific potential transmission pathway can be expressed.

The susceptibility of a particular species to infection with *Mycobacterium bovis* is a major factor determining its importance in the epidemiology of bovine tuberculosis. Schliesser (1985) produced a susceptibility ranking for a number of domestic and laboratory animals. He suggests that chimpanzees, rhesus monkeys, rabbits and guinea pigs are highly susceptible to infection with *Mycobacterium bovis*. Cattle, sheep, goats, pigs, cats are susceptible, horses, dogs and mice are not very susceptible and rats and poultry are not susceptible to infection. Schliesser based this information on data from the literature as well

as his own experience. He mentions that a comparison of susceptibility between species is quite difficult as it depends on the doses which were used in the individual experiments. In terms of a genetic influence on susceptibility of a particular species, Schliesser came to the conclusion that this may have an effect on the pathogenesis of tuberculosis in the species, but is unlikely to influence initial susceptibility to infection.

There is only limited information available regarding the susceptibility of wild animals to infection with *Mycobacterium bovis*. This is further complicated by the fact that many wild animal species are likely to show a different immune response in captivity compared with the response they would show in the wild. This is particularly evident in possums where Corner and Presidente (1980, 1981) found that these animals in captivity succumbed very quickly to experimental infection with *Mycobacterium bovis*. Buddle *et al* (1992) used lymphocyte stimulation using T-cell mitogen as an indicator of the effect of stress on cellular immunity and estimated that a minimum of 4 weeks of cage adaptation after capture was required before possums could be used for experimental studies, to avoid producing atypical fulminating disease in stressed possums.

Reliable estimates on the prevalence of bovine tuberculosis in wild animal species is scarce due to the fact that these species are not normally included in national bovine tuberculosis surveillance programs. In general it has been considered that the occurrence of *Mycobacterium bovis* in wild animals was dependent on the presence of infection in domestic animal species. Schliesser (1985) considered that in Europe as long as the disease remained in domestic animals, sporadic cases were reported in wild animal species such as deer, elk, chamois, pigs, foxes, hares, beaver and hedgehogs. He mentions that in zoo animals bovine tuberculosis occurs to a significant extent even in countries where the disease has been eradicated from domestic animals. He adds that the available data on infection in zoo animals is likely to be an underestimate of the true bovine tuberculosis prevalence as the majority of communal and private zoos, wildlife parks and pet shops would not report occurrences of the disease.

It is of note that the susceptibility of the three major wildlife reservoirs which have been of concern in tuberculosis eradication programs in recent years - possums, badgers and deer - was established, as for numerous other species, some years before evidence began to accumulate that they could act as maintenance hosts. It is uncertain for badgers and deer whether the recognition of an emerging problem signified increased occurrence or improved detection, but in the case of the possum the evidence seems unequivocal that a new reservoir host had been established.

The second major factor influencing whether a wildlife host is of practical importance in tuberculosis control is the effectiveness of transmission pathways within the species, and between the potential maintenance host and domestic stock. In this regard, there are a number of similarities between badgers and possums, which are the two species studied in most detail.

Both species can develop lymph node abscesses which discharge through the skin and hence provide a mechanism of transmission between animals of the same species, and a source for contamination of the environment. They both develop tuberculous lesions predominantly in the lung which is likely to result in excretion of bacteria via the respiratory route. There is evidence in the case of both the badger and the possum that 'pseudo-vertical' transmission from an infected mother to her young may be of considerable importance for maintenance of disease within an area, as well as for spread of infection between populations. After young animals become independent of their mothers some of them will disperse in order to establish a new home range. A proportion of these animals may be infected with *Mycobacterium bovis* and would therefore be able to set up new disease foci.

The issue of how possums and badgers transmit infection to domestic stock is still currently being resolved, since it has so far proved impossible to answer this question directly, and indirect evidence has been used to infer how transmission takes place. Early evidence on badgers gave emphasis to aerosol transmission, but later reports have emphasized the fact that excretion in urine is common, and hence have suggested pasture contamination as the main route. In possums the reverse trend has occurred, with early papers focussing on transmission through contaminated pasture, and later work favouring aerosol transmission as the central method. With regard to badgers, the published evidence shows that urinary excretion occurs commonly, but it does not show unequivocally that this is how transmission commonly occurs, rather than by aerosol. It may be that in both species aerosol transmission is the pathway of greatest practical importance (Morris *et al* 1994).

Therefore the transmission mechanisms exist in possums and badgers for them to maintain infection in an endemic state within the species, and to transmit infection to the important domestic animal host species.

There is also now good evidence (Paterson 1993) that the third component of a successful maintenance host occurs in the possum, in that behaviour of both possums and domestic stock facilitate transmission of infection. Terminally tuberculous possums show changed behaviour in that they wander around during the day, stay closer to feeding areas such as pasture and do not avoid cattle as actively as healthy animals. They are likely to attract the attention of and be investigated by domestic animals, which will provide excellent opportunities for transmission of infection. There is circumstantial evidence that a comparable behavioural pattern facilitating exposure may occur in the badger as well as in the possum, although this has not been demonstrated under controlled conditions.

The aspect in which there is an important difference between badgers and possums is related to the social behaviour patterns of the two species, and why they can both act as reservoir hosts when they have such different social structures. Badgers are social animals and live in social groups which occupy exclusive territories. Each territory contains a number of underground burrows termed setts, the main one in a territory being very extensive. Within a

social group they will share setts, and sleep in close contact with each other. A badger sett provides good conditions both for survival of organisms and for aerosol transmission, which would greatly facilitate dissemination of tuberculosis within a social group.

In contrast possums are not considered to be true social animals, nor do they occupy exclusive territories. Close contact sufficient for direct animal-to-animal transmission would be likely to occur under much more limited circumstances - mainly between mother and young during the rearing period, between adult males and females during courting and mating, between adults (males in particular) during competitive and agonistic behaviour, and during simultaneous den sharing by two or more animals (excluding mother-joey sharing, which is stated above).

However possums occur at much higher population density per square kilometer than badgers, and it would seem reasonable that at possum densities transmission mechanisms which have a relatively low probability of success per encounter are effective because of the high number of other animals to which each possum is potentially exposed, whereas in badgers the number of contacts is far smaller but because of the degree of social interaction transmission is as successful as in the possum.

Thus it would appear in retrospect that the necessary factors were present to allow both possums and badgers to act as successful wildlife reservoirs for tuberculosis, and might perhaps on some future occasion allow other species to convert from occasional hosts to reservoir hosts if circumstances are right. In this connection it would be very informative to understand exactly how the possum became endemically infected with tuberculosis in New Zealand but did not in Australia. At this stage it is possible to put forward reasoned conjectures, but there is inefficient solid evidence to formulate a firm hypothesis.

EPIDEMIOLOGICAL COMPARISONS OF TUBERCULOSIS AND OTHER WILDLIFE DISEASES

The three diseases chosen for comparison with tuberculosis were deliberately selected to cover a spectrum of the epidemiological patterns and trends found in diseases which involve wildlife, not because they are all epidemiologically similar to tuberculosis. All three are viral rather than bacterial diseases and the disease process is generally more acute than for tuberculosis, but nevertheless useful comparisons can be made. Since some essential pieces of epidemiological information required for the design of tuberculosis control programs which would be effective in the presence of a wildlife reservoir are not yet available, there is merit in looking for parallels with other diseases which might offer clues to the best chances of making progress.

Of the three diseases, the one which shows closest parallels to tuberculosis is rabies, because despite the fundamental differences in the nature of the diseases there are ecological factors which produce intriguing similarities in the epidemiology of the two diseases. Firstly, in the form of interest here, tuberculosis is maintained principally in a single reservoir species

(possum or badger), with infection in the domestic host species being largely of the spillover type. There tend also to be low levels of what appears to be spillover of infection into predator and scavenger species in particular, though producing sometimes higher prevalence in these species than in the reservoir species, due to the concentration effects of such animals consuming large numbers of the reservoir species. Rabies shows the interesting feature of typically having one primary maintenance host in most areas, but with lower incidence in one or more other hosts - which might become important as a residual source of infection if a control program is limited to the principal maintenance host. In some cases there is a second apparently independent cycle of infection being maintained in another species. The issue of whether any wildlife hosts other than the possum can genuinely act as maintenance hosts either in small foci or nationally remains controversial but a very open question. It is unlikely to be decided for some years, but requires continuing evaluation. Some new evidence is expected to arise from later stages of the longitudinal study described in this thesis. To date the only wildlife species in New Zealand that has been shown to be a true maintenance host is the possum, and the same would appear to be true with regard to the badger in the United Kingdom and Ireland. In New Zealand feral deer may also qualify as maintenance hosts in that they appear to maintain infection within the species without the need for cross-infection from other species, and may perhaps infect possums in previously clear areas on occasions. Although arguments have been presented that other species are also acting as maintenance hosts, there is little firm evidence that this is true, and it seems likely that if other species do fill this role it is at most in isolated areas with special epidemiological features. So as for rabies, in the case of bovine tuberculosis in the United Kingdom, Ireland and New Zealand there is typically only a single important maintenance host, although many more species can be shown to be infected.

Temporal trends also show intriguing similarities between tuberculosis and fox rabies. In both cases the annual maximum falls in spring, but there is no apparent common ecological reason for this and it is probably a chance similarity. Both badger tuberculosis and rabies show multi-year cycles in incidence, which probably does result from common factors related to the generation length of the host species and the number of susceptibles in the population at particular times. It is not yet clear whether there is any long-term cyclicity in possum tuberculosis, but the short generation length of possums makes major multi-year cyclic patterns less likely to be important. There has been no published evidence of specific weather influences on rabies of the kind provisionally identified in the longitudinal study for possum tuberculosis. This suggests that rabies transmission and pathogenesis are little influenced by short term weather patterns because there is not a stress component to the determinants of the rate of pathogenesis, whereas in possum tuberculosis the evidence from the longitudinal study suggests that pathogenesis is variable in length and shortened by weather or nutritional stress.

Fox rabies and tuberculosis in badgers and possums show spatial similarities with regard to the spread of infection to new populations, which in all three species is a result of

dispersal of juveniles approaching maturity. In all three cases this is critical to control success, and is a major factor inhibiting development of improved control policies for tuberculosis, whereas the use of oral vaccination of fox populations has made a major difference to the success of control by substantially reducing outward spread of infection through dispersers. The argument of Carey (1985) that rabies in the eastern US persisted best in rugged terrain with a mixture of farms and forest has its parallel in possum tuberculosis with the bush-pasture margin as the area of prime importance for maintenance and transmission of tuberculosis. Spatial patchiness is a notable feature of both diseases, although because of the differences between the diseases in the lengths of both incubation period and clinical period, plus the different susceptibility to environmental influences, patchiness in tuberculosis is a much more pronounced and locally persistent feature of the disease.

There are few "models" which can be used as a guide in thinking about the likely effectiveness of control policies for possum tuberculosis, and it would appear that fox rabies comes closest to providing such a model, despite the obvious differences. If it does represent a useful parallel example, then it certainly emphasizes the difficulty of achieving effective control by a policy which relies solely on large scale periodic population reduction, and the accumulating evidence in New Zealand suggests that additional control procedures will be necessary to make greater progress in control. The notable success of wildlife vaccination as a control strategy in fox rabies supports the importance of pursuing this control option in wildlife tuberculosis as a long term control option, and the computer simulation model should in future be enlightening in evaluating this strategy.

African swine fever and rinderpest clearly do not have such strong epidemiological similarities to tuberculosis as rabies, because as shown in the consideration of these diseases the determinants of spatial and temporal patterns of disease transmission between wildlife and domestic hosts are quite different, so control policies need to be different as well. Thus consideration of wildlife disease epidemiology must take fully into account the ecology of the wildlife host(s) and the opportunities for behavioural interaction between the wildlife and at-risk domestic stock.

The main insights which these two comparisons offer relate to the flows of infection between wildlife and domestic stock. In rinderpest early concern about wildlife reservoirs of infection has given way to a dominant view that in this particular case domestic stock are the reservoirs and wildlife are the spillover hosts. This has greatly simplified regional control of the disease. African swine fever offers salutary lessons of a disease which was initially easy to limit to the wildlife reservoir species, but which for various reasons established a new and quite different transmission cycle which made it totally independent of the wildlife mammalian reservoirs and allowed it to spread to new countries and cause outbreaks. These have been persistent and difficult to control in the presence of a suitable tick vector, but easier to control in countries where there was no tick vector. Possum tuberculosis is a very clear example of a disease establishing in an entirely new maintenance host, which happens to be a

very successful coloniser of diverse habitats, being probably the most geographically widespread wild animal in both Australia and New Zealand, and one of the most numerous animals in both countries. Both population control and habitat management are therefore not easy to achieve for the possum as ways of controlling tuberculosis, and much more subtle combined control strategies will be necessary. There also remains a risk that with infection occurring widely in the possum, that one or more of the current spillover hosts could in future become a maintenance host. It can be conjectured that feral deer might the original maintenance wildlife hosts in New Zealand, and that the possum was a spillover host which for ecological and epidemiological reasons became a very successful maintenance host. If this were true, then the sparseness of feral deer in Australia would help explain why the possum did not become a reservoir species there. For New Zealand the implications would be the importance of preventing any further species from becoming maintenance hosts, and the possible need to see feral deer tuberculosis control as an essential adjunct to possum tuberculosis control.

Parallels can be drawn with the resurgence of human tuberculosis over the last decade. Until then tuberculosis control had been one of the success stories of human medicine. Since the middle of this century the disease had been largely confined to populations living in underprivileged conditions in industrialized as well as developing countries. It could be argued that the advent of diseases such as AIDS in combination with specific behavioural patterns and environmental conditions has created an epidemiological situation which facilitates maintenance of *Mycobacterium tuberculosis* infection in specific subgroups of the population.

Relatively few of the diseases which involve transmission flows between domestic and wildlife hosts have been investigated in sufficient epidemiological detail to enable detailed comparisons to be made. The three examples chosen here for comparison were selected both because they are among the best understood, and because they represent a spectrum from rabies (which offers some of the best insights into the problems which must be faced in controlling possum tuberculosis), through African swine fever (which has parallels mainly with regard to the risks of new epidemiological patterns emerging) to rinderpest (which is at the opposite end of the epidemiological spectrum, in that wildlife are spillover hosts, and control in the wildlife is far less important than control in domestic stock). This comparison shows where tuberculosis fits in the spectrum of diseases shared between wildlife and domestic stock, and although analogies must be treated with considerable caution, the comparison does help to identify epidemiological issues which should be borne in mind in thinking about control policies.

EPIDEMIOLOGICAL STUDY METHODS FOR DISEASES IN WILDLIFE

Diseases in wildlife provide a major challenge to modern veterinary medicine. It should be noted that on a world-wide scale diseases which involve wildlife reservoir species such as

rabies, bovine tuberculosis and trypanosomiasis are amongst those animal diseases which have provided modern veterinary medicine with the most difficult challenges when attempting to achieve control or eradication. This is an area where a sound epidemiological approach is required in order to make progress. Over the last 20 to 30 years the methodology used in veterinary epidemiology has become very advanced and has been used successfully in dealing with diseases in domestic animals. Very sophisticated and successful animal disease surveillance systems have been developed for the control of diseases in domestic animals. It is now clear that these methods cannot be applied to wild animal populations without considerable modification and refinement. With minor modifications, the reduction of the number of animals has been the major method used in the control of diseases in wild animal populations. In some circumstances this can change the balance sufficiently to bring a disease under control, especially during the peak of an epidemic - when other factors also favour a reduction in incidence. However for endemic diseases the ecological forces which exert strongest influence tend to be those favouring rebuilding of the host population and maintenance of the disease within the rebuilding population, which contains a high proportion of susceptibles. Thus the more endemically stable a disease is in the wildlife population (and clearly tuberculosis in possums fits this situation), the less effective large area population control is likely to be in producing a stable low prevalence of the disease. Refinements can be introduced which improve the effectiveness of population control by differentially reducing infected populations, dispersing animals and other high risk groups. The example of fox rabies also shows the major benefits of reducing the proportion of susceptibles in the population through wildlife vaccination.

Epidemiological studies of diseases in wild animal populations require a different methodology than studies of diseases in domestic animals largely because the information which can be collected on the population as a whole as well as on individuals is subject to a number of limitations. These include difficulties in accessibility, identification, examination and follow-up of individuals. It can be very difficult to make observations on the interaction between individuals in the population as well as between them and other wild and domestic species. For these reasons it is very challenging to conduct longitudinal or cohort studies which are among the most powerful tools which can be used by epidemiologists in wild animal populations. The data collected in most epidemiological studies of wild animal populations will be subject to sampling error or measurement bias to a larger extent than in the case of the study of human or domestic animal populations. Often there are many unknown risk factors as well as confounding factors which may influence the results, but cannot be measured and controlled for in the analysis. For these reasons, despite their major limitations in providing an understanding of the dynamics of the disease, cross-sectional studies have been widely used to study the epidemiology of diseases in wild animals - primarily because they are relatively easy to conduct. The pathogenesis of diseases in wildlife is usually studied under experimental conditions, which means the findings will often

misrepresent the course of disease in the wild. Epidemiologically sound observational studies are therefore necessary to obtain information on the pathogenesis of the disease under uncontrolled conditions. Ecologists are used to dealing with the problems involved with studying wildlife populations. Some of those methods are likely to be applicable in veterinary wildlife epidemiology.

EVALUATION OF THE STUDY METHODS ADOPTED FOR TUBERCULOSIS

Longitudinal Study of Bovine Tuberculosis in Possums

The longitudinal study approach is one of the most powerful techniques which can be used in wildlife disease epidemiology. It is possible to estimate the incidence of disease, and the temporal relationship between hypothesized causes and their effects can be tested. The problems involved with this methodology include difficulties in the follow-up of individual animals. They are also very costly and labour intensive. One of the major difficulties in the study reported in this thesis was related to the diagnostic methods, which were poorly sensitive for detecting possums infected with *Mycobacterium bovis* and only moderately sensitive for detecting clinically diseased possums.

The results of the data analysis for the longitudinal study showed that prevalence and incidence levels of clinical disease in a possum population with endemic infection vary significantly between seasons and between years. Adverse environmental conditions such as cold and rainy weather, limited availability of food resources and suitable den sites resulted in an increase of the incidence of clinical disease. These factors also limit survival of clinically diseased possums. A possum which develops clinical disease under rather favourable environmental conditions may be able to survive for extended periods. If it is exposed to rain, wind and low environmental temperature, it would not be able to survive for more than maybe 1 or 2 months from the onset of clinical disease. Therefore, during cold and rainy weather there could be many clinically diseased and infectious animals in the populations for a relatively short duration. Under more favourable conditions only a few clinical cases would be present, but they could survive for extended periods. It appears from the longitudinal study that this variation in survival is an important feature of the dynamics of endemic infection in possum populations. Prior to this study information from experimental studies suggested that the period between the onset of clinical disease is very short (O'Hara *et al* 1976, Corner and Presidente 1980, 1981). The pathology of the disease could not be studied in detail, but many animals with clinical disease had lymph node abscesses which discharged through the skin. Most tuberculous possums had lesions in the lung tissue and /or peripheral lymph nodes.

The data collected during the study provided basic information on the importance of the different potential disease transmission paths. The evidence suggests that transmission from infected adult female possums to their offspring is one of the major factors in the epidemiology of the disease. Pseudo-vertical transmission could be responsible to a significant extent for maintenance of the disease within a possum population as well as for the

spread of infection to other populations. Young infected animals can remain subclinically infected for an extended period, the length of which remains quite uncertain. It can commonly be a number of months, and may in some cases be as long as 1 to 2 years - or even longer. Infected animals appear likely to develop clinical disease under conditions of stress induced by the environment, feed shortages or through behavioural stresses such as those occurring at mating or dispersal. Once they are clinically diseased these possums can infect other animals, including both domestic stock and other wild animals as well as possums. Especially during the mating season a clinically diseased adult male possum can readily transmit infection to susceptible adult female animals in association with the courting and mating process, as well as to susceptible adult males during agonistic behaviour. From the data of the longitudinal study it appears that these patterns of social interaction comprise the main transmission mechanisms in the epidemiology of *Mycobacterium bovis* in possums. Indirect transmission - on pasture during feeding, during sequential sharing of den sites and through contamination of sites used for marking - probably occurs, but on the evidence from the longitudinal study seems to be of much more limited importance than direct methods of transmission.

The longitudinal study also provided information on transmission of infection to other domestic and wild animals. The two restriction-endonuclease strains of *Mycobacterium bovis* which dominated in possums were also found in cattle and wild pigs, and a minor strain was found in a ferret. It unlikely that during the study infection was transmitted from any of these species to possums. In this scenario cattle, wild pigs and ferrets are more likely to be spillover hosts. Evidence from the study also suggests that terminally ill and possibly dead tuberculous possums are important sources of infection for spillover hosts including cattle.

Cross-sectional Study of Bovine Tuberculosis in Possums

Cross-sectional studies can be used to obtain baseline epidemiological information, but they can only provide a static picture of the disease situation. They can be relatively easily conducted in a wildlife situation. The Hauhungaroa study had been conducted at relatively low intensity over too large an area, which resulted in a loss of detail of information at the farm-level. Also the different areas which were included in the study were quite heterogeneous in that some of them had been subject to possum population control, the habitat was different and the possum populations were sampled at different seasons, without regard for possible seasonal variation in prevalence. Especially the last factor complicated the analysis considerably in that the possum populations were compared at different stages of their yearly biological cycle. The diagnostic method for identification of tuberculous possums was poorly sensitive because laboratory confirmation was not based on culture, which is the most sensitive method available in routine diagnosis.

The results of the cross-sectional study suggest that on a larger geographical scale about 2% of possums have clinical tuberculosis. Prevalence in local clusters can be up to 20%. Tuberculosis prevalence was higher in adult possums than in immature possums. In immature possums males were more likely to show clinical disease than females, which was not the case

for adults. Breeding female animals were more likely to have tuberculous lesions than adult females without a pouch young. A large proportion of possums with clinical disease had lesions in the respiratory tract and/or peripheral lymph nodes. The presence of localized tuberculous lesions did not appear to affect condition to a significant extent. The study also allowed a statistical examination of the relationship between possum tuberculosis prevalence and cattle tuberculosis incidence. The correlation between the two measures was poor. This result should be interpreted with care, because it was not exactly known when and how long cattle had been kept in paddocks adjacent to the areas which had been sampled for the presence of tuberculous possums. Also, the data collected did not allow possum tuberculosis prevalence to be estimated for a particular farm, because in most cases only a small area had been sampled. What can be said is that cattle from farms adjacent to possum populations with endemic tuberculosis were more likely to have reactors than cattle from properties where no tuberculosis was found in the associated possum sample.

The longitudinal and the cross-sectional study complement each other. The longitudinal study provides detailed information about the epidemiology of *Mycobacterium bovis* infection in a possum population. It is possible to obtain information about the infection dynamics and transmission patterns. On the other hand the cross-sectional study provides an impression of the epidemiology of the disease at a larger geographical scale.

Both studies come to similar conclusions on points which they could both measure - such as that the disease is clustered in space and that the predominant sites for tuberculous lesions include the respiratory tract and peripheral lymphnodes. The results of the longitudinal study did not confirm that immature male possums were more likely to show clinical lesions than immature females. A meaningful conclusion cannot be drawn from this finding in the longitudinal study because only few immature animals with clinical tuberculosis were identified.

Case-control Study of Tuberculosis Breakdowns in Cattle Herds

A case-control study approach is typically used when studying rare diseases. The number of breakdowns in cattle herds due to tuberculosis infection is still a relatively rare occurrence and in a case-control study it was possible to include the maximum number of case herds in order to achieve meaningful results in the analysis. Matching of cases and controls allows the control of confounding factors. In this study the presence of infection in local possum populations and the type of farm enterprise were considered major confounding factors. Therefore whereas a typical case-control study has all controls chosen on the same basis, in this case two differently chosen controls were used for each case. Each case herd was matched with one control herd of any enterprise type from the surrounding region (random control) and a second nearby control farm with the same type of enterprise (matched control). The matched case-control approach should allow the analysis to minimise the confounding effects of the factors which have been used for matching. Controlling for the risk of infection from local possum populations by selecting controls from the vicinity of case herds may not

have been a very effective way of matching because tuberculosis infection in possum populations is extremely clustered in space and the risk of infection for local herds may vary considerably between neighbouring herds. However without extending the study to include examination of possum populations on candidate farms, this was the best matching which could be attempted.

The study provided some insight into the importance of transmission between and within species, for both possums and cattle. The results suggest that in the Waikato area possum to cattle transmission may be of importance for herds which are located closer to the tuberculosis endemic area. In these herds it is more likely that infection is present in the local possum population. But it is also possible that these farmers were more likely to have bought animals from the tuberculosis endemic area which may have been infected with *Mycobacterium bovis*. It appears that cattle-to-cattle transmission by contact between neighbouring herds (contiguous spread) is unimportant under the management conditions in the Waikato area. No inferences can be drawn regarding the importance of domestic and wild deer as no suitable data was available which could have been included into the analysis.

The study had been conducted because there had been a number of breakdowns in the area and it had not been possible to explain them by demonstrating the presence of tuberculous possums on the farm, since extensive cross-sectional studies had failed to detect any tuberculous possums, despite a strong suspicion that they were present in the area. (Subsequently infected possums were found in the area). The results of this study suggest that exposure to infection in local possum populations is not the dominant cause of breakdowns in these herds and that the occurrence of clusters of outbreaks does not demonstrate that they were caused by infection in local possum populations. Farmers who had a breakdown were more likely to be among those who followed herd management practices considered to put farms at risk of buying in infection in purchased stock. This finding and the fact that many farmers had only limited knowledge about the disease suggests that there is a need for an educational effort and incentives for farmers to change their purchase policies in order to minimise the risk of introducing infection. The case-control approach used here provided a method of provisionally distinguishing the relative importance of the two major transmission hypotheses fairly quickly and inexpensively - something which would have been much more costly to do by any alternative investigational approach.

RESEARCH TECHNIQUES

It was possible to apply a number of research techniques to the analysis of the data collected in the various studies included in this project which have not previously been used widely for epidemiological investigations. Based on the type of data they can be broadly grouped into techniques which can be used in the analysis of difficult longitudinal data, data including a spatial component and/or temporal component, and multivariate analysis techniques.

Analysis of Difficult Longitudinal Data

Data collected during a longitudinal study of diseases in wildlife populations is characterized by losses to follow-up and by new recruitments. Losses to follow-up can represent deaths as well as emigration. New recruitments can be animals which were born locally or can be animals which immigrated into the study area. In ecology, capture-mark-recapture techniques are used to estimate parameters describing the dynamics of such populations. The main techniques which were used in this analysis include a set of models developed by Otis *et al* (1978) and the Jolly-Seber model (1982). Both techniques allow estimation both of population size for each point in time when the population was sampled and of survival between sequential visits during data collection. Capture-mark-recapture techniques are finding wider use in epidemiology, and have been used recently to estimate population at risk for sexually transmitted diseases (Rubin *et al* 1992). In the longitudinal study reported here they were essential to reduce bias in estimating the population at risk and entry/removal rates.

Analysis of Data including a Temporal Component

Data with a true temporal component is collected typically during a follow-up study and possibly during a retrospective study. The variable measured is the time elapsed until occurrence of a particular event of interest. This type of data may be complicated by four factors: loss of individuals during follow-up (right censoring), individuals which do not develop the outcome of interest during the period of the study (right censoring), new recruitments during follow-up (left censoring or staggered entry) and change of attributes of an individual during follow-up (time-dependent variables). Survival analysis (or event-history analysis, as it is called in the social sciences) provides a methodology which can take account of these factors. Cox's proportional hazards regression model adds a powerful multivariate analysis tool to survival analysis. These techniques have proved valuable both in the longitudinal and the modelling study.

Analysis of Data including a Spatial Component

Proximity is one of the major factors in the epidemiology of contagious disease processes. Over the last 5 years the storage and management of spatial information in a computer has become more practical with the advent of software termed geographic information systems (GIS). It is now possible to use these systems to provide data which can be used in epidemiological analyses. The main areas of application include the preparation of descriptive maps of disease occurrence and of relevant features in the environment such as topography and vegetation, as well as the quantitative description of spatial relationships between features. The descriptive mapping component of such systems was used in the longitudinal study to display and compare the overlap of the areas which were used by possums infected with individual strains of *Mycobacterium bovis*. Distances to features of potential importance such as herds with tuberculosis infection or the distance to the tuberculosis endemic area in the case-control study were used as potential risk factors in the multivariate analysis of the particular study. In the longitudinal study analyses were conducted to identify risk factors

which differentiate den sites used by possums with tuberculosis and den sites only recorded as used by non-tuberculous possums. These risk factors included locational characteristics such as slope and height above sea level, which were retrieved using spatial database management techniques such as overlay operations from the relevant information layers.

Analysis of Data including Both a Temporal and Spatial Component

Clustering of disease occurrence in time and space is typical for a infectious disease process. The management of joint temporal and spatial data is extremely complex. It is an area which is still under development. At the moment methods such as Mantel's time-space regression method can be used to investigate such relationships. The general view appears to be that this technique is not very sensitive. In this analysis the use of different transformations of the time and space variables resulted in inconsistent results. The results of the analysis were quite difficult to interpret. The use of time-space interaction analysis gave hints of transmission associations which were helpful in formulating an overall hypothesis of disease transmission, but it is considered that at this stage of development of the techniques they must be used very cautiously as purely an exploratory tool.

Analysis of Multivariate Data

A number of multivariate analysis techniques were used in the different studies included in this project. Correspondence analysis and multidimensional scaling both allow the visual description of relationships between variables. Correspondence analysis is only applicable to categorical data. It places each category as a point in a type of scatterplot or 'map'. The relative positions of the category points in this map indicate certain levels of similarity or association between categories. Multidimensional scaling is used for the analysis of preference data such as used in the case-control study to describe the interviewee's self concept. Both techniques are only useful for descriptive analysis and the interpretation of the distances between points and the meaning of the axes in the map largely depends on the judgment of the reader.

In veterinary epidemiology multivariate data is commonly analysed using a two step technique. In a univariate analysis the data is first screened for statistically significant relationships between the dependent variable and each potential risk factor. This step is then followed by a multivariate analysis which includes the variables which were statistically significantly associated with the outcome variable in the univariate analysis. The multivariate analysis can be conducted in a number of ways. One option is a stepwise regression approach which results in a final regression model purely based on statistical grounds. These models do not provide a comprehensive view of the causal web they are trying to describe. Path analysis is a technique for multivariate data analysis which allows the researcher to combine biological understanding of the problem under consideration with the power of statistical techniques. Both path analysis techniques which were used in the case-control study, standard regression path analysis and structural equation modelling, involve the development of a hypothetical path model by the researcher which is then tested using statistical methods. Both path analysis

approaches are very useful for exploring the causal structure of the data. Compared with standard regression path analysis, the structural equation model is more powerful and has more options. Its advantages are that it provides a quantitative estimate of overall fit of the path model, latent variables can be included, the model does not have to be recursive and direct as well as indirect effects can be quantified. Its disadvantages include the complexity of the modelling process compared with regression path analysis, the methodology for dealing with categorical variables is not yet fully developed and sample sizes have to be quite large to estimate stable and meaningful path models. Classification tree analysis is another method which can be used in the multivariate data analysis step. This statistical technique is used to develop an hierarchical representation of the data space using a binary tree. This "decision tree" can be readily interpreted and integrated into a decision framework. Classification tree analysis produces the best results when large sample sizes are available. It is of limited use for causal analysis.

COMPUTER SIMULATION MODELLING AS A TOOL IN DISEASE CONTROL

Computer simulation modelling provides an essential tool for investigating the epidemiology of a disease and for comparing potential disease control strategies. As pointed out by Martin *et al* (1987), in addition to its other uses the modelling exercise brings to light important deficiencies in the available body of data. Consequently a model can be used to demonstrate the importance of the missing data and to direct data collection efforts (Hurd and Kaneene 1993). A simulation model which provides an adequate representation of the epidemiology of a disease can be used to test the effect of different control strategies. Anderson (1991) emphasizes that the transmission dynamics of the infectious agent in question and its distribution and abundance within the host population, which can both be represented in simulation models, have a major influence on the intensity and frequency of control intervention required in order to halt transmission. The effectiveness of new methods of disease control can be assessed in order to decide which of a number of options should be considered for medium- or long-term research efforts.

One of the objectives of this epidemiological project was to develop a computer simulation model describing the epidemiology of endemic bovine tuberculosis infection in a local possum population. A systems analysis approach targeted at generating knowledge about the system under study was adopted in order to provide the information required for the development of the simulation model. At the beginning of the study a general overview of the system was obtained by conducting a literature review, and by communication with people working in the subject area. This general information was then used to design and implement the observational field studies as the next stage in the system analysis. These sources of information generated the knowledge which was used to develop the simulation model. The model is the synthesis of the information available through the two first stages of the system analysis. Once the model is developed a feedback of information to the two lower levels can occur, resulting in design of studies to test hypotheses generated by the model or formulation

of hypotheses which can be tested directly by the simulation model. A number of studies have already commenced as a result of data requirements identified as necessary for the model (e.g. survival of *Mycobacterium bovis* in the environment, and behavioural interaction between possums and domestic livestock).

As explained above, the knowledge about the system currently implemented in the model is based on a detailed literature review and analysis of data from a longitudinal and a cross-sectional study of *Mycobacterium bovis* infection in possum populations. In combination, both field studies provided an epidemiological framework for generating hypotheses about important factors and their interaction in the dynamics of tuberculosis infection in wild possum populations. The development of the simulation model required qualitative knowledge about these factors as well as parameter estimates describing quantitative relationships between factors. The longitudinal study provided this information. This epidemiological knowledge was used to design a Monte-Carlo simulation model as described by Morris (1976). The model adequately represents the epidemiology of bovine tuberculosis infection in local possum populations. Some of the quantitative estimates of the model parameters may have to be refined as soon as new information from other field studies becomes available. Currently there is no independent field data available, against which the model could be validated. A number of options for disease control have been tested using the model, but these results have to be interpreted keeping in mind the early stage of model development which has so far been reached and uncertainty about some of the parameter estimates. The model will be developed further in order to simulate the impact of control at a larger geographical scale and eventually will form part of a planned decision support system for tuberculosis control in New Zealand.

Simulation and Disease Control in Animal Populations

A major objective of developing a disease simulation model is to evaluate the effect of different control options on the pattern of disease within a population. A number of simulation models have been developed for the epidemiology of diseases in wildlife. Results from a wide range of models on epidemiology of rabies in fox populations have been published. These include a small number of stochastic models using a modelling approach similar to that used for the development of the current model. A model developed by Preston (1973) was used to investigate the possible interactions in a rabies controlled population. It included a spatial component, but did not allow extensive evaluations of disease control strategies. Voigt *et al* (1985) developed a stochastic simulation model of rabies (Ontario Rabies Model) which simulates the spatial and social behaviour of red foxes. It was specifically designed in order to assess control strategies and evaluate tactics of oral vaccination. This model has been used extensively for planning rabies control operations in the Canadian province of Ontario (MacInnes *et al* 1988). Experiments with the Ontario Rabies model have improved the understanding of temporal and spatial patterns of rabies incidence (Tinline 1988). Smith and Harris (1989) used the Ontario Rabies Model to investigate the potential pattern of spread of

rabies in Britain from a single source of infection. They came to the conclusion that they had to develop a new model, because the Ontario Rabies Model could not be applied to the detailed simulations required in the British situation. Smith and Harris (1991) reviewed 18 of the available rabies models and noted that most of the models were designed to reproduce various factors involved in the spread of rabies, rather than attempt to examine possible means of controlling the disease. Based on this experience they developed a discrete time simulation model with monthly steps modelling the spatial and social behaviour of individual foxes. This model uses numeric maps of the simulation area defining topographical barriers and locations of fox families. It is also possible to define the areas where fox control will occur. In their paper the authors concluded that their model provides a valuable tool in planning rabies control operations and helps to unite the bodies of theoretical and practical knowledge on rabies spread.

Various tuberculosis models have also been developed. A deterministic simulation model of the epidemiology of bovine tuberculosis infection in badger populations has been developed by Anderson and Trewhella (1985). Barlow (1991a)developed a deterministic simulation model with stochastic components for bovine tuberculosis in New Zealand possum populations. He used this model to evaluate a number of possible control measures (Barlow 1991b).

Strategies for Pest Management

An assessment of methods for control of possum populations requires some background information on the theory and practice of pest management. Putman (1989) reviewed approaches which have been taken to pest management. He writes that a population reduction (short of extermination of the species which causes the damage) is bound to have only short-lived success. Therefore it must be sustained and possibly repeated year after year. He adds that artificial population reduction may release the density-dependent brake on population growth resulting in increased reproduction and reduced natural mortality. Putman writes that even if we cannot prove density-dependent effects on recruitment and survival, local reduction of the species in one area is rapidly compensated by immigration from outside. In many situations control of a pest problem by direct reduction of population size may be the only realistic option available and may prove effective, if carried out intelligently and with full understanding of the underlying dynamics of the pest population.

Fischman (1985) points out that for control of rabies in wildlife, classic population reduction methods including trapping, poisoning, gassing and hunting have proven expensive, objectionable, often not effective and, even where effective, require periodic repetition. He advocates the use of chemosterilants as offering an attractive alternative which would also be much more acceptable to the general public. Fischman suggests that if a wildlife population is experiencing a rabies epidemic, a bait should be delivered containing an oral vaccine to halt the epidemic and an antifertility compound lowering the fertility at the next breeding season.

The antifertility compound would have to be used repeatedly to keep the population at a predetermined level.

Conway (1976) described the following major techniques for vertebrate pest control: culling (use of chemical compounds, trapping or shooting to kill or remove pests), biological control (use of natural enemies, either by augmenting those present or by introducing new species), habitat manipulation (use of agricultural or other practices to change habitat available to pests), exclusion (use of fences or other barriers to prevent entry) and plant or animal resistance (breeding of animals and crop plants for resistance to pests). More recently fertility control has been taken into consideration as a more humane method of population reduction (Bomford 1990).

Pest Control and Animal Population Dynamics

In order to make a decision on the optimal control strategy it is necessary to understand the mechanisms which determine the population dynamics of the species to be controlled. This includes knowledge about the following characteristics of a population: its increase and decrease, its fertility, mortality, dispersal and immigration rates. The effect of perturbations on the population dynamics has to be understood. It has been demonstrated that conventional control by increasing mortality results in increased reproduction and survival in the remaining animals, and possibly increased immigration and decreased dispersal. The population would therefore recover relatively quickly to its original density.

MacArthur and Wilson (1967) and Pianka (1970) defined the *r*- *K* continuum to categorize species according to the bionomic strategies reflected in their population dynamics. *r*- strategists are opportunists, selected for maximum food intake by the exploitation of their ephemeral habitats. Southwood (1977) writes that in contrast *K*-strategists maintain a steady population at or near the carrying capacity of the habitat; they are in equilibrium with their resources, whose renewal they do not adversely affect. Recruitment, migration and mortality rates are low, and if their numbers are reduced to low levels, they become extinct. On the other hand the population dynamics of *r*-strategists are characterized by 'boom and bust' as Southwood writes. This strategy is dominated by large-scale migration, and new populations are continually developing from a handful of colonisers. Southwood adds that a species may be able to move along the *r*-*K* continuum to a limited extent, if the ecosystem it lives in changes. He defines in addition to *r*- and *K*- pests the class of intermediate pests who are generally held at a level lower than carrying capacity of their habitat by the presence of natural enemies. These animals will achieve pest status if they are introduced into new areas free of their natural enemies (for example the gypsy moth after introduction to North America). Southwood writes that *r*-pests are always so numerous that they may in certain places destroy their habitat (he cites the example of smallpox virus and *Bacillus anthracis*). They typically show a high migratory tendency, essential for their movement from one 'dying habitat' to a new one. *K*-pests usually are relatively large organisms such as the African elephant which began to damage its habitat after man confined the species to game parks. The

African trypanosomes and their vectors, tsetse flies, are considered to be typical examples of *K*-pests. Pianka (1983) emphasizes that no organism is completely *r*- or *K*-selected. The *r* - *K* selection should be thought of as a continuum and an organism's position along it can only be determined in a particular environment at a given point in time. The concept of *r* and *K* selection has been criticized by a number of authors as too simplistic and too inadequate to explain variation in life-history traits in nature (Fleming 1979).

Southwood (1977) postulates that for *r*-pests a powerful control strategy is to decrease immigration. Population levels of these species are always fluctuating. It is the nature of *r*-strategists that after reduction control the population will quickly 'bounce back'. Southwood *et al* (1974) write that *r*-strategists have very efficient mate finding tactics at all densities, and therefore have a very low extinction point. Stenseth (1981) developed a conceptual framework for formulating pest control strategies on the basis of knowledge about the pest's demography, its habitat, and the control strategies available. He considers the extremes in the *r-K* spectrum of population selection. Stenseth concludes that if there are methods for reducing immigration into empty patches by almost 100%, regardless of the population demography of the pest most of the available resources should be spent on reducing immigration. If there is no particularly effective method for reducing immigration available, different methods should be used for *r*- and *K*- selected species in the same environment and habitat. With *r*-selected species most economic resources should be devoted mainly towards reducing reproduction rather than increasing mortality. For *K*- selected species Stenseth recommends directing most resources towards reducing dispersal but some for increasing mortality. In particular reduction in reproduction is likely to be inefficient for controlling *K*- strategists. Whereas Southwood (1977) recommended the use of pesticides for controlling *r*-selected species, Stenseth suggests that because most pests are *r*-selected this may explain why the extensive application of pesticides has proven inefficient in protecting the world's food supply against destruction.

Tyndale-Biscoe (1979) classifies the brushtail possum as an *r*-selected species, as it is extremely adaptable and occurs in a wide range of habitats. How (1978) reviewed the population strategies of four species of Australian possums and he considered *Trichosurus vulpecula* Kerr to be closer to the *r*- end of the *r-K* continuum than the other three species. Given the above theories about population strategies it would appear that for possum population control population reduction clearly is unlikely to be fully effective alone.

Modelling of Infectious Diseases

Models of infectious diseases are frequently used to test the effectiveness of disease control strategies in animals and humans. Most of these models are based on sets of abstract mathematical equations. In order to understand the problems involved with developing and using such *mathematical* models it is necessary to describe their main components.

Mathematical models depend to a large extent on an estimate of the basic reproductive rate of disease $\mathbf{R_0}$ which is defined as the average number of secondary infections attributable

to a single infectious case introduced into a fully susceptible population. This principle is one of the most important concepts in mathematical epidemiology (Anderson 1991). Under the theoretical 'mass action' assumption $\mathbf{R_0}$ is a function of population size or density. The goal of mathematical modelling is to estimate a threshold population density for disease maintenance (Fine *et al* 1982). Given this figure it is possible to calculate minimum host densities required for the disease to remain endemic within a population. Using this approach Anderson *et al* (1981) estimated threshold values for maintenance of rabies infection within fox populations. Voigt *et al* (1985) found that in Canada rabies was persisting at fox population levels lower than the ones where it disappeared in Europe. MacDonald and Voigt (1985) point out that the contact rate is the most fundamental ingredient of a rabies model. It is not a constant, but a complex function of the social organisation and density of the vectors, and thus the frequency of meetings amongst them. They write that in the stochastic world of complex animal populations, measurement of contact rate is notoriously difficult; the frequency of meeting between individuals (and hence the potential contact rate of disease) is a reflection of their population density, social organization and their ecology. MacDonald and Voigt add that it is known that the spatial organization of vectors influences the local rate of contact, the reintroduction and the long-range dispersal of vectors. Mollison (1987) emphasizes that the major problem in developing a mathematical model for disease dynamics lies in estimating the value of $\mathbf{R_0}$ even in undisturbed populations or worse in predicting its new value under some control strategy. He writes that in the case of rabies in foxes because of a social disruption caused by culling part of the population, $\mathbf{R_0}$ may not change in proportion to population density. In a more detailed discussion of the implications of control on model parameters Mollison (1985) points out that on the one hand, by reducing fox density competition for available food will be reduced and therefore contact will be less common. But on the other hand, as mentioned above, families will be broken up by the culling. This social disturbance will result in more contacts. He adds that with diseases such as measles and whooping cough where the population density is not affected by the disease the reproductive rate can be extremely low and the effects of heterogeneous mixing gain importance. Mollison (1986) writes that estimating $\mathbf{R_0}$ resists any general quantitative approach. He states that species do not have an absolute value of $\mathbf{R_0}$ but a value relative to a particular habitat or ecosystem.

Anderson (1985) developed models for both rabies in foxes and for bovine tuberculosis in badgers. He writes that both diseases show very different epidemiological patterns within their respective host populations. Rabies is an epidemic disease exhibiting large fluctuations in prevalence from year to year, with an overall relatively low number of rabid animals. In contrast, bovine tuberculosis in badgers appears to be stable, with the prevalence remaining relatively constant through time and a high standing crop of infected animals. Anderson suggests that the characteristics of the epidemiology of rabies in foxes would suggest that control should be relatively easy by taking advantage of the instability of infection within host

populations. But this can only be achieved given an intense and sustained control effort in areas of good fox habitat to maintain fox populations at a sufficiently low level of abundance to eradicate disease. Ideally control should be applied between epidemic intervals. In the case of bovine tuberculosis in badger populations Anderson suggests that eradication of infection may require the eradication of the host species. Summarizing, he writes that rabies control in fox populations requires less intensive control effort at one point in time, but sustained effort at frequent intervals. On the other hand bovine tuberculosis in badgers needs intense control effort to substantially reduce host abundance, applied at relatively infrequent intervals. The discussion earlier in this chapter of this same issue suggests that although there are differences between rabies and tuberculosis in wildlife, there are also important epidemiological similarities and that these two diseases merit continuing comparison since investigation of each can provide useful insights into control options for the other.

For rabies in foxes, Tinline (1988) used the 'Ontario Rabies Model' and came up with some factors which he suggests may affect persistence of infection within southern Ontario. He firstly hypothesizes the existence of 'rabies units' which form distinct spatial clusters and temporal patterns of infection. He suggests that there must be a balance between good and poor habitat to maintain spread but slow it down sufficiently to allow populations to rebuild behind epidemics. The conclusion was that by concentrating control on particular units with good habitat it may be possible to break the chain of infection. He called this spatial heterogeneity at a macroscale, which he thinks could well be the primary mechanism causing persistence of rabies in southern Ontario. Tinline postulates a threshold size for rabies to persist in certain areas. On a macro scale he found that using the 'Ontario Rabies Model' he could only simulate an endemic situation if the area being modelled was at least 4000km^2. Other factors which Tinline investigated using the 'Ontario Rabies Model' included the interaction between reproduction and mortality, the incubation period and the interaction between contact rate and population density. Obviously the epidemiology of fox rabies is distinct from the epidemiology of bovine tuberculosis in possums, but this example shows how a stochastic simulation model can be used to improve the understanding of the epidemiology of a disease in wildlife populations to a significant extent.

Modelling and Planning for the Control of Bovine Tuberculosis in New Zealand

Norton (1988) advocates a decision analysis / systems analysis approach to vertebrate pest management. The method includes two key concepts. A decision model describing the four major factors affecting decision making - the pest problem itself, the control options available, the decision maker's perception of the problem and his or her objectives. Each of these factors has to be considered during the actual decision making process. The second key concept concerns development pathways. Norton sees the direction of the agricultural and pest management development process as influenced by factors such as economic forces, agricultural policy, technological as well as previous development.

In New Zealand the decision process for implementing control of tuberculosis in possum populations was mainly based on the finding that once a local possum population with endemic tuberculosis infection had been reduced, the incidence of infection in cattle grazing in habitat overlapping with possum habitat dropped significantly. It was noted that a few years after possum population control, cattle reactor numbers increased again. It was therefore concluded that repeated culling of possums was a suitable method for controlling the disease locally. The other dimension of the problem was that infection was also spreading between local possum populations. It was realized that it was very difficult if not impossible to contain the disease within local possum populations. Given the cost of these possum control operations the Ministry of Agriculture and Fisheries decided that the primary objective of tuberculosis disease control was the containment of *Mycobacterium bovis* within endemic areas. From a population theorists' point of view, both the rapid recovery of population numbers and the fact that possums are a good vehicle for spreading a disease spatially are characteristics which are probably fairly typical of *r*-selected species.

Given the above situation, mathematical models were developed to provide information for guiding and justifying disease control management decisions at a national level. Although various models have been developed to represent aspects of possum tuberculosis in New Zealand, the one which comes by far the closest to representing the field control problem and the only one which has been used in practical policy formulation is the model developed by Barlow (1991a, 1991b) This model has evolved substantially over a period of years, and has proved very informative as a guide to development of control policies. The first version was used to lay out a strategy for local possum control and for preventing spread of infection from endemic areas. For local possum control Barlow writes that the results of simulation analyses suggest that widespread poisoning operations, or single intensive control operations followed by 'maintenance control' are highly effective and offer the only means for rapidly reducing tuberculous possum density. He suggests that sterilization as a control measure is less effective than culling, and vaccination would be least promising. It was also concluded that as long as diseased possums can immigrate, it is impossible to eradicate the disease. But Barlow suggests that the disease will not spread if the population is maintained below the threshold for disease persistence. This is a conclusion which has also been used extensively to justify destruction of foxes for rabies control. Yet control of fox populations through population reduction has not been successful. Barlow also states that immigration of susceptibles has relatively little effect on the disease recovery rate following control. Stochastic effects at a small scale may have a large impact on the outcome of a control operation. Therefore it seems appropriate to express the results of comparisons of control options using a simulation model in terms of probabilities of achieving control/eradication, as well as expected prevalence, which hides the stochastic variability in the system. This would give disease control managers critical information which can be used in the decision process. This type of information is

difficult to provide using deterministic mathematical models, but can be easily produced by a Monte-Carlo simulation model.

There is some evidence from a major field study of post-control population and tuberculosis dynamics which suggests that a control strategy combining an initial population reduction of at least 70% with regular maintenance control as recommended by Barlow cannot maintain low possum numbers for long enough to prevent recovery of tuberculosis disease levels in some areas (Hickling 1991). In this particular study tuberculosis prevalence dropped from pre-control levels of 2.3% (N=830) to 1.2% (N=489) for aggregated data from the post-control surveys. This reduction was not statistically significant (p=0.17). Tuberculous possums were generally found in the same locations as during the pre-control survey. In a different type of habitat with a population free from tuberculosis infection it was found that after a control operation which produced an 88% successful population reduction, the local possum population appears to require at least 8 years to reach pre-control levels. This agrees with predictions of Barlow's mathematical model (Brockie *et al* 1991).

Barlow (submitted) developed a variation of the basic model which allows the simulation of spread of bovine TB between local possum populations. One of the major conclusions from this model was that a 3km 'buffer' zone with reduced possum density surrounding an endemic area would reduce the number of possums dispersing into the non-endemic area by effectively moving the source of possums 3 km further back. Knowledge about dispersal behaviour of possums with regard to distance and the role of local density as a factor influencing direction is still very limited. But it has been suggested by Cowan and Rhodes (1988) that areas of reduced population density while not necessarily exhibiting a 'vacuum effect' may in fact promote improved survival of infected dispersing juveniles.

One of the major conclusions from Barlow's model was that 'spot control' of locations in non-endemic areas, where cattle test positive for tuberculosis, in combination with a low possum population density buffer zone prevents spread of the disease. Cattle reacting to the tuberculin test would be used as indicators for the presence of tuberculous possums. The assumption behind this strategy is that the probability for transmission from possums to cattle is more than 0.10. Yet, the validity of this assumption has not been adequately verified. Before these conclusions are used as the basis for policy decisions, the sensitivity of the model using a range of transmission probabilities from possums to cattle should be tested. If it were that spatial spread of *Mycobacterium bovis* infection within possum populations does progress faster than local cattle populations can pick up the disease from infected possums, then relying on "spot-control" would be very dangerous. At present the answer to this question is simply not available. It may also be that spot control offers benefits, but for reasons which differ substantially from those which underlie the model analysis. For the case of fox rabies Bacon (1981) points out the dangers associated with the delay in detection of rabid foxes which may lead to misjudging the progress of the 'infection wave front'. Mollison

(1986) notes that occasional long distance dispersers have a disproportionate importance for spread and therefore for attempting control.

Past experience with the maintenance of infection within endemic areas and the spread of bovine tuberculosis infection in New Zealand has shown that the conclusions which can be drawn based on mathematical models are limited. Development of a disease simulation model requires converting a biological system into an abstraction of the same system which can be implemented on a computer. Commonly the mathematical researchers who develop the model do not have direct field experience with the disease problem. In such cases, validity of the model structure can only be ensured if model logic and mechanisms can be explained to non-mathematicians, and evaluated in detail for biological validity. With mathematical models it is often attempted to represent a complex biological system with an abstract set of mathematical equations, which non-mathematicians find very difficult to understand. Some of the problems with false predictions produced by Barlow's earlier models probably are attributable to these difficulties which are inherent to mathematical modelling process. Also, when Barlow developed his first models, the understanding of the epidemiology of tuberculosis infection in possum populations was much more limited than it is today. There is thus a need to ensure that any models used for policy formulation adequately represent biological reality, and that both developers and users understand each other very fully. Even more important, when technical knowledge of a disease is fairly limited it is only possible to build models which reflect that level of knowledge, and to evaluate control options in broad terms. As knowledge grows, policy decisions can become more precise and the models used to support such decisions must reflect the increased level of understanding.

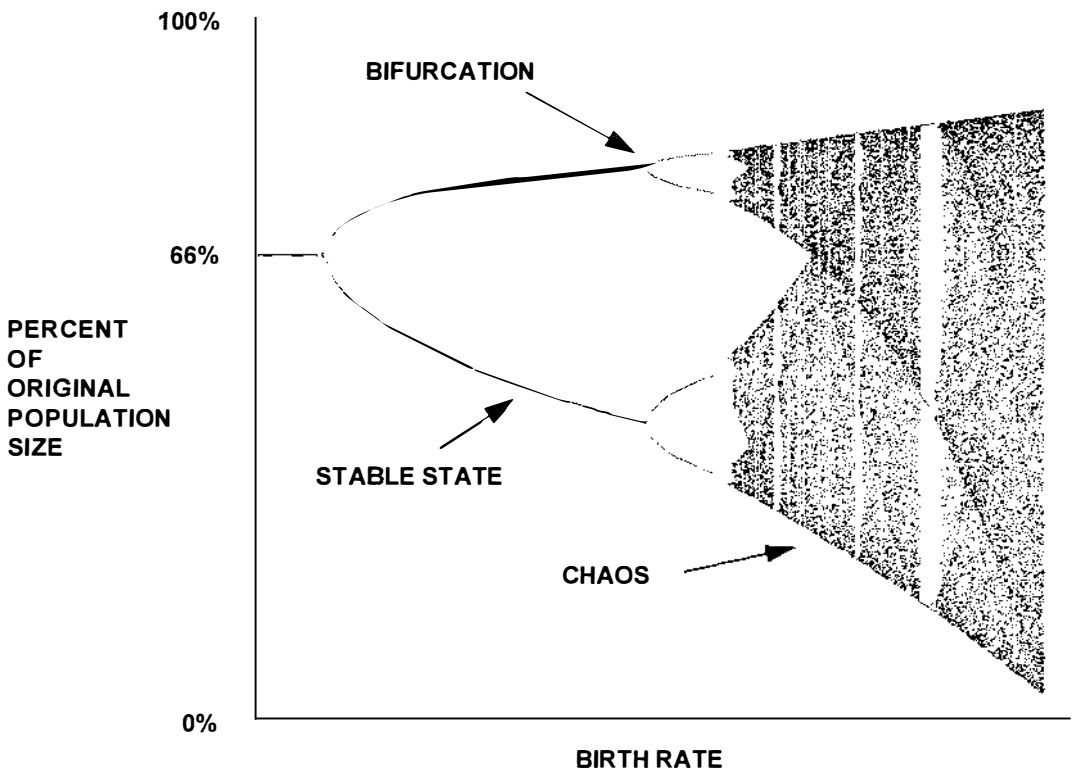
The model described in this thesis does not yet allow valid comparisons of disease control strategies. The simulation results so far have shown the importance of sensitivity analysis in combination with model verification/validation. Yet, the structure of the model does appear to represent the current understanding of the epidemiology of tuberculosis infection in possum populations adequately. Using sensitivity analysis it was possible to clearly identify the areas which need improved estimates.

Simulation Models of Infectious Diseases in Epidemiological Research

As has been pointed out by Bradley (1982), the conclusion both from reviewing the literature and from experience with developing this simulation model is that for real progress to be made, both the mathematical modeller and the epidemiologist must have "mud on their boots". It is one of the dangers with using models in an operational setting that they are expected to produce accurate predictions of what is going to happen in the future. Nonlinear modelers have shifted their emphasis away from prediction towards varying different variables in order to learn about a system's critical points and its resistance to change (Briggs and Peat 1989). Prigogine and Stengers (1984) point out that individual events can easily affect the direction of the behaviour of a system at a macroscale. They refer to bifurcation regions such as the ones produced by logistic growth equations where the behaviour of one

individual can upset the global state of the system and produce unpredictable behaviour (see figure 109). Assuming average behaviour for individuals who are part of a system as is done in mathematical modelling disregards the importance of variation in effects at a microscale. Monte-Carlo models such as the one developed for this thesis are sensitive to stochastic events at a small scale. By using the results from repeated runs they can provide the decision makers with success probabilities for particular disease control strategies. Such models have the disadvantage that they are more complex and development takes longer than for mathematical models. Using both types of approaches to model a single system would combine the strengths of the approaches. Monte-Carlo techniques could be used to represent smaller-scale and mathematical modelling to represent large-scale effects.

Figure 109: Map of bifurcating attractors and underlying structure of chaos for a non-linear birthrate equation



In conclusion, the development of this model has provided valuable insights into the epidemiology of *Mycobacterium bovis* infection in possum populations. The structure of the model allows its mechanisms to be explained and discussed with its potential users without requiring advanced mathematical training. The model provides an example of an integrated approach where a epidemiological field study was used as the basis for model development. The model is unique by incorporating a strong geographical component. This allows it to be used for analysing the epidemiology of bovine tuberculosis infection in possum populations in

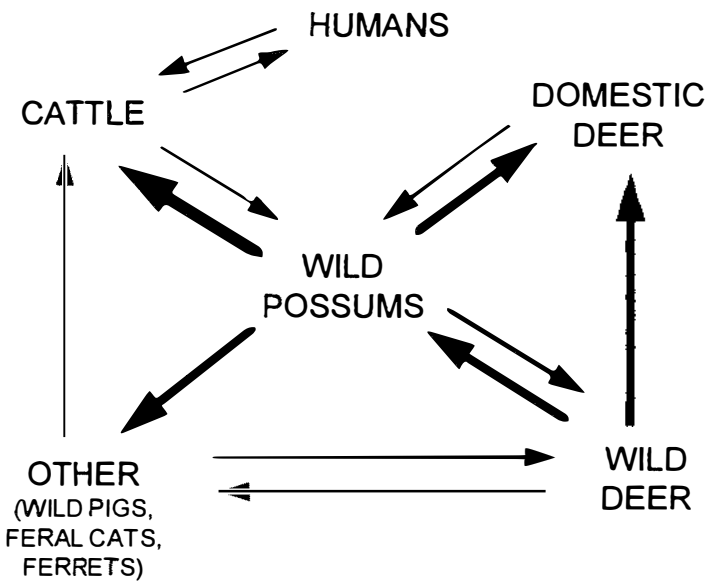
different geographical location of the country. Such models however require more resources than mathematical models since their structure is more complex, and this particular model will continue to evolve for some considerable time, taking advantage of new data emerging from current epidemiological studies. The objective of this stage of development was to formulate an initial model structure, not to complete development and validation of a full model.

BOVINE TUBERCULOSIS DISEASE CONTROL

The success of disease control strategies depends to a significant extent on an adequate understanding of the epidemiology of the disease. The wider the host spectrum of an infectious organism the more difficult it is to gain sufficient insight into its epidemiology. If the major hosts include wild animal species as maintenance hosts, the problem becomes even more challenging. In such a situation control of the disease in domestic animals, which is the primary objective of most disease control programs, cannot be achieved without controlling disease in wild animal reservoir species. Most industrialised countries which successfully eradicated diseases in domestic animals did not have densities of wild animals large enough to become a significant reservoir of infection, with limited exceptions such as badgers in the south west of England and Ireland. In many other countries, where there is a potential for wild animals being a significant reservoir of infection, economic and social reasons may have prevented disease control programs from reaching the stage where domestic animals become a spillover host of infection in wild animals, or where this becomes recognized as a residual problem, when direct transmission within the domestic population declines to a very low level. This is what occurred with tuberculosis in New Zealand. In yet other cases, such as the example of rinderpest cited earlier, it is the domestic stock which are the reservoir and the wildlife merely spillover hosts.

The epidemiology of *Mycobacterium bovis* infection in New Zealand is very complex and it now appears that over the last three decades the bovine tuberculosis disease control program has been successful in changing the cattle population from a maintenance host to a spillover host of infection from wild animal reservoir species. Figure 110 shows a systems diagram representing the importance of the different species involved in the epidemiology of bovine tuberculosis in New Zealand and transmission between species. Current understanding strongly suggests that the brushtailed possum is the major reservoir species for the disease in New Zealand. Cattle, feral pigs, feral and wild carnivores can be considered spillover hosts. The importance of domestic and wild deer is not yet clear, and they may either be spillover hosts or low level maintenances hosts. Both feral and domestic deer could conceivably provide a source of infection for possums. This may not be a frequent event, but could be the cause for the development of new, isolated disease foci within the country.

Figure 110: Species involved in the epidemiology of *Mycobacterium bovis* in New Zealand (arrows representing direction and importance of transmission between species)



The evidence suggests that the control of bovine tuberculosis in domestic cattle can be achieved using the standard test-and-slaughter policy based on the tuberculin skin test. For domestic deer the national disease control scheme employs a skin test as well as serological tests to diagnose the disease. Both, domestic cattle and deer are part of an intensive disease surveillance scheme which allows continuous monitoring of the effectiveness of the program. Although there clearly are cases of infected animals which are not detected by the test and legal or illegal movements of infected animals, this is unlikely to be a major cause for continuing occurrence of the disease in some areas of the country. The situation is different for disease control in wild animal populations, especially the brushtailed possum. The standard technique for controlling bovine tuberculosis in possum populations is currently based on population reduction through culling. This method has shown its effectiveness through a reduction of numbers of cattle reacting to the tuberculin test following possum population control operations. However, large scale possum population control operations can only be seen as a short-term measure because they are unlikely to achieve eradication and have to be repeated periodically. Also, there is some evidence which suggests that possum population reduction may not be sufficiently effective in limiting the spread of infection between possum populations or maintaining an insignificant level of disease in the local possum population. Hence, the tools for disease control which are currently available will reduce cattle reactor numbers within areas of endemic bovine tuberculosis infection, but at substantial cost and they are unlikely to fully prevent expansion of these endemic areas. It is therefore necessary to develop new methods of control which are more effective, less costly and also more acceptable to the public and New Zealand's overseas trading partners.

Martin *et al* (1987) provide a list of the activities which can be used in preventing, controlling or eradicating disease. They include slaughter, quarantine, reduction of contact, chemical use, modification of host resistance, environment and/or management control, education and finally biological control. The areas of modification of host resistance and biological control could be applicable for the control of bovine tuberculosis in possums. Both would require a long-term research effort before they could be applied in the field. In the short-to mid-term domestic cattle and deer will have to be protected against transmission of infection from local possum populations by possum population reduction through culling, with the addition of new techniques involving education of farmers leading to management changes on individual farms and possibly to environmental control.

Strategies available in the Short-Term

There are a number of improvements to the current disease control scheme which can be made immediately. These new strategies can be targeted at changes in regional policies used by disease control authorities and at initiatives to be taken by individual farmers on their property. Regional control policies currently implemented by the Ministry of Agriculture and Fisheries do not sufficiently take account of the epidemiology of bovine tuberculosis infection in possum populations. In the light of the new findings in the different studies of this project it appears that the effectiveness of possum cull operations in reducing possum tuberculosis depends significantly on the timing, the frequency and the geographical selection of target areas for such operations. Given the seasonality of overall mortality, incidence of clinical tuberculosis and of hypothesized major transmission mechanisms (mating and pseudo-vertical transmission), in any given geographical location it could be argued in principle that possum cull operations are likely to be most effective during times when possums are ranging over large areas (as they are more likely to encounter a poison bait) and when their populations are at peak densities. This would be the case during the summer/autumn period. Also it would appear that in terms of bovine tuberculosis epidemiology it would be an advantage to reduce population density during the times of mating when social interaction between animals is at a maximum. The time of March/April would satisfy both criteria. Any control operation conducted during winter (which is currently the case) would have the disadvantage that adult, pregnant females are more likely to avoid poison baits. Also, during winter time there will be more clinically diseased possums in the population and if these animals are late in the disease process they may be less likely to take a poison bait. The issue of whether the carcases of such animals act as a source of infection for cattle or deer if they die on open pasture is also not fully resolved. In winter, contaminated carcases will take longer to decompose and bacteria will be able to survive longer than during summer.

There is a lot of scope for initiatives to be taken by farmers. Given sufficient background information on the epidemiology of the disease farmers may well know which are the areas on their property where contact with tuberculous possums is most likely to occur. It should be possible to devise management strategies adapted to the conditions of individual

farms which minimise contact between domestic animals and infected possums populations, and this can be combined with local possum population control operations targeted at the problem locations within the farm. Farmers may be prepared to adjust their domestic stock management and clear vegetation which provides den sites for possums. They could conduct local, very targeted possum kill operations using bait stations which remain permanently in the problem areas and are used during specific times of the year in consultation with epidemiological advisers from the Ministry of Agriculture and Fisheries.

Strategies available in the Medium-Term

In the medium term it may be possible to develop a vaccination strategy for possums, domestic cattle and deer. At the moment the BCG vaccine which has been used extensively in the control of human tuberculosis is being evaluated by a number of research groups for its usefulness to control bovine tuberculosis in any of the above target species. The outcome of such evaluations depends on the degree of protection which is required in these species. In domestic animals the efficacy of the vaccine would have to be considerably higher than in wild animals in order to be epidemiologically more effective than a test-and-slaughter strategy. Typically in cattle herds, reactor numbers are low which is indicative of the status of a spillover host, and a vaccine would have to be extremely effective to prevent this from happening. The test-and-slaughter strategy performs quite adequately in minimising lateral spread within herds. But in possums, tuberculosis prevalence is comparatively high and the objective of a vaccine would be to break the cycle of infection within the population -which may not require a very high protection level. Therefore, for this and other reasons it would seem most appropriate that any vaccine strategy should have the possum population as its main target.

Tuberculosis vaccination of domestic animals would require acceptance in the international community and is likely to produce responses to the tuberculin test in vaccinated but uninfected animals. Vaccination of possums could be used in combination with population reduction methods. Currently efforts are made in selected parts of New Zealand to prevent the spatial spread of infection by placing "buffers" between 3 to 5 km wide of low possum population density around the fringes of tuberculosis endemic areas. A vaccination buffer may be more effective and would not influence immigration of possums.

Large-scale oral vaccination against rabies has had considerable success in preventing the spread of rabies infection in fox populations (Brochier *et al* 1991, Winkler and Bögel 1992). In relation to the large-scale vaccine trial which had been conducted in Belgium (Brochier *et al* 1991), Anderson (1992) describes a number of problems which have to be addressed before this strategy can be widely adopted. First, mass immunization of wildlife is very costly. Second, Anderson raises some questions regarding the interpretation of the trials which have been conducted to evaluate the effects of vaccination. The design of the Belgian trial did not include a comparable control area in which vaccine baits were not distributed. It is known that rabies incidence is typically cyclical in fox populations, with an inter-epidemic

period of 3-6 years depending on fox density. If the trial happened to be carried out during a period of low incidence, the results would be quite misleading. Anderson also points out that the level of vaccination coverage achieved in the Belgian trial was in accord with the level required to block transmission in a fox population of a given density according to epidemiological theory. He concludes that above all the development of a method for disease control not involving slaughter of wildlife would be only too welcome in the conservation-conscious climate of the 1990s.

Strategies available in the Long-Term

There are a number of control options which may provide the answer to the bovine tuberculosis problem in New Zealand but would require a medium- to long-term research effort. Vaccination could be one of these methods, either using BCG or development of a new and superior vaccine. A major disadvantage of vaccination of possums is that it does not help solving the immense problems these animals are causing to native flora and fauna. Biological population control through introduction of natural enemies would provide a solution to both problems, bovine tuberculosis and environmental damage. The requirements for a suitable biological control agent are that it has to be host specific and it should pose no threat to the native flora and fauna of the country of introduction, particularly to its endangered species (Waage and Greathead 1988). Biological control of rabbits through introduction of the Myxomavirus causing myxomatosis was initially considered successful in Australia, but changes in the virus and other factors have led to a rebuilding of rabbit populations, and myxomatosis is no longer effective as a control method. In New Zealand this method was recently rejected as an option for rabbit control.

Another method for population control which has received a lot of publicity is fertility control. Bomford (1990) conducted a major review on the role of fertility control in wildlife management. She came to the conclusion that currently no method for effective fertility control in wildlife management is available. Bomford provides the following list of research directions which show promise of pay-off in the future: the development of delivery techniques for drugs that control fertility, the development of drugs which cause permanent, humane, non-toxic sterility in both sexes of target wildlife species; investigation into fertility control to prevent recovery growth of populations reduced by other means; and investigation into the development of genetically engineered viruses to spread sterility-inducing agents through pest populations. She suggests that methods which require continuous or repeated oral dosing over extended periods, which involve surgical implantation and which only affect males show little or no promise for the regulation of abundant or widespread wildlife.

In evaluating any of the above methods it will be necessary to assess their effects not just on the individual in terms of its susceptibility to infection or its reproductive capacity. It will be at least as important to test the effect of the technique on the population dynamics of the target wildlife species. Hone (1992) assessed the potential of fertility control on population dynamics using mathematical methods. He came to the conclusion that

effectiveness declines if a reduction in fecundity increases survivorship and that fertility control of pests may be most effective in a pest population previously reduced in abundance. Caughley *et al* (1992) point out that a knowledge of the social structure and mating system of the host species is important before an attempt is made to suppress female fertility.

It appears that a long-term research effort should be directed towards a method of fertility control in possums. There are a number of options which will have to be evaluated. They include the use of chemosterilants causing permanent or temporary sterility in either sex, or alter the fertility of offspring produced. Another method which has been discussed is immuno-contraception.

DIRECTIONS FOR FUTURE RESEARCH

The studies which are part of this project provide an indication for future directions of research. The results of the longitudinal study suggest that more information is required on the behavioural interaction between possums and between possums and domestic animals. Possum tuberculosis epidemiology can be divided in two main research areas, the dynamics of infection within a local population and the spatial dynamics of infection between local populations.

The longitudinal study has focused on the epidemiology of bovine tuberculosis in population with endemic infection. The results of the study provide an overview of the epidemiology and suggest a number of areas which require further research. More detailed knowledge on the social interaction between possums during the mating period is required. Some of the questions which would have to be answered include how long do male and female mating partners stay together, do they form a consort relationship and is there significant aggressive interaction between males during mating time. Currently no information about interaction in relation to den sites and surrounding areas is available.

The cross-sectional study provided a crude impression of the dynamics of *Mycobacterium bovis* on a larger scale. It did only provide limited data on the spatial dynamics of infection between local populations. The influence of habitat on a larger scale on infection dynamics will have to be investigated. The importance of patch dynamics on the epidemiology of bovine tuberculosis in possums should be assessed. Rather than looking at the effect of individual local habitat patches, their locational relationship within a mosaic of habitat patches - their sizes, shapes, arrangement and connectedness - may be an important factor in the spatial dynamics of *Mycobacterium bovis* infection. In the case of rabies in fox populations it has been found that the heterogeneity of patchiness influenced the persistence of rabies (Tinline 1988).

The modelling approach provides a powerful tool for the longer term both for optimising use of existing control methods, and for evaluating the expected benefits of new ones. Some of the control options outlined above require careful evaluation within the model,

to determine whether they perform as well in the model where feedback loops and other interactions can be represented, as they might be expected to do from general principles.

SYNTHESIS

This project provides an example for an epidemiological approach to studying the epidemiology of an infectious disease which is endemic in a wildlife population. Data collection and analysis techniques which are used by animal ecologists have been applied for both, the cross-sectional and longitudinal field study. The complexity of the data which had to be analysed required the use of a wide range of statistical analysis techniques including survival analysis, path analysis and time-series analysis. The spatial aspects of disease occurrence were investigated using geographical information systems and a range of statistical techniques for spatial analysis.

The results from this study provide a basis for further investigations into the epidemiology of *Mycobacterium bovis* in New Zealand. The study concentrated mainly on the dynamics of tuberculosis infection within feral populations of Australian brushtail possums. Current understanding suggests that this species is of principal importance in the epidemiology of *Mycobacterium bovis* in New Zealand. Further investigations will be required to determine the importance of other wildlife species such as deer, pigs and small carnivores, and to evaluate the expected benefits of potential new control options both in the simulation model and in the field.

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APPENDIX

APPENDIX I: FORM FOR RECORDING OF DATA COLLECTED DURING CLINICAL EXAMINATION OF POSSUMS

POSSUM CARD

DATE ☐☐ ☐☐ ☐☐ OBSERVER ☐☐☐☐☐☐☐☐

POSSUM No. ☐ ☐☐☐☐

TRAP No. ☐☐☐☐ Cage (M,D,E) ☐

TATTOO ☐☐☐ EAR NOTCHED ☐

COLOUR: BLack,BrownBlack,BRown,GreyBrown,GRey ☐☐

WEIGHT (kg) ☐☐

LENGTH (cm) Tail ☐☐ Total ☐☐

CONDITION: Good, Average, Poor ☐

Male, Female ☐ Mature, Immature ☐

TESTIS WIDTH (mm) ☐☐

POUCH YOUNG: Present, Absent ☐ Lactating (Yes/No) ☐

Tag No. ☐☐☐☐ Sex ☐

Head length (mm) ☐☐ Weight (g) ☐☐☐

PALPATION: (+, −) ☐

ANAESTHETIC: Ketamin ☐☐ ml Other: ☐☐ ml
(specify)

BLOOD: + o − ☐ ☐☐ ml

TOOTH WEAR CLASS: (based on upper left first molar)

COMMENT:

APPENDIX II: FORM FOR RECORDING TRAP CATCH DATA

OBSERVER:

TRAP-CATCH-CARD I

Trap No.	Date M					

CODES:

Catch method: (column M) MAF-cage -> "M", DSIR-cage-> "D", DSIR2-cage -> "E"

Catch result:
- Possum -> Possum Tag No. + "R" for recaptures + "N" for new
- Non-target -> "N" + species
- Trap sprung -> "S"
- Bait gone -> "B"
- Other -> "O"
- Undisturbed -> "/"

POSSUM POST MORTEM CARD

DATE ☐☐ ☐☐ ☐☐ OBSERVER

POSSUM No. ☐ ☐☐☐☐

LOCATION (trap in vicinity or other)

CAPTURE (C), FOUND (F) ☐

Male, Female ☐ Mature, Immature ☐

POUCH YOUNG: Present, Absent ☐ Tag No. ☐ ☐☐☐

Head length (mm) ☐☐ Weight (g) ☐☐☐

LENGTH (cm) Tail ☐☐ Total ☐☐

WEIGHT (kg) ☐☐

TESTIS WIDTH (mm) ☐☐

COLOUR: BLack,BrownBlack,BRown,GreyBrown,GRey ☐☐

CONDITION: Good, Average, Poor ☐

CAUSE OF DEATH: Natural, Euthanasia ☐

CONDITION OF CARCASS (FResh,BLoated,DEcay,DRy) ☐☐

TOOTH WEAR CLASS: (based on upper left first molar)

1 2 3 4 5 6 7

RIGHT MANDIBLE (and upper left first molar) Yes/No ☐

POST MORTEM OBSERVATIONS: not possible ☐

n.p.f. ☐ TB suspect lesions ☐ other ☐

SAMPLES TAKEN Yes/No ☐ Specify under comments

COMMENTS:

DETAILS ON POST MORTEM FINDINGS

1. EXTERNAL EXAMINATION
   Open lesions present? Yes/No ☐
   Describe:

2. ABDOMEN
   Renal Lnn.:
   Mesenterical Lnn.:
   Liver:
   Spleen:
   Kidney:
   Other:

3. THORAX
   Mediastinal Lnn.:
   Lung:

4. RETROPHARYNGEAL LNN.:

5. SUPERFICIAL LNN.:
   Axillary Lnn.:
   Inguinal Lnn.:

CODES:

0 = no pathological findings

Size of lesions
1 = few miliary lesions
2 = many 1 – 2 mm lesions
3 = up to 5 mm lesions
4 = more than 5 mm lesions

Consistency
F = fluid, mucous
C = caseous

Position of organ
L = left
R = right
B = both

Colour
Y = yellowish
G = greenish

APPENDIX IV: FORM FOR RECORDING OF DEN SITE TRACKING DATA

POSSUM DEN SITES

Observer:
Date:

Possum Tag	Radio Transmitter	Code Den Site	Trap Site in Proximity	Description of Trap Site

Comments:

APPENDIX V: QUESTIONNAIRE FOR CASE FARMS INCLUDED IN TUBERCULOSIS CASE-CONTROL STUDY

QUESTIONNAIRE FOR WAIKATO-WEST TUBERCULOSIS CASE-CONTROL STUDY

PROJECT MAFQual - MASSEY UNIVERSITY

VERSION FOR CASE HERDS

QUESTIONNAIRE NO. [ ]

General Information

1. Date of interview: _____
2. Name of Interviewer: _____
3. Name of interviewee:
   (note: where possible this should be the person directly managing the herd at the time of TB breakdown)

   _____

4. Address and location of farm:

   _____

   _____

5. Address of interviewee (*if different from farm address*):

   _____

   _____

   Telephone No.:_____

6. Name of **farm** owner (if not interviewee):

   _____

7. Address of farm owner (*if different from farm address*):

   _____

   _____

8. Name of **herd** owner (if not interviewee):

   _____

9. Address of herd owner (*if different from farm address*):

   _____

   _____

Farm Specific Information

We would now like to get some general information about your farm. We are interested in the situation at the time when the TB breakdown occurred.

10. What type of operation was your farm at the time of the breakdown?
(if more than one answer, give approximate percentage of **total** farm income)

[ ] cattle _____
[ ] sheep _____
[ ] deer _____
[ ] goats _____
[ ] pigs _____
[ ] other (specify: _____)

11. What type of operation was the cattle related part of your farm?
(if more than one answer, give approximate percentage of **total** farm income)

[ ] dairy _____
  [ ] factory supply _____
  [ ] town supply _____
[ ] beef _____
  [ ] breeding _____
  [ ] dry stock - fattening _____
  [ ] dry stock - dairy type _____
[ ] other (specify: _____)

12. Did the ownership of the **farm** change during the 5 years prior to the date of the breakdown?

[ ] no
[ ] yes (specify how many times: _____)

13. Did the ownership of the **herd** change during the 5 years prior to the date of the breakdown?

[ ] no
[ ] yes (specify how many times: _____)

14. Did the management system of the farm change in a major way during the 5 years prior to the date of the breakdown?

[ ] no
[ ] yes (specify: _____)

15. What was the size of the farm at the time of the breakdown?
(specify vegetation areas in hectares)

Total farm size _____

crops _____ (specify type)
forest _____
willows _____
scrub/bush _____
gorse _____
water _____
pasture _____
non- grazeable area (not included above) _____

other, specify: _____

16. How would you describe the predominant topography of your farm?

[ ] flat
[ ] rolling
[ ] steep

17. Do you have any of the listed surface waters on your farm?
(if possible, give rough estimate of length or area; include boundary)

[ ] river/canal_____
[ ] creek _____
[ ] lake _____
[ ] swamp _____
[ ] open drain_____
[ ] flood prone area _____

18. What is the average rainfall per year on your farm? _____

General Information on Interviewee

We would now like to ask you some questions about your background and working situation.

19. Sex:

[ ] male
[ ] female

20. In what year were you born? _____
21. What is your ethnic background? _____
22. Do you live on the farm?

[ ] no
[ ] yes

23. Do you have any other employment commitments?

[ ] no

[ ] yes (specify: _____)

24. Can you cover your living expenses from your farm income?

[ ] no

[ ] yes

25. What is your relation to the property?

[ ] owner

[ ] share milker

[ ] 50%

[ ] 39%

[ ] 29%

[ ] manager

[ ] lessee

[ ] other (specify: _____)

If interviewee is the owner, go to question no. 27.

26. What is your relation to the owner?

[ ] parent

[ ] relative

[ ] none of the above

27. Which persons are involved in decisions concerning herd management?

[ ] only interviewee

[ ] others (specify: _____)

If interviewee is the owner, go to question no.31.

28. How long have you been working on this farm? _____

29. Does the owner live on the farm?

[ ] no

[ ] yes

30. How frequent is contact between the owner and you?

[ ] daily

[ ] at least once a week

[ ] at least once month

[ ] less than once a month

31. How many years have you worked in farming? _____

32. How many years have you worked in non-farm jobs? _____

(note: total of question 31 and 32 should equal years of work)

33. What is your farming background?

[ ] started farm job without having any farming background

[ ] brought up on farm

[ ] other (specify: _____)

34. What level of formal education do you have?

[ ] lower than school certificate

[ ] school certificate

[ ] university entrance

[ ] technical institute

[ ] university

35. What kind of farm-specific qualification do you have?

[ ] none

[ ] technical agricultural institute

[ ] agricultural diploma

[ ] bachelor of agriculture

[ ] other (specify: _____)

36. Where do you seek advice concerning the herd management on your farm?
(give ratings in order of importance)

[ ] none

[ ] family, relatives

[ ] owner or other workers on farm

[ ] neighbours

[ ] farm discussion group

[ ] stock agent

[ ] salesmen

[ ] MAF consultant

[ ] private consultant

[ ] veterinarian

[ ] magazines

[ ] books

[ ] other (specify: _____)

37. How many farm workers do you have on the farm?
(give approximate labour units according to the following categories including yourself)

[ ] none

[ ] permanent

[ ] temporary

[ ] family

38. What kind of aids do you use to muster your animals?

[ ] none
[ ] motor bike
[ ] tractor
[ ] 4 wheel drive vehicle
[ ] dog
[ ] horse
[ ] other (specify: _____)

39. Have any capital investments been made on your farm during last two years?

[ ] none
[ ] minor (< $ 5000)
[ ] major

General Stock Information

We would now like to get some general information on your stock especially numbers, purchases, sales and agistments. We are interested in the situation at the present time.

40. Do you have any animals in the following categories?
(give approximate numbers)

[ ] dairy cattle

weaners _____
yearlings _____
cows _____
bulls _____

[ ] beef cattle

heifers _____
cows _____
steers _____
bulls _____

[ ] deer

yearlings _____
hinds _____
stags _____

[ ] sheep (excluding lambs) _____

[ ] goats (excluding kids) _____

[ ] pigs (excluding unweaned piglets) _____

[ ] others, specify: _____

41. Have you introduced any animals on to your farm during the last 2 years?
(if not, go to question no.52)

[ ] yes

[ ] no

[ ] do not know

42. How many animals of the following stock categories have you bought during the last two years?

sheep _____

goats _____

pigs _____

others, specify: _____

43. Have you bought cattle during the last two years?
(if not, go to question no.48)

[ ] yes

[ ] no

[ ] do not know

44. What type of cattle did you buy in the last 2 years?
(give approximate numbers)

[ ] bobby calves

[ ] weaners

[ ] heifers

[ ] cows

[ ] bulls

[ ] steers

[ ] do not know

45. From how many different herds have you bought cattle during the last 2 years?

[ ] 1 - 3 herds

[ ] more than 3 herds

46. What are your main reasons for deciding to buy cattle?
(give order of priority)

[ ] for trading purposes

[ ] need for breeding herd replacements

[ ] improvement of herd

[ ] breeding bull

[ ] increase herd size

[ ] other (specify: _____)

[ ] do not know

47. Where have you bought cattle?
(give rating according to importance)

[ ] sale yards
[ ] through stock agent
[ ] private
[ ] clearing sale
[ ] other (specify: _____)
[ ] do not know

48. Have you bought or captured deer during the last two years?
(if not, go to question no.52)

[ ] yes
[ ] no
[ ] do not know

49. Where have you bought or captured deer?
(give rating according to importance)

[ ] sale yards
[ ] through stock agent
[ ] private
[ ] bush capture (specify location: _____)
[ ] other (specify: _____)
[ ] do not know

50. From how many properties have you bought or captured deer during the last two years?

_____

51. What type of deer have you bought or captured?
(give numbers)

[ ] fawns _____
[ ] hinds _____
[ ] stags _____
[ ] do not know

52. What measures do you take to avoid the introduction of infectious diseases through purchased stock?
(if more than one answer, give order of priority)

[ ] none
[ ] do not buy
[ ] buy only from safe farms
[ ] buy only from farms after checking status with MAF
[ ] keep new stock separate
[ ] prior examination by veterinarian
[ ] get animals tested for (specify: _____)
[ ] other (specify: _____)

53. Have you grazed or leased any cattle belonging to other farmers on your farm during the last two years?
(if not, go to question no.58)

[ ] yes
[ ] no
[ ] do not know

54. From how many herds did you keep cattle belonging to other farmers on your farm during the last two years? _____

55. What type of cattle belonging to other farmers did you keep?

[ ] weaners
[ ] heifers
[ ] cows
[ ] bulls
[ ] steers
[ ] do not know

56. Where did the cattle belonging to other farmers come from?
Specify location:

_____

57. Did you require any animal health tests prior to accepting the cattle?

[ ] yes (specify: _____)
[ ] no

58. Have you grazed or leased any deer belonging to other farmers on your farm during the last two years?
(if not, go to question no.62)

[ ] yes
[ ] no
[ ] do not know

59. From how many herds did you keep groups of deer belonging to other farmers on your farm during the last two years? _____

60. Where did the deer belonging to other farmers come from?
Specify location:

_____

61. Did you require any animal health tests prior to accepting the deer?

[ ] yes (specify: _____)
[ ] no

Stock Management Information

We would now like to get some information about your methods for handling your stock. The questions are intended to relate to the time of the TB breakdown.

The first group of questions will be dealing with grazing management and the situation on the paddocks.

62. What system of cattle grazing management did you use?
(if more than one answer, give order of priority and reason)

[ ] rotational grazing _____
[ ] strip/block grazing _____
[ ] set stocking _____
[ ] open gate _____
[ ] wintering pad/sacrifice paddock _____
[ ] other (specify: _____)

63. What kind of fertilizer did you apply? (give order of priority and frequency of application per year)

[ ] super phosphate _____
[ ] lime _____
[ ] organic fertilizer _____
[ ] nitrogen _____
[ ] other (specify: _____)
[ ] none
[ ] do not know

64. Did stock have access to the following water sources?
(give order of priority)

[ ] river
[ ] creek
[ ] lake
[ ] pond
[ ] dam
[ ] bore/well
[ ] other (specify: _____)

65. Did you have problems with water supply for your stock during any period of the year?

[ ] yes (give reasons: _____)
[ ] no

66. Did you have a regular problem with flooding of pasture?
(if not, go to question no.68)

[ ] yes
[ ] no

67. Give a rough estimate of hectares of pasture that was most likely to be affected by floods:

_____

68. Did you have difficulties in achieving complete herd mustering?

[ ] yes (give reasons: _____)

[ ] no

69. How often did stock from neighbouring farms break into your property? (specify species)

[ ] at least once or twice a month _____

[ ] at least once or twice a year _____

[ ] rarely, if ever _____

70. How quickly would you have moved neighbour's stock out?

[ ] same day

[ ] same week

[ ] later

71. Did your cattle have access to bush or forest?
(if not, go to question no.74)

[ ] yes

[ ] no

72. Where was this area?

[ ] on farm

[ ] off farm

73. For what reasons did you graze your cattle in bush or forest?

[ ] part of paddock

[ ] difficult to prevent

[ ] additional food supply when required

[ ] other (specify: _____)

74. What type of fencing system did you use?
(give order of priority, code **B** for farm boundaries and **O** otherwise)

[ ] permanent electric fences _____

[ ] wire and batten fences _____

[ ] barbed wire fences _____

[ ] hedges _____

[ ] movable electric fences _____

[ ] other (specify: _____)

75. If you found that your fences were not stock proof, when did you fix them?

[ ] same day

[ ] within same week

[ ] later

76. Have you cleared any paddocks from bush/forest in the last 5 years?
(if not, go to question no. 78)

[ ] yes
[ ] no
[ ] do not know

77. What cover or debris remained after clearing?
(give order of importance)

[ ] trees
[ ] logs
[ ] bush or scrub
[ ] uncleared gullies
[ ] pasture only
[ ] other (specify: _____)

78. Where did your cattle graze during the 2 years before TB breakdown?
(give order of importance)

[ ] main farm
[ ] run-off
[ ] graze on other farm
[ ] graze in forest/bush off farm
[ ] other, specify: _____

79. Did you graze the road frontage?

[ ] frequently
[ ] rarely
[ ] never

If no run-off was used or cattle were not grazed on other farm, go to question no.84.

80. What class of cattle grazed on run-off or other farm during the 2 years before TB breakdown?

[ ] weaners
[ ] yearlings
[ ] hold-over dry cows
[ ] steers
[ ] others (specify: _____)

81. What was the location of run-off or other farm?

_____

82. Did stock grazing on the run-off or other farm have the chance for frequent contact with cattle or deer belonging to other farmers?

[ ] yes (specify species: _____)
[ ] no
[ ] do not know

83. Describe the run-off or other farm in terms of the size of the following types of cover (in hectares):

Total size _____

crops _____
forest _____
willows _____
scrub _____
gorse _____
water _____
pasture _____
other non-grazeable area _____

[ ] do not know

84. Did you keep permanent separate mobs of animals in your cattle or deer herd?

[ ] yes
[ ] no

85. Did you graze different species in the same paddock?

[ ] yes, specify:

_____

_____

[ ] no

The next group of questions is related to herd management. It is divided into sub groups for the different types of cattle operations.

86. What were your reasons for culling cattle in order of importance?

[ ] age
[ ] health problems
[ ] fertility/empty cow
[ ] production
[ ] other (specify: _____)
[ ] do not cull

87. Where did you get your replacements for your cattle herd from in order of priority?

[ ] purchase
[ ] lease
[ ] rearing

88. What kind of individual animal records did you keep?

[ ] diary/notebook
[ ] individual record cards for each animal
[ ] computerized herd records (e.g. Livestock Improvement Association)
[ ] other (specify: _____)
[ ] none

89. Did you identify your cattle individually?
(if not, go to question no.91)

[ ] yes
[ ] no

90. What was your method of identification?

[ ] eartags
[ ] earmarks
[ ] hide brand
[ ] tattoo
[ ] other (specify: _____)

If farmer did not keep fattening cattle, go to question no.92.

91. What method did you use for assessing weight gain in your fattening cattle?

[ ] scale
[ ] weighband
[ ] condition scoring (numerical)
[ ] eye appraisal
[ ] other (specify: _____)

If farmer did not keep dairy or beef breeding cattle, go to question no.100.

92. What kind of breeding information did you record regularly?

[ ] calving dates
[ ] dates on heat
[ ] mating dates
[ ] pregnancy diagnosis
[ ] production
[ ] animal health
[ ] other (specify: _____)

93. Which of the listed breeding methods did you use?

[ ] only artificial insemination
[ ] artificial insemination followed by natural breeding for clean-up
[ ] natural breeding

94. If a beef herd, what was the calving rate during 1987? _____

95. What method did you use for calf rearing?
(number in order of importance)

[ ] suckle mother
[ ] suckle cows other than mother
[ ] bulk milk (from herd)
[ ] milk replacer
[ ] other (specify: _____)
[ ] do not rear calves

96. Did you provide any supplementary feeding to your cattle?

[ ] hay
[ ] silage
[ ] crops (specify according to following categories)

[ ] lucerne
[ ] concentrates
[ ] others (specify: _____)

97. Did you buy any hay during the last 2 years?

[ ] yes (specify location: _____)
[ ] no

If farmer did not keep dairy cattle, go to question no.100.

98. Did you record milk production?

[ ] yes
[ ] no

99. Which of the following methods did you use for mastitis control?
(if applicable, tick more than one)

[ ] none
[ ] teat spray
[ ] Individual Cow Somatic Cell Counts
[ ] dry cow therapy
[ ] other (specify: _____)

100. What method did you use for cattle effluent disposal?

[ ] nothing
[ ] settlement pond
[ ] spray on pasture
[ ] other (specify: _____)

The following questions are concerned with the animal health situation and management of your herd.

101. What are the four most important animal health problems in cattle and/or deer on your farm? (list in order of importance)

1. _____
2. _____
3. _____
4. _____

102. Do you drench your cattle herd regularly and why? (give order of importance)

[ ] no
[ ] bloat
[ ] facial eczema
[ ] hypomagnesaemia
[ ] internal parasites
[ ] others (specify: _____)

103. What do you vaccinate your cattle for?

[ ] leptospirosis
[ ] blackleg/clostridial diseases
[ ] salmonellosis
[ ] Johne's disease
[ ] other (specify: _____)
[ ] no vaccination

104. How often does a private veterinarian visit your farm during calving and mating season?

[ ] about once a week
[ ] about once a month
[ ] about once every 2-3 months
[ ] rarely

105. Do you consider any animal diseases when purchasing or leasing animals?

[ ] yes (specify: _____)
[ ] no
[ ] do not know

Tuberculosis Information

In this section we would like to ask you some questions seeking your opinion about and experiences with tuberculosis and tuberculosis control.

106. Do you think New Zealand should be willing to allow tuberculosis to be present permanently in the country, provided it is not on your farm? (give reasons)

[ ] yes _____

[ ] no _____

[ ] do not know

107. Would you be willing to allow TB to be present permanently on your farm? (give reasons)

[ ] yes _____

[ ] no _____

[ ] do not know

108. What methods does MAF use to control bovine TB?

1. _____
2. _____
3. _____
4. _____

[ ] do not know

109. Do you believe that overall these methods are effective?

[ ] yes, give reasons:

_____

_____

_____

[ ] no, give reasons:

_____

_____

_____

[ ] do not know

110. What do you think are the main reasons why tuberculosis has not been fully controlled in New Zealand?

1. _____
2. _____
3. _____
4. _____

[ ] do not know

111. If you were in charge of TB control, what approach would you use to control the disease?

1. _____
2. _____
3. _____
4. _____

[ ] do not know

112. What do you consider an appropriate percentage of animal value to use in compensating owners for TB infected cattle that are slaughtered?

If the government pays: _____

If farmer funds must be used: _____

[ ] do not know

113. Do you think that you personally can do anything to keep your herd clear from TB?

[ ] yes, specify:

_____

_____

_____

[ ] no, give reasons:

_____

_____

[ ] do not know

114. Does tuberculosis affect any other species than cattle?

[ ] yes, specify:

_____

_____

[ ] no

[ ] do not know

115. Could you specify how the disease is spread from cattle to humans?

[ ] yes, specify in order of importance:

1. _____
2. _____
3. _____
4. _____
5. _____

[ ] no

116. Do you think you are working in a situation where you could catch the disease from your cattle, if they were infected?

[ ] yes, specify:

_____

_____

[ ] no

[ ] do not know

117. What do you think are the possible means for introduction of TB into a cattle herd? (give order of importance)

1. _____
2. _____
3. _____
4. _____
5. _____

[ ] do not know

118. Could you describe how the disease is spread between cattle?

[ ] yes, specify in order of importance:

1. _____
2. _____
3. _____
4. _____
5. _____

[ ] no

119. Are any wild animals important in the spread of TB?

[ ] yes, specify in order of importance:

_____

_____

[ ] no

[ ] do not know

120. What is the degree of contact between the following **wild** animals and your cattle? (code answer: 0=no, 1=possible, 2=very likely, 9=do not know)

[ ] possums

[ ] wild pigs

[ ] wild deer

[ ] wild cattle

[ ] wild goats

[ ] others (specify: _____)

121. Have there been TB infected wild or domesticated species identified in the neighbourhood of your farm during the last 5 years?
(if not, go to question no.123)

[ ] yes, specify species and location:

_____

_____

[ ] no

[ ] do not know

122. If yes, did you take any precautions for your herd?

[ ] yes, specify:

_____

_____

[ ] no, give reasons:

_____

_____

123. Has there been any possum control on your farm over the last 5 years?
(if not, go to question no.129)

[ ] yes (specify the last time: _____)

[ ] no

[ ] do not know

124. Why has possum control been done?

specify:

_____

_____

[ ] do not know

125. Who did the possum control?

[ ] you

[ ] friend, relative

[ ] commercial trapper

[ ] part time trapper

[ ] MAF

[ ] pest destruction board

[ ] local/county ranger

[ ] do not know

126. What methods have been used?

[ ] 1080 poisoning
[ ] cyanide poisoning
[ ] shooting
[ ] trapping
[ ] other (specify: _____)
[ ] do not know

127. How many times has it been done during the last 5 years? _____

128. Has any **major** possum control operation been done before TB breakdown?

[ ] yes (specify when: _____)
[ ] no
[ ] do not know

129. What are your views about the use of **large scale** possum control operations for TB?

_____
_____
_____
_____

130. Which particular form of possum control to you consider best?

_____
_____
_____

[ ] do not know

131. Do you have any other comments on TB control in general, or in relation to your farm?

_____
_____
_____
_____

Farmer's self concept

132. This is the final question and here we would like you to give us an assessment of your personal characteristics. For this purpose please try to describe yourself on the scale in relation to the following characteristics.

Not easy going	[ ] [ ] [ ] [ ] [ ]	Easy going
Meek	[ ] [ ] [ ] [ ] [ ]	Not meek
Patient	[ ] [ ] [ ] [ ] [ ]	Impatient
Unsociable	[ ] [ ] [ ] [ ] [ ]	Sociable
Not modest	[ ] [ ] [ ] [ ] [ ]	Modest
Persevering	[ ] [ ] [ ] [ ] [ ]	Giving up Easily
A worrier	[ ] [ ] [ ] [ ] [ ]	Not a worrier
Cheerful	[ ] [ ] [ ] [ ] [ ]	Grumpy
Talkative	[ ] [ ] [ ] [ ] [ ]	Not talkative
One who speaks one's mind	[ ] [ ] [ ] [ ] [ ]	One who keeps quiet
Difficult to get on with	[ ] [ ] [ ] [ ] [ ]	Easy to get on with
Lacking confidence	[ ] [ ] [ ] [ ] [ ]	Confident
Liking change	[ ] [ ] [ ] [ ] [ ]	Suspicious of change
Forceful	[ ] [ ] [ ] [ ] [ ]	Giving in easily
One who prefers machinery	[ ] [ ] [ ] [ ] [ ]	One who prefers cows
One who prefers buying a new machine	[ ] [ ] [ ] [ ] [ ]	One who prefers choosing a new bull
Unwilling to learn	[ ] [ ] [ ] [ ] [ ]	Very willing to learn
Still learning	[ ] [ ] [ ] [ ] [ ]	Very knowledgeable
One who likes to avoid hard work	[ ] [ ] [ ] [ ] [ ]	One who values hard work
One who dislikes using records	[ ] [ ] [ ] [ ] [ ]	One who likes using records
One who values traditional ways	[ ] [ ] [ ] [ ] [ ]	One who likes adopting new ideas
One who does not like to set targets	[ ] [ ] [ ] [ ] [ ]	One who likes setting targets for him/herself
One who likes to look after his/her favourite animals a bit better than the rest	[ ] [ ] [ ] [ ] [ ]	One who likes to strictly monitor performance of the herd

MAF Tuberculosis Test Data

The following information will be available through MAF.

133. Record Type: _____
134. Report number: _____
135. Herd number: _____
136. County: _____
137. Livestock officer area: _____
138. Case Control Status:

    [ ] Case

    [ ] Management control

    [ ] Random control

139. TB possum risk area of farm location:

    [ ] Endemic

    [ ] Fringe

    [ ] Non-endemic

    [ ] Surveillance

140. Approximate elevation above sea level of farm:

    _____

141. Date of herd establishment

    cattle _____

    deer _____

142. Date of herd accreditation

    cattle _____

    deer _____

143. Date of TB breakdown: _____
144. Results of last four herd tests for cattle and deer:

SPECIES	DATE	TYPE OF TEST	NO ANIMALS TESTED	NO OF CF/CC REACTORS	PM RESULT	CULTURE

145. Have there been difficulties in complete herd mustering? _____
146. TB possum risk area of location given in answer to question no.56:

    _____

    _____

147. TB possum risk area of location given in answer to question no.60:

    _____

148. What is the TB test status of the herd where the introduced deer originated? (refers to question no.60)

149. TB possum risk area of location given in answer to question no.81:

150. TB possum risk area of location in answer to question no.97:

151. MAF information to question no.121:

152. MAF information about major possum control operations including location of farm: (refers to question no.128)

153. Comments of the interviewer:

Please attach copy of MAF assessment of TB breakdown investigation.

APPENDIX VI: TURBO PASCAL FOR WINDOWS PROGRAM CODE FOR SIMULATION MODEL

```
program PossumPopulation;
{$D+}{$L+}{$R tbmodel.res}{$G+}{$N+}{$F-}{$B-}{$W-}{$A+}{$S-}{$I-}{$V-}{$X+}
uses
  WObjects, WinProcs, WinCrt, WinDos, WinTypes, OPString, Strings, ListObj, OpDate, Ultrafx;
const
  Male     = 1;
  Female   = 0;
  MaxAge   = 6;
  pHealthy  = 0;
  pInfected = 1;
  pClinical = 2;
  m = 2147483647;
  WindowSize: TPoint = (x:400;y:200);}
var
  DailyImmigrMale, DailyImmigrFemale  : real;
  ControlInterval, ControlPeriod, NextControl, BackToNormal, code : integer;
  SplashRect: TRect;
  FirstSeed : longint;
  SurvAdultSeed, SurvImmatureSeed, MatingSeed, TBSurvAdultSeed, TBSurvImmSeed, TBImmigrSeed, SubToClinSeed,
  PopDensEmigrSeed, BirthSexSeed, TBPrevSeed, JoeySurvSeed, TBDenSeed, TBBufferSeed, DenRejectSeed, TBMatingSeed,
  AgeIndepSeed1, AgeIndepSeed2, AgeMatureSeed1, AgeMatureSeed2, DailyImmigrMaleSeed, DailyImmigrFemaleSeed,
  SurvAdultSeedOrg, SurvImmatureSeedOrg, MatingSeedOrg, TBSurvAdultSeedOrg, TBSurvImmSeedOrg, TBImmigrSeedOrg,
  SubToClinSeedOrg, PopDensEmigrSeedOrg, BirthSexSeedOrg, TBPrevSeedOrg, JoeySurvSeedOrg, TBDenSeedOrg,
  TBBufferSeedOrg, DenRejectSeedOrg, TBMatingSeedOrg, AgeIndepSeed1Org, AgeIndepSeed2Org, AgeMatureSeed1Org,
  AgeMatureSeed2Org, DailyImmigrMaleSeedOrg, DailyImmigrFemaleSeedOrg  : extended;
  DenSummaryFile, NonDenSummaryFile: text;
  PopFile,DenMapFile,ControlFile,ParamFile, YorN : string;
  PossumControl : boolean;
type
  SexType = byte;
  LocationType = longint;
TMyApplication = object(TApplication)
FirstApp : Boolean;

procedure InitMainWindow; virtual;
end;

PMyWindow = ^TMyWindow;
TMyWindow = object(TWindow)
  MainFontRec : TLogFont;
  TheDC : HDC;
  procedure Paint(PaintDC : HDC; var PaintInfo: TPaintStruct);virtual;
  procedure MakeFont; virtual;
  constructor Init(AParent: PWindowsObject; ATitle: PChar);
  procedure GetWindowClass(var WndClass : TWndClass); virtual;
  procedure About(var Msg:TMessage); virtual cm_First + 101;
  procedure RunModel(var Msg : TMessage); virtual cm_First + 201;
end;

ModelObj = object
ParamFile     : string;
AdultSurvival : array [1..12, Female..Male] of real;
ImmatureSurvival : array[1..12, Female..Male] of real;
JoeySurvival  : array [0..8] of real;
Immigration   : array [1..12, Female..Male] of integer;
MatingProb    : array [1..12] of real;
MaleBirthProb : real;
MatingBuffer  : integer; {Radius around female where to select mate from}
MatingTBProb  : real;
BackgroundTBProb  : array [1..12] of real;
ImmigrantTBProb   : array [1..12] of real;
ClinicalAdultSurvival : array [1..12] of real;
ClinicalImmatureSurvival : array [1..12] of real;
GoClinicalProb : array [1..12] of real;
DenTBPeriod : integer;  {Number of days TB remains active in den}
DenTBProb : real;       {Probability of possum catching TB in a clinical den}
DenTBBuffer : integer;  {Radius of TB active buffer zone around clinical den}
```

```
BufferTBProb : real ;  {Probability of catching TB in the buffer zone}
MaxDenTravel  : integer;
DenRejectProb  : real;
UseDenMemory  : boolean;
ResidentDenThreshold  : integer;
NoDenMortality       : array [1..12] of real;
ImmigrantDenThreshold : integer;
ResidentDenWindow    : integer;
InitInfectProb : real;
constructor Init;
destructor Done; virtual;
procedure Edit;
procedure Go;
procedure WriteToFile(FileName : string);
procedure SetFromFile(FileName : string);
function SaveChange : boolean;
procedure Load;
procedure Save;
end;

TimeObj = object
StartDate  : Date;
CurrentDate : Date;
constructor Init(InitDay, InitMonth, InitYear : integer);
destructor Done; virtual;
procedure Increment(IncDay, IncMonth, IncYear : integer);
function Day     : integer;
function Month   : integer;
function Year    : integer;
function Days    : integer;
function Months  : integer;
function Years   : integer;
function Season  : string;
function MonthStr : string;
function EndOfMonth : boolean;
procedure SaveState(FileName : string);
end;

AgeArray  = array [0..MaxAge, Female..Male] of longint;
SummaryPtr = ^Summary;
Summary = record
Sum   : AgeArray;
SumSq  : AgeArray;
Next  : SummaryPtr;
end;

PossumPtr = ^Possum;

PossumListPtr = ^PossumList;

PossumList = object(List)
  constructor Init;
  destructor Done; virtual;
  procedure AddPossum(NewPossum : PossumPtr);
end;

PopulationPtr = ^Population;
Population = object(PossumList)
AgesHealthy : array [0..MaxAge, Female..Male] of byte;
AgesClinical : array [0..MaxAge, Female..Male] of byte;
AgesInfected : array [0..MaxAge, Female..Male] of byte;
NewClinical  : array [0..2, Female..Male] of byte; { Incidence }
NewInfected  : array [0..2, Female..Male] of byte; { arrays    }
AveNilDens : real;
NoInAve   : integer;
constructor Init;
destructor Done; virtual;
procedure Fill;
procedure DeletePossum(N : NodePtr);
procedure Immigration;
procedure UpdateAges;
procedure UpdateAveNilDens(NewValue : integer);
```

```
procedure Grow;
procedure GoClinical;
procedure Mating;
function  Fecundity : real;
procedure TB;
procedure Move;
procedure Death;
procedure DensityDynamics;
function Size     : integer;
function NoMales(Status : byte) : integer;
function NoFemales(Status : byte) : integer;
function NilDens : integer;
procedure PrintAges;
function AgeSummary : string;
function DenSummary : string;
function NonDenSummary : string;
procedure PrintList;
procedure PrintDenSummary;
procedure PrintNonDens;
procedure SaveState(FileName : string);
end;

DenListPtr = ^DenList;

DenPtr = ^DenObj;
DenObj = object
id       : word;
X        : LocationType;
Y        : LocationType;
Occupants  : PossumListPtr;
CloseDens  : DenListPtr;
DateLastTB : Date;
constructor Init(Initid : word; InitX, InitY : LocationType);
destructor Done; virtual;
function Distance(RefX, RefY : LocationType) : real;
function Empty : boolean;
procedure CloseDensList;
function LoadNextDen : SNodePtr;
procedure CalculateGeography(Dens : DenListPtr);
procedure WriteGeography;
procedure ReadGeography(Dens :DenListPtr);
procedure Draw(OriginX, OriginY : LocationType);
function Clinical : boolean;
end;

DenList = object(SList)
MaxX, MinX, MaxY, MinY : LocationType;
constructor Init;
destructor Done; virtual;
procedure SetFromFile(FileName : string);
procedure CalculateGeography;
procedure LoadGeography;
procedure Draw;
procedure AddDen(Den : DenPtr);
function ClinicalDens : integer;
procedure ClearInfection;
end;

Possum = object
Birthday,
DateIndependent,
MaturityDate    : Date;
Sex        : SexType;
Joey       : PossumPtr;
Mother     : PossumPtr;
Pregnant   : boolean;
DenMemory  : DenListPtr;
Den        : DenPtr;
NoDenList  : SListPtr;
Immigrant  : boolean; {Once an animal dens in the area it is no longer considered an immigrant Joeys
                       are considered to be immigrants}
DateInfected : Date;
```

```
DateClinical : Date;
constructor Init(InitBirthday : Date; InitSex : SexType; InitDens : DenListPtr);
destructor Done; virtual;
procedure Reproduce;
procedure Independence;
function Live   : boolean;      {Grow older or die!}
function Mature : boolean;
function Age    : integer;
function AgeCode: byte;
procedure FindDen;
procedure LeaveDen;
procedure Infect;
function Clinical : boolean;
function Infected : boolean;
end;

var
  Time     : TimeObj;
  Model    : ModelObj;
  Dens     : DenList;
  Pop      : Population;
  GraphDriver : integer;
  GraphMode   : integer;
  ErrorCode   : integer;

function FileExists(Filename : string) : boolean;
var
  f : file;
begin
  {$I-}
  Assign(f, FileName);
  Reset(f);
  Close(f);
  {I+}
  FileExists := (IOResult = 0) and (FileName <> '');
end;

(*******************************************************)
(******************  MODEL  ********************)
(*******************************************************)

constructor ModelObj.Init;
var
  i : integer;
begin
  FillChar(Self, SizeOf(Self), char(0));
  if ParamCount <> 7 then
      begin
          repeat
              write('Enter name of file for Start Population: ');
              readln(PopFile);
              if not fileexists(PopFile) then
                  writeln('File does not exist, please reenter');
          until fileexists(PopFile);
          repeat
              write('Enter name of file for den site locations: ');
              readln(DenMapFile);
              if not fileexists(DenMapFile) then
                  writeln('File does not exist, please reenter');
          until fileexists(DenMapFile);
          repeat
              write('Enter name of parameter file: ');
              readln(ParamFile);
              if not fileexists(ParamFile) then
                  writeln('File does not exist, please reenter');
          until fileexists(ParamFile);
           PossumControl := false;
          YorN := 'N';
          write('Do you want to implement control operations? (Y/N): ');
          readln(YorN);
          if UpCase(YorN[1]) = 'Y' then
              begin
```

```
                    PossumControl := True;
                    write('At what intervals do you want to control? (in days): ');
                    readln(ControlInterval);
                    write('Duration of control operation? (in days): ');
                    readln(ControlPeriod);
                    repeat
                        write('Which parameter file stores effect of control? : ');
                        readln(ControlFile);
                        if not fileexists(ControlFile) then
                            writeln('File does not exist, please reenter');
                    until fileexists(ControlFile);
                end;
        end
    else
        begin
            PopFile := ParamStr(1);
            DenMapFile := ParamStr(2);
            ParamFile := ParamStr(3);
            write('Start Population File: ');
            writeln(PopFile);
            write('Parameter File: ');
            writeln(ParamFile);
            write('Den Map File: ');
            writeln(DenMapFile);
            YorN :=ParamStr(4);
            write('Simulate Possum Control Operations :');
            writeln(YorN);
            if UpCase(YorN[1]) = 'Y' then
                begin
                    PossumControl := True;
                    val(ParamStr(5), ControlInterval, code);
                    val(ParamStr(6), ControlPeriod, code);
                    ControlFile :=ParamStr(7);
                    write('Interval between control operations: ');
                    write(ControlInterval);
                    writeln(' days');
                    write('Duration of possum control operation: ');
                    write(ControlPeriod);
                    writeln(' days');
                    write('Paramter file for control operation: ');
                    writeln(ControlFile);
                end;
        end;
    SetFromFile(ParamFile);
    YorN := 'N';
end;

destructor ModelObj.Done;
begin
end;

procedure ModelObj.SetFromFile(FileName : string);
var
    FileVar : text; : integer;
    UseDenMemoryCh : char;
begin
    Assign(FileVar, FileName);
    Reset(FileVar);
    for i := 1 to 12 do
        read(FileVar, AdultSurvival[i, Male]);
    for i := 1 to 12 do
        read(FileVar, AdultSurvival[i, Female]);
    for i := 1 to 12 do
        read(FileVar, ImmatureSurvival[i, Male]);
    for i := 1 to 12 do
        read(FileVar, ImmatureSurvival[i, Female]);
    for i := 1 to 12 do
        read(FileVar, Immigration[i, Female]);
    for i := 1 to 12 do
        read(FileVar, Immigration[i, Male]);
    for i := 1 to 12 do
        read(FileVar, MatingProb[i]);
```

```
  for i := 1 to 12 do
      read(FileVar, ClinicalAdultSurvival[i]);
  for i := 1 to 12 do
      read(FileVar, ClinicalImmatureSurvival[i]);
  for i := 1 to 12 do
      read(FileVar, BackgroundTBProb[i]);
  for i := 1 to 12 do
      read(FileVar, ImmigrantTBProb[i]);
  for i := 1 to 12 do
      read(FileVar, GoClinicalProb[i]);
      readln(FileVar, ResidentDenThreshold, ResidentDenWindow);
  for i := 1 to 12 do
      read(FileVar, NoDenMortality[i]);
      readln(FileVar, ImmigrantDenThreshold);
      readln(FileVar, MaleBirthProb, InitInfectProb);
  for i := 0 to 8 do
      read(FileVar, JoeySurvival[i]);
      readln(FileVar, DenTBPeriod, DenTBProb);
      readln(FileVar, DenTBBuffer, BufferTBProb);
      readln(FileVar, MaxDenTravel, DenRejectProb);
      readln(FileVar, UseDenMemoryCh);
  if UseDenMemoryCh = 'Y' then UseDenMemory := True
  else UseDenMemory := False;
  readln(FileVar, MatingBuffer, MatingTBProb);
  Close(FileVar);
end;

procedure ModelObj.Go;
const
  uiQuit = 0; { Global constants for user interupt routine }
  uiSave = 1;
  uiNull = 2;
var
  NoDays, Day: longint;
  AntiTheticRun, AT, Run, NoRuns, Year, NoYears, Month, NoMonths, PrevYear : integer;
  Age, Sex, NilDens : integer;
  YorN, FileName, DenMapFile, PopFile : string;
  AgeFile,DenFile, DenSummaryFile : text;
  DayPrint, MonthPrint, YearPrint, PrintAge, PrintDens, NonDens, AntiThetic : boolean;
  SummaryP, SummaryStart, PrevS : SummaryPtr;
  GraphicalDisplay : boolean;
  Finished : boolean;
  Ch : char;
  EnterSeed, DumpDens, DumpDensDaily, DumpCurrentDens: boolean;
  oldx, oldy : byte;

procedure PrintAgeSummary;
begin
  writeln('Run : ', Run, '  Year : ', Time.Year,
  Time.MonthStr:10, '  ', Pop.Size, '  Healthy Males : ', Pop.NoMales(pHealthy),  Healthy Females : ', Pop.NoFemales(pHealthy));
  writeln('              ','Clinical Males : ', Pop.NoMales(pClinical),'  Clinical Females : ', Pop.NoFemales(pClinical),
          '   Clinical Dens : ', Dens.ClinicalDens);
  writeln('              ', 'Infected Males : ', Pop.NoMales(pInfected),'  Infected Females : ', Pop.NoFemales(pInfected),
          '  Infected Dens : ', Dens.ClinicalDens);
  writeln('              ', '  Fecundity : ', Pop.Fecundity:5:3);
end;

procedure WriteDens(DayorMonth : integer);
var
  DenNode : SNodePtr;
  Den     : DenPtr;
begin
  DenNode := Dens.First;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          if (DumpCurrentDens and Den^.Clinical) or ((not DumpCurrentDens) and  (Den^.DateLastTB <> BadDate)) then
              writeln(DenFile, DayorMonth:5,',',Den^.ID:5);
          DenNode := DenNode^.Next;
      end;
end;
```

```
function UserInterupt: byte;
const
  DisplayText = 'Pause...press Q to quit, S to save, other to continue';
begin
  case ReadKey of
      #27, 'Q', 'q' : UserInterupt := uiQuit;
      'S', 's'      : UserInterupt := uiSave;
  else
      begin
          writeln(DisplayText);
          while not KeyPressed do
              begin
              end;
          case ReadKey of
              #27, 'Q', 'q' : UserInterupt := uiQuit;
              'S', 's'      : UserInterupt := uiSave;
          end;
      end;
  end;
end;
begin
  clrscr;
  writeln( 'Simulation Model of a Possum Population');
  writeln;
  NoRuns := 1;
  NoDays := 10000;
  write(' Enter number of days: ');
  readln(NoDays);
  writeln;
  write(' Print summary each day (Y/N; N=default) ');
  readln(YorN);
  DayPrint := False;
  if UpCase(YorN[1]) = 'Y' then DayPrint := True;
  write(' Print summary each month (Y/N; N=default) ');
  readln(YorN);
  MonthPrint := False;
  if UpCase(YorN[1]) = 'Y' then MonthPrint := True;
  if not MonthPrint then
      begin
          write(' Print summary each year (Y/N; N=default) ');
          readln(YorN);
          YearPrint := False;
          if UpCase(YorN[1]) = 'Y' then YearPrint := True;
      end;
  write(' Print monthly age distribution to file (Y/N; N=default) ');
  readln(YorN);
  PrintAge := False;
  if UpCase(YorN[1]) = 'Y' then PrintAge := True;
  if PrintAge then
      begin
          write(' Enter filename to use : ');
          readln(FileName);
          Assign(AgeFile, FileName);
          ReWrite(AgeFile);
          writeln(AgeFile, 'Day,Month,Year,NoDays,MM,MF,IM,IF,JM,JF,IMM,NIMM,IMF,NIMF,IIM,NIIM,IIF,
                  NIIF,IJM,NIJM,IJF,NIJF,CMM,NCMM,CMF,NCMF,CIM,NCIM,CIF,NCIF,CJM,NCJM,CJF,NCJF');
      end;
  write(' Print monthly den distribution to file (Y/N; N=default) ');
  readln(YorN);
  PrintDens := False;
  if UpCase(YorN[1]) = 'Y' then PrintDens := True;
  YorN := 'N';
  if PrintDens then
      begin
          write(' Enter filename to use : ');
          readln(FileName);
          Assign(DenSummaryFile, FileName);
          ReWrite(DenSummaryFile);
          writeln(DenSummaryFile, 'Day,Month,Year,NoDays,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20');
      end;
  write(' Print monthly distribution of non-denning possums to file (Y/N; N=default) ');
  readln(YorN);
```

```
NonDens := False;
if UpCase(YorN[1]) = 'Y' then NonDens := True;
YorN := 'N';
if NonDens then
    begin
        write(' Enter filename to use : ');
        readln(FileName);
        Assign(NonDenSummaryFile, FileName);
        ReWrite(NonDenSummaryFile);
        writeln(NonDenSummaryFile, 'Day,Month,Year,NoDays,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20');
    end;
write(' Dump coordinates of clinical dens (Y/N; N=default) ');
readln(YorN);
DumpDens := False;
if UpCase(YorN[1]) = 'Y' then DumpDens := True;
if DumpDens then
    begin
        DumpCurrentDens := true;
        write(' Current or All (C/A; C=default) ');
        readln(YorN);
        if UpCase(YorN[1]) = 'A' then DumpCurrentDens := false;
        DumpDensDaily := false;
        write(' Daily or Monthly (D/M; M=default) ');
        readln(YorN);
        if UpCase(YorN[1]) = 'D' then DumpDensDaily := true;
        write(' Enter filename to use : ');
        readln(FileName);
        Assign(DenFile, FileName);
        ReWrite(DenFile);
        if DumpDensDaily then write(DenFile, 'Day ')
         else
            write(DenFile, 'Month, ');
            writeln(DenFile, 'DenID, X, Y');
        end;
        YorN := 'Y'; {default setting}
        write(' Do you want to use antithetic variates (Y/N; Y=default): ');
        readln(YorN);
        AntiThetic := true;
        AntiTheticRun := 2;
        if UpCase(YorN[1]) = 'N' then
            begin
                AntiThetic := False;
                AntiTheticRun := 1;
            end;
        YorN := 'N';
        write(' Enter random number seed (N = randomize (default)) : ');
        readln(YorN);
        EnterSeed := false;
        if UpCase(YorN[1]) = 'Y' then EnterSeed := True;
        if EnterSeed = False then
            begin
                randomize;
                FirstSeed := RandSeed;
                rinit(RandSeed,7654321);
                write(' This is the Seed for the Random Number Generators :');
                writeln(FirstSeed);
                SurvAdultSeed := i31bit;
                SurvImmatureSeed := i31bit;
                MatingSeed := i31bit;
                TBSurvAdultSeed := i31bit;
                TBSurvImmSeed := i31bit;
                TBImmigrSeed := i31bit;
                SubToClinSeed := i31bit;
                PopDensEmigrSeed := i31bit;
                BirthSexSeed := i31bit;
                TBPrevSeed := i31bit;
                JoeySurvSeed := i31bit;
                TBDenSeed := i31bit;
                TBBufferSeed := i31bit;
                DenRejectSeed := i31bit;
                TBMatingSeed := i31bit;
                AgeIndepSeed1 := i31bit;
```

```
            AgeIndepSeed2 := m - AgeIndepSeed1;
            AgeMatureSeed1 := i31bit;
            AgeMatureSeed2 := m - AgeMatureSeed1;
            DailyImmigrFemaleSeed := i31bit;
            DailyImmigrMaleSeed := i31bit;
        end;
    if EnterSeed = True then
        begin
            write(' Enter Seed for Random Number Generation : ');
            readln(FirstSeed);
            RandSeed := FirstSeed;
            rinit(RandSeed,7654321);
            write(' This is the Seed for the Random Number Generators :');
            writeln(FirstSeed);
            SurvAdultSeed := i31bit;
            SurvImmatureSeed := i31bit;
            MatingSeed := i31bit;
            TBSurvAdultSeed := i31bit;
            TBSurvImmSeed := i31bit;
            TBImmigrSeed := i31bit;
            SubToClinSeed := i31bit;
            PopDensEmigrSeed := i31bit;
            BirthSexSeed := i31bit;
            TBPrevSeed := i31bit;
            JoeySurvSeed := i31bit;
            TBDenSeed := i31bit;
            TBBufferSeed := i31bit;
            DenRejectSeed := i31bit;
            TBMatingSeed := i31bit;
            AgeIndepSeed1 := i31bit;
            AgeIndepSeed2 := m - AgeIndepSeed1;
            AgeMatureSeed1 := i31bit;
            AgeMatureSeed2 := m - AgeMatureSeed1;
            DailyImmigrFemaleSeed := i31bit;
            DailyImmigrMaleSeed := i31bit;
            randomize
        end;
    SurvAdultSeedOrg :=   SurvAdultSeed;
    SurvImmatureSeedOrg := SurvImmatureSeed;
    MatingSeedOrg := MatingSeed;
    TBSurvAdultSeedOrg := TBSurvAdultSeed;
    TBSurvImmSeedOrg := TBSurvImmSeed;
    TBImmigrSeedOrg  := TBImmigrSeed;
    SubToClinSeedOrg := SubToClinSeed;
    PopDensEmigrSeedOrg := PopDensEmigrSeed;
    BirthSexSeedOrg  := BirthSexSeed;
    TBPrevSeedOrg := TBPrevSeed;
    JoeySurvSeedOrg := JoeySurvSeed;
    TBDenSeedOrg := TBDenSeed;
    TBBufferSeedOrg := TBBufferSeed;
    DenRejectSeedOrg := DenRejectSeed;
    TBMatingSeedOrg := TBMatingSeed;
    AgeIndepSeed1Org := AgeIndepSeed1;
    AgeIndepSeed2Org := AgeIndepSeed2;
    AgeMatureSeed1Org := AgeMatureSeed1;
    AgeMatureSeed2Org := AgeMatureSeed2;
    DailyImmigrMaleSeedOrg := DailyImmigrMaleSeed;
    DailyImmigrFemaleSeedOrg := DailyImmigrFemaleSeed;
    for Run := 1 to NoRuns do
        begin
            for AT := 1 to AntiTheticRun do
                begin
                    if AntiTheticRun = 2 then
                        begin
                            SurvAdultSeed :=  m -  SurvAdultSeedOrg;
                            SurvImmatureSeed := m - SurvImmatureSeedOrg;
                            MatingSeed := m - MatingSeedOrg;
                            TBSurvAdultSeed := m - TBSurvAdultSeedOrg;
                            TBSurvImmSeed := m - TBSurvImmSeedOrg;
                            TBImmigrSeed  := m - TBImmigrSeedOrg;
                            SubToClinSeed := m - SubToClinSeedOrg;
                            PopDensEmigrSeed := m - PopDensEmigrSeedOrg;
```

```
            BirthSexSeed  := m - BirthSexSeedOrg;
            TBPrevSeed := m - TBPrevSeedOrg;
            JoeySurvSeed := m - JoeySurvSeedOrg;
            TBDenSeed := m - TBDenSeedOrg;
            TBBufferSeed := m - TBBufferSeedOrg;
            DenRejectSeed := m - DenRejectSeedOrg;
            TBMatingSeed := m - TBMatingSeedOrg;
            AgeIndepSeed1 := m - AgeIndepSeed1Org;
            AgeIndepSeed2 := m - AgeIndepSeed2Org;
            AgeMatureSeed1 := m - AgeMatureSeed1Org;
            AgeMatureSeed2 := m - AgeMatureSeed2Org;
            DailyImmigrMaleSeed := m - DailyImmigrMaleSeedOrg;
            DailyImmigrFemaleSeed := m - DailyImmigrFemaleSeedOrg;
        end;
    Init(31, 12, 1989);    { The initial population is given }
    clrscr;
    writeln('The simulation begins !!!');
     Pop.Init;              { one movement to simulate a dynamic }
    writeln('The population is initialized.');
     Pop.Fill;              { population, since non-denning animals }
    writeln('Dens have been allocated.');
    Time.Increment(1, 0, 0);    { are considered to be immigrants }
    PrevYear := Time.Year;
    NoMonths := 0;
    Finished := false;
    writeln('Amount of free memory: ',memavail);
    write('Starting Random Seed: ');
    writeln(FirstSeed);
    if NoDays = 0 then NoDays := maxint;
    for Day := 1 to NoDays do
        begin
            if PossumControl = true then
                begin
                    if Day = ControlInterval then
                        begin
                            SetfromFile(ControlFile);
                            NextControl := Day + ControlInterval;
                            BackToNormal := Day + ControlPeriod;
                            writeln;
                            write('Begin Possum Control Operation on Day :  ');
                            writeln(Day);
                        end;
                    if Day = NextControl then
                        begin
                            SetfromFile(ControlFile);
                            NextControl := Day + ControlInterval;
                            BackToNormal := Day + ControlPeriod;
                            writeln;
                            write('Begin Possum Control Operation on Day :  ');
                            writeln(Day);
                        end;
                    if Day = BackToNormal then
                        begin
                            SetfromFile(ParamFile);
                            writeln;
                            write('End Possum Control Operation on Day :  ');
                            writeln(Day);
                        end;
                end;
            if MatingProb[Time.Month] > 0 then Pop.Mating;
             Pop.Immigration;
             Pop.Move;
             Pop.TB;
             Pop.DensityDynamics;
            if DumpDens and DumpDensDaily then WriteDens(Day);
            NilDens := Pop.NilDens;
             Pop.UpdateAveNilDens(NilDens);
            write('.');
            if Time.Day = 1 then
                begin
                    Pop.GoClinical;
                end;
```

```
                                if Time.Day = 15 then
                                    begin
                                        Pop.Grow;
                                    end;
                                { End of Month }
                                if Time.EndOfMonth then
                                    begin
                                        writeln;
                                        inc(NoMonths);
                                        if (not DayPrint) and (not GraphicalDisplay) then writeln;
                                        if MonthPrint then
                                            begin
                                                writeln;
                                                PrintAgeSummary;
                                            end;
                                        if PrintAge then
                                        writeln(AgeFile, DateToDateString('DD,MM,YY', Time.CurrentDate),',',
                                                Time.CurrentDate - Time.StartDate,',', Pop.AgeSummary);
                                        if PrintDens then writeln(DenSummaryFile, DateToDateString('DD,MM,YY',
                                        Time.CurrentDate),',', Time.CurrentDate - Time.StartDate,',', Pop.DenSummary);
                                        if NonDens then
                                            writeln(NonDenSummaryFile, DateToDateString('DD,MM,YY',
                                                    Time.CurrentDate),',',Time.CurrentDate - Time.StartDate,',',
                                                    Pop.NonDenSummary);
                                        if DumpDens and (not DumpDensDaily) then WriteDens(NoMonths);
                                    end; { of Month }
                                if Time.Year <> PrevYear then
                                    begin
                                        PrevYear := Time.Year;
                                        if (not MonthPrint) and (YearPrint) then
                                            begin
                                                Pop.PrintDenSummary;
                                                PrintAgeSummary;
                                            end;
                                    end; { of Year }
                                 Pop.UpdateAges;
                                if KeyPressed then
                                     begin
                                        case UserInterupt of
                                            uiQuit : Day := NoDays;
                                            uiSave : SaveState;
                                        end;
                                    end;
                                Time.Increment(1, 0, 0);
                            end; { of Day}
                        writeln;
                        PrintAgeSummary;
                         Pop.Done;
                        writeln('Pop.Done');
                        Time.Done;
                        Dens.ClearInfection;
                    end; { end of Antithetic run}
            end; { of Run }
        if PrintAge then Close(AgeFile);
        if Printdens then Close(DenSummaryFile);
        if NonDens then close(NonDenSummaryFile);
        if DumpDens then Close(DenFile);
        writeln;
        write('End of Simulation, press Return.');
        readln;
end;

{Random Number Generators }

FUNCTION SurvAdultRandom(VAR SurvAdultSeed: extended): extended;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
```

```
  Temp := a *  SurvAdultSeed;
  Temp := Temp / m;
  SurvAdultSeed := Int(m*Frac(Temp) + 0.5);
  SurvAdultRandom := SurvAdultSeed * rm;
END;

FUNCTION SurvImmatureRandom(VAR SurvImmatureSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  SurvImmatureSeed;
  Temp := Temp / m;
  SurvImmatureSeed := Int(m*Frac(Temp) + 0.5);
  SurvImmatureRandom := SurvImmatureSeed*rm;
END;

FUNCTION MatingRandom(VAR MatingSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  MatingSeed;
  Temp := Temp / m;
  MatingSeed := Int(m*Frac(Temp) + 0.5);
  MatingRandom := MatingSeed*rm;
END;

FUNCTION TBSurvAdultRandom(VAR TBSurvAdultSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBSurvAdultSeed;
  Temp := Temp / m;
  TBSurvAdultSeed := Int(m*Frac(Temp) + 0.5);
  TBSurvAdultRandom := TBSurvAdultSeed*rm;
END;

FUNCTION TBSurvImmRandom(VAR TBSurvImmSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBSurvImmSeed;
  Temp := Temp / m;
  TBSurvImmSeed := Int(m*Frac(Temp) + 0.5);
  TBSurvImmRandom := TBSurvImmSeed*rm;
END;

FUNCTION SubToClinRandom(VAR SubToClinSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  SubToClinSeed;
  Temp := Temp / m;
```

```
  SubToClinSeed := Int(m*Frac(Temp) + 0.5);
  SubToClinRandom := SubToClinSeed*rm;
END;

FUNCTION PopDensEmigrRandom(VAR PopDensEmigrSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  PopDensEmigrSeed;
  Temp := Temp / m;
  PopDensEmigrSeed := Int(m*Frac(Temp) + 0.5);
  PopDensEmigrRandom := PopDensEmigrSeed*rm;
END;

FUNCTION BirthSexRandom(VAR BirthSexSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  BirthSexSeed;
  Temp := Temp / m;
  BirthSexSeed := Int(m*Frac(Temp) + 0.5);
  BirthSexRandom := BirthSexSeed*rm;
END;

FUNCTION TBPrevRandom(VAR TBPrevSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBPrevSeed;
  Temp := Temp / m;
  TBPrevSeed := Int(m*Frac(Temp) + 0.5);
  TBPrevRandom := TBPrevSeed*rm;
END;

FUNCTION JoeySurvRandom(VAR JoeySurvSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  JoeySurvSeed;
  Temp := Temp / m;
  JoeySurvSeed := Int(m*Frac(Temp) + 0.5);
  JoeySurvRandom := JoeySurvSeed*rm;
END;

FUNCTION TBDenRandom(VAR TBDenSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBDenSeed;
  Temp := Temp / m;
  TBDenSeed := Int(m*Frac(Temp) + 0.5);
  TBDenRandom := TBDenSeed*rm;
```

```
END;

FUNCTION TBBufferRandom(VAR TBBufferSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBBufferSeed;
  Temp := Temp / m;
  TBBufferSeed := Int(m*Frac(Temp) + 0.5);
  TBBufferRandom := TBBufferSeed*rm;
END;

FUNCTION DenRejectRandom(VAR DenRejectSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  DenRejectSeed;
  Temp := Temp / m;
  DenRejectSeed := Int(m*Frac(Temp) + 0.5);
  DenRejectRandom := DenRejectSeed*rm;
END;

FUNCTION TBMatingRandom(VAR TBMatingSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBMatingSeed;
  Temp := Temp / m;
  TBMatingSeed := Int(m*Frac(Temp) + 0.5);
  TBMatingRandom := TBMatingSeed*rm;
END;

FUNCTION TBImmigrRandom(VAR TBImmigrSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  TBImmigrSeed;
  Temp := Temp / m;
  TBImmigrSeed := Int(m*Frac(Temp) + 0.5);
  TBImmigrRandom := TBImmigrSeed*rm;
END

FUNCTION MyRandom(VAR MySeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  MySeed;
  Temp := Temp / m;
  MySeed := Int(m*Frac(Temp) + 0.5);
  MyRandom := MySeed*rm;
END;
```

```
FUNCTION AgeMatureRandom1(var AgeMatureSeed1 : extended): extended;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;    (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  AgeMatureSeed1;
  Temp := Temp / m;
  AgeMatureSeed1 := Int(m*Frac(Temp) + 0.5);
  AgeMatureRandom1 := AgeMatureSeed1 * rm;
END;

FUNCTION AgeMatureRandom2(var AgeMatureSeed2 : extended): extended;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;    (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  AgeMatureSeed2;
  Temp := Temp / m;
  AgeMatureSeed2 := Int(m*Frac(Temp) + 0.5);
  AgeMatureRandom2 := AgeMatureSeed2 * rm;
END;

function AgeMatureNormal(Mean, StdDev, Max, Min : real) : real;
var
  Result1,Result2,w,w1,w2,c : real;
begin
  repeat
      repeat
          w1 := 2 * AgeMatureRandom1(AgeMatureSeed1) - 1;
          w2 := 2 * AgeMatureRandom2(AgeMatureSeed2) - 1;
        w  := w1 * w1 + w2 * w2;
      until w < 1;
      c :=  sqrt(-2*ln(w)/w);
      Result1 := w1 * c;
      Result2 := Mean + Result1 * StdDev
  until (Result2 <= Max) and (Result2 >= Min);
  AgeMatureNormal := Result2;
end;

FUNCTION AgeIndepRandom1(var AgeIndepSeed1 : extended): extended;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;    (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  AgeIndepSeed1;
  Temp := Temp / m;
  AgeIndepSeed1 := Int(m*Frac(Temp) + 0.5);
  AgeIndepRandom1 := AgeIndepSeed1 * rm;
END;

FUNCTION AgeIndepRandom2(var AgeIndepSeed2 : extended): extended;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;    (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  AgeIndepSeed2;
  Temp := Temp / m;
  AgeIndepSeed2 := Int(m*Frac(Temp) + 0.5);
  AgeIndepRandom2 := AgeIndepSeed2 * rm;
END;
```

```
function AgeIndepNormal(var AgeIndepSeed1, AgeIndepSeed2 : extended; Mean, StdDev, Max, Min : real) : real;
var
  Result1,Result2,w,w1,w2,c : real;
begin
  repeat
      repeat
          w1 := 2 * AgeIndepRandom1(AgeIndepSeed1) - 1;
          w2 := 2 * AgeIndepRandom2(AgeIndepSeed2) - 1;
         w  := w1 * w1 + w2 * w2;
      until w < 1;
      c :=  sqrt(-2*ln(w)/w);
      Result1 := w1 * c;
      Result2 := Mean + Result1 *StdDev ;
  until (Result2 <= Max) and (Result2 >= Min);
  AgeIndepNormal := Result2;
end;

FUNCTION DailyImmigrMaleRandom(VAR DailyImmigrMaleSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  DailyImmigrMaleSeed;
  Temp := Temp / m;
  DailyImmigrMaleSeed := Int(m*Frac(Temp) + 0.5);
  DailyImmigrMaleRandom := DailyImmigrMaleSeed*rm;
END;

FUNCTION DailyImmigrFemaleRandom(VAR DailyImmigrFemaleSeed: extended): real;
CONST
  m=2147483647;
  a=742938285;
  rm= 0.4656612875246e-9;     (* 1.0/m *)
VAR
  Temp : extended;
BEGIN
  Temp := a *  DailyImmigrFemaleSeed;
  Temp := Temp / m;
  DailyImmigrFemaleSeed := Int(m*Frac(Temp) + 0.5);
  DailyImmigrFemaleRandom := DailyImmigrFemaleSeed*rm;
END;

function DailyImmigrMalePoisson(DailyImmigrMale : real; var DailyImmigrMaleSeed : extended) : integer;
var
Count : integer;
ProbZero, Product : real;
begin
  Count := 0;
  Product := DailyImmigrMaleRandom(DailyImmigrMaleSeed);
  ProbZero := exp(-DailyImmigrMale);
  while Product > ProbZero do                    { Poisson Distribution}
      begin
          Count := Count + 1;
          Product := Product * DailyImmigrMaleRandom(DailyImmigrMaleSeed);
      end;
  DailyImmigrMalePoisson := Count;
end;

function DailyImmigrFemalePoisson(DailyImmigrFemale : real; var DailyImmigrFemaleSeed : extended) : integer;
var
  Count : integer;
  ProbZero, Product : real;
begin
  Count := 0;
  Product := DailyImmigrFemaleRandom(DailyImmigrFemaleSeed);
  ProbZero := exp(-DailyImmigrMale);
  while Product > ProbZero do                    { Poisson Distribution}
      begin
```

```
            Count := Count + 1;
            Product := Product * DailyImmigrFemaleRandom(DailyImmigrFemaleSeed);
        end;
  DailyImmigrFemalePoisson := Count;
end;

{ End of Random Number Generator Section }

(*****************************************************)
(********          TIME          *********)
(*****************************************************)

constructor TimeObj.Init (InitDay, InitMonth, InitYear : integer);
begin
  StartDate   := DMYToDate(InitDay, InitMonth, InitYear);
  CurrentDate := StartDate;
end;

destructor TimeObj.Done;
begin
end;

function TimeObj.Day : integer;
var
  D, M, Y : integer;
begin
  DateToDMY(CurrentDate, D, M, Y);
  Day := D;
end;

function TimeObj.Month : integer;
var
  D, M, Y : integer;
begin
  DateToDMY(CurrentDate, D, M, Y);
  Month := M;
end;

function TimeObj.Year : integer;
var
  D, M, Y : integer;
begin
  DateToDMY(CurrentDate, D, M, Y);
  Year := Y;
end;

function TimeObj.Days : integer;
var
  D, M, Y : integer;
begin
  DateDiff(StartDate, CurrentDate, D, M, Y);
  Days := D;
end;

function TimeObj.Months : integer;
var
  D, M, Y : integer;
begin
  DateDiff(StartDate, CurrentDate, D, M, Y);
  Months := M;
end;

function TimeObj.Years : integer;
var
  D, M, Y : integer;
begin
  DateDiff(StartDate, CurrentDate, D, M, Y);
  Years := Y;
end;

procedure TimeObj.Increment (IncDay, IncMonth, IncYear : integer);
begin
```

```
  CurrentDate := IncDate(CurrentDate, IncDay, IncMonth, IncYear);
end;

function TimeObj.Season : string;
begin
  case Month of
       12, 1, 2 : Season := 'Summer';
         3..5   : Season := 'Autumn';
         6..8   : Season := 'Winter';
         9..11  : Season := 'Spring';
end;
 end;

function TimeObj.MonthStr : string;
begin
  case Month of
          1  : MonthStr := 'January';
          2  : MonthStr := 'February';
          3  : MonthStr := 'March';
          4  : MonthStr := 'April';
          5  : MonthStr := 'May';
          6  : MonthStr := 'June';
          7  : MonthStr := 'July';
          8  : MonthStr := 'August';
          9  : MonthStr := 'September';
         10 : MonthStr := 'October';
         11 : MonthStr := 'November';
         12 : MonthStr := 'December';
  end;
end;

function TimeObj.EndOfMonth : boolean;
var
  D, M, Y : integer;
begin
  DateToDMY(CurrentDate+1, D, M, Y);
  if M <> Month then EndOfMonth := true else EndOfMonth := false;
end;

procedure TimeObj.SaveState(FileName : string);
var
  FileVar : text;
  i       : integer;
begin
  Assign(FileVar, FileName);
  ReWrite(FileVar);
  writeln(FileVar, CurrentDate);
  Close(FileVar);
end;

(*******************************************************)
(**************        DEN         *************)
(*******************************************************)

constructor DenObj.Init( Initid : word; InitX, InitY : LocationType);
begin
  id := Initid;
  X  := InitX;
  Y  := InitY;
  Occupants := New(PossumListPtr,Init);
  CloseDens := New(DenListPtr, Init);
  DateLastTB := BadDate;
end;

destructor DenObj.Done;
begin
  Occupants^.Done;
  CloseDens^.Done;
end;

function DenObj.Distance( RefX, RefY : LocationType) : real;
begin
```

```
  Distance := sqrt(((RefX - X) * (RefX - X))+ ((RefY - Y) * (RefY - Y)));
end;

function DenObj.Empty : boolean;
begin
  if Occupants^.First = nil then Empty := true else Empty := false;
end;

procedure DenObj.CloseDensList;
var
  tmp, dist : string;
  DenNode : SNodePtr;
  Den : DenPtr;
begin
  tmp := '';
  DenNode := CloseDens^.First;
  while (DenNode <> nil) and (length(tmp) < 250) do
      begin
          Den := DenNode^.Data;
          writeln(Den^.id:4, Distance(Den^.X, Den^.Y):4:0);
          DenNode := DenNode^.Next;
      end;
  readln;
end;

procedure DenObj.CalculateGeography(Dens : DenListPtr);
var
  Node, SearchNode : SNodePtr;
  OtherDen, SearchDen, FarDen : DenPtr;
  DistanceToOther : real;
  Finished : boolean;
begin
  CloseDens^.Done;
  CloseDens^.Init;
  Node := Dens^.First;
  while Node <> nil do
      begin
          OtherDen := Node^.Data;
          DistanceToOther := sqrt(((OtherDen^.X - X) * (OtherDen^.X - X))+ ((OtherDen^.Y - Y) * (OtherDen^.Y - Y)));
          if DistanceToOther < Model.MaxDenTravel then
              begin
                  if (CloseDens^.Length = 0) then { First Den in list }
                      CloseDens^.AddNode(New(SNodePtr, Init(OtherDen)))
                  else
                      begin
                          FarDen := CloseDens^.Last^.Data;
                  if (DistanceToOther > FarDen^.Distance(X, Y)) then
                      begin
                          { Add to end of close den list }
                          CloseDens^.AddNode(New(SNodePtr, Init(OtherDen)));
                       end
                  else
                      begin
                          if (DistanceToOther < DenPtr(CloseDens^.First^.Data)^.Distance(X, Y)) then
                              begin
                                  { Add to begining }
                                  CloseDens^.InsertAfter(New(SNodePtr, Init(OtherDen)), nil);
                               end
                          else
                              begin
                                  { Insert somewhere in middle }
                                  SearchNode := CloseDens^.First;
                                  { Check each den in CloseDen list to determine position of TestDen }
                                  Finished := false;
                                  while (not Finished) and (SearchNode^.Next <> nil) do
                                      begin
                                          SearchDen := SearchNode^.Next^.Data;
                                          if SearchDen^.Distance(X, Y) > DistanceToOther then
                                              begin
                                                  CloseDens^.InsertAfter(New(SNodePtr, Init(OtherDen)), SearchNode);
                                                  Finished := true;
                                               end
```

```
                                        else
                                            SearchNode := SearchNode^.Next;
                                    end; { While }
                                end;
                            end;
                        end;
                end;
    Node := Node ^.Next;
    end;
end;

procedure DenObj.WriteGeography;
var
    F : text;
    idstr : string;
    DenNode : SNodeptr;
    Den : DenPtr;
begin
    str(id, idstr);
    Assign(F, 'cd' + idstr + '.dat');
    ReWrite(F);
    DenNode := CloseDens^.First;
    while DenNode <> nil do
        begin
            Den := DenNode^.Data;
            writeln(F, Den^.id:4, ' ', Distance(Den^.X, Den^.Y):5:0);
            DenNode := DenNode^.Next;
        end;
    Close(F);
end;

procedure DenObj.ReadGeography(Dens : DenListPtr);
var
    F : text;
    idstr, Filename : string;
    CloseId : integer;
    DenNode : SNodeptr;
    Den : DenPtr;
    Found : boolean;
    DistanceToDen : integer;
begin
    CloseDens^.Done;
    CloseDens^.Init;
    str(id, idstr);
    Filename := 'cd' + idstr + '.dat';
    if FileExists(FileName) then
        begin
            Assign(F, FileName);
            ReSet(F);
            readln(F, CloseId, DistanceToDen);
            while (not eof(F)) and (DistanceToDen <= Model.MaxDenTravel) do
                begin
                    { Find pointer to den }
                    DenNode := Dens^.First;
                    Found := false;
                    while (DenNode <> nil) and (not Found) do
                        begin
                            Den := DenNode^.Data;
                            if (Den^.id = CloseId) then Found := true else DenNode := DenNode^.Next;
                        end;
                    if DenNode <> nil then
                        begin
                            CloseDens^.InsertAfter(New(SNodePtr, Init(Den)), CloseDens^.Last);
                        end;
                    readln(F, CloseId, DistanceToDen);
                end;
            Close(F);
        end;
end;

function DenObj.LoadNextDen : SNodePtr;
{ This function loads the next closest den into the CloseDens list, and returns the pointer to this den}
```

```
var
  F      : text;
  strid  : string;
  count  : integer;
  Denid  : integer;
  Den    : DenPtr;
  DenNode : SNodePtr;
  Found  : boolean;
begin
  str(Id, strid);
  Assign(F, 'CD'+strid+'.dat');
  Reset(F);
  DenNode := nil;
  Count := I;
  while (not Eof(f)) and (DenNode = nil) do
      begin
          readln(F, Denid);
          if Count > CloseDens^.Length then
              begin
                  { Find pointer to den }
                  DenNode := Dens.First;
                  Found := false;
                  while (DenNode <> nil) and (not Found) do
                      begin
                          Den := DenNode^.Data;
                          if (Den^.id = DenId) then Found := true else DenNode := DenNode^.Next;
                      end;
                  if DenNode <> nil then
                      begin
                          DenNode := New(SNodePtr, Init(Den));
                          if DenNode = nil then OutOfMemory('LoadNextDen');
                          CloseDens^.InsertAfter(DenNode, CloseDens^.Last);
                       end
                  else
                      begin
                          writeln('LoadNextDen: Error loading den ',id,', den ',Denid,' not found in Dens');
                          halt(1);
                      end;
               end
           else
              inc(Count);
      end; { While }
  if eof(F) then
      begin
          writeln('LoadNextDen: Error end of file in den ',id,' CloseDens.Length=',CloseDens^.Length);
          writeln(Model.DenTBBuffer);
          halt(1);
      end;
  LoadNextDen := DenNode;
  Close(F);
end;

function DenObj.Clinical : boolean;
begin
  if (DateLastTB = BadDate) or ((Time.CurrentDate - DateLastTB) > Model.DenTBPeriod) then
      Clinical := false
   else
      Clinical := true;
  end;

(*************************************************************)
(************          DENLIST          **************)
(*************************************************************)

constructor DenList.Init;
begin
  SList.Init;
  MaxX := 0;
  MaxY := 0;
  MinX := 0;
  MinY := 0;
end;
```

```
procedure DenList.SetFromFile(FileName : string);
var
  F : text;
  id  : word;
  X, Y : LocationType;
begin
  Assign(F, FileName);
  Reset(F);
  readln(F, id, Y, X);
  MaxX := X; MinX := X; MaxY := Y; MinY := Y;
  repeat
      AddDen(New(DenPtr, Init(id, X, Y)));
      if X > MaxX then MaxX := X;
      if X < MinX then MinX := X;
      if Y > MaxY then MaxY := Y;
      if Y < MinY then MinY := Y;
      readln(F, id, Y, X);
  until eof(F);
  Close(F);
end;

destructor DenList.Done;
begin
  SList.Done;
end;

procedure DenList.CalculateGeography;
var
  DenNode : SNodePtr;
  Den : DenPtr;
  TmpMax : integer;
begin
  DenNode := First;
  TmpMax := Model.MaxDenTravel;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          writeln('Calculating for den ', Den^.Id);
          Den^.CalculateGeography(Addr(Self));
          Den^.WriteGeography;
          Den^.ReadGeography(Addr(Self));
          DenNode := DenNode^.Next;
      end;
  Model.MaxDenTravel := TmpMax;
  LoadGeography;
end;

procedure DenList.LoadGeography;
var
  DenNode : SNodePtr;
  Den : DenPtr;
  Tmp : integer;
begin
  DenNode := First;
  Tmp := Model.MaxDenTravel;
  Model.MaxDenTravel := 0;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          Den^.ReadGeography(Addr(Self));
          DenNode := DenNode^.Next;
      end;
  Model.MaxDenTravel := Tmp;
end;

procedure DenList.AddDen(Den : DenPtr);
begin
  AddNode(New(SNodePtr, Init(Den)));
end;

function DenList.ClinicalDens : integer;
```

```
var
  DenNode : SNodePtr;
  Den : DenPtr;
  Tmp : integer;
begin
  tmp := 0;
  DenNode := First;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          if (Den^.Clinical) then inc(tmp);
          DenNode := DenNode^.Next;
      end;
  ClinicalDens := tmp;
end;

procedure DenList.Draw;
var
  DenNode : SNodePtr;
  Den : DenPtr;
begin
  DenNode := First;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          Den^.Draw(MinX, MinY);
          DenNode := DenNode^.Next;
      end;
end;

procedure DenList.ClearInfection;
var
  DenNode : SNodePtr;
  Den : DenPtr;
begin
  DenNode := First;
  while DenNode <> nil do
      begin
          Den := DenNode^.Data;
          Den^.DateLastTB := BadDate;
          DenNode := DenNode^.Next;
      end;
end;

(************************************************)
(***************    POSSUM    ***************)
(************************************************)

constructor Possum.Init(InitBirthday : Date; InitSex : SexType; InitDens : DenListPtr);
begin
  Birthday := InitBirthday;
  Sex    := InitSex;
  if InitDens = nil then DenMemory := New(DenListPtr, Init)
   else
      DenMemory := InitDens;
  Den     := nil;
  Joey    := nil;
  Mother  := nil;
  Pregnant := false;
  DateInfected := BadDate;
  DateClinical := BadDate;
  {Calculate maturity date}
  MaturityDate := Birthday + round(AgeMatureNormal(547,46,730,365));
  DateIndependent := BadDate;
  NoDenList := New(SListPtr, Init);
  Immigrant := true;
end;

destructor Possum.Done;
var
  SN : SNodePtr;
  DP : ^Date;
```

```
begin
  if Joey <> nil then Dispose(Joey, Done);
  if Den <> nil then LeaveDen;
  Dispose(DenMemory, Done);
  SN := NoDenList^.First;
  while SN <> nil do
      begin
          DP := SN^.Data;
          Dispose(DP);
          SN^.Data := nil;
          SN := SN^.Next;
      end;
  Dispose(NoDenList, Done);
end;

procedure Possum.Reproduce;

function DetermineSex : SexType;
const
  MaleProb  = 0.5;
begin
  if BirthSexRandom(BirthSexSeed) < Model.MaleBirthProb then
      DetermineSex := Female
   else
      DetermineSex := Male;
  end;
  { DetermineSex }

begin
  if Pregnant then
      begin
          Joey := New(PossumPtr, Init(Time.CurrentDate, DetermineSex, nil));
          Joey^.DateIndependent := Joey^.Birthday + round(AgeIndepNormal(AgeIndepSeed1, AgeIndepSeed2,150,10,180,120));
          Joey^.Mother := Addr(Self);
          Pregnant := false;
      end;
end; { Possum.Reproduce }

procedure Possum.Independence;
var
  MothersDen : SNodePtr;
begin
  if (DateIndependent <> BadDate) and (DateIndependent <= Time.CurrentDate) then
      begin
          { Give female joey den memory of mother }
          if Sex = Female then Den := Mother^.Den;
          if Model.UseDenMemory then
              begin
                  MothersDen := Mother^.DenMemory^.First;
                  while MothersDen <> nil do
                      begin
                          DenMemory^.AddDen(DenPtr(MothersDen^.Data));
                          MothersDen := MothersDen^.Next;
                      end;
              end;
          {Remove Mother-Joey link}
          Mother^.Joey := nil;
          Mother := nil;
          DateIndependent := BadDate;
          if Live then Pop.AddPossum(Addr(Self));
      end;
end;

function Possum.Live : boolean;  { Returns false if possum dies }
var
  LiveTmp : boolean;
begin
  LiveTmp := True;
  if DateClinical = BadDate then
      begin
          {for non-clinical possums}
          if Mature then
```

```
                begin
                    if (SurvAdultRandom(SurvAdultSeed) > Model.AdultSurvival[Time.Month, Sex]) then
                        LiveTmp := False;
                end
            else
                begin
                    if (SurvImmatureRandom(SurvImmatureSeed) > Model.ImmatureSurvival[Time.Month, Sex]) then
                        LiveTmp := False;
                end;
        end
    else
        begin
            if Mature then
                begin
                    if (TBSurvAdultRandom(TBSurvAdultSeed) > Model.ClinicalAdultSurvival[Time.Month]) then
                        LiveTmp := False;
                end
            else
                begin
                    if (TBSurvImmRandom(TBSurvImmSeed) > Model.ClinicalImmatureSurvival[Time.Month]) then
                        LiveTmp := False;
                end;
        end;
    if (Joey <> nil) and ((not LiveTmp) or (JoeySurvRandom(JoeySurvSeed) > Model.JoeySurvival[Joey^.Age div 30])) then
        begin
            Dispose(Joey, Done);
            Joey := nil;
        end;
    Live := LiveTmp;
end;

function Possum.Mature : boolean;
begin
    if MaturityDate <= Time.CurrentDate then
        Mature := true
    else
        Mature := false;
end;

function Possum.Age : integer;
begin
    Age := Time.CurrentDate - Birthday;
end;

function Possum.AgeCode : byte;
begin
    if Mother <> nil then
        AgeCode := 0
    else if Mature then
        AgeCode := 2
        else AgeCode := 1;
end;

procedure Possum.Infect;
{ Infect a possum }
var
    AgeStatus : byte;
begin
    if (DateInfected = BadDate) then
        begin
            DateInfected := Time.CurrentDate;
            inc(Pop.NewInfected[AgeCode,Sex]);
        end;
end;

function Possum.Infected : boolean;
begin
    if (DateInfected <> BadDate) then
        Infected := true
    else
        Infected := false;
end;
```

```
function Possum.Clinical : boolean;
begin
  if (DateClinical = BadDate) or (DateClinical > Time.CurrentDate) then
      Clinical := false
  else
      Clinical := true;
end;

procedure Possum.FindDen;
var
  Found, Found2 : boolean;
  Count : longint;
  DenNode : SNodePtr;
  OldDen  : DenPtr;
  TotalTravel : integer;
  DensTried : DenList;

function NodeInList( First, Node : SNodePtr) : boolean;
var
  Found : boolean;
  TriedNode : SNodePtr;
begin
  TriedNode := First;
  Found := false;
  while (not Found) and (TriedNode <> nil) do
      begin
          if TriedNode^.Data = Node^.Data then Found := true;
          TriedNode := TriedNode^.Next;
      end;
  NodeInList := Found;
end;

procedure FindEmpty(FirstProspect : SNodePtr; var Den : DenPtr; var Found : boolean; var TotalTravel : integer; UseCloseDens :
boolean);
var
  ClosestDen,                    { Closest den to target }
  Target : DenPtr;     { Last den visited }
  Distance, ShortestDistance : integer;
  ProspectiveNode, TriedNode : SNodePtr;
  Finished : boolean;
  NewDate : ^Date;
begin
  Target := Den;
  if FirstProspect <> nil then Finished := false else Finished := true;
  Found := false;
  while (not Finished) do
      begin
          ClosestDen := nil;
          ShortestDistance := maxint;
          if UseCloseDens then
              ProspectiveNode := Target^.CloseDens^.First
          else
              ProspectiveNode := FirstProspect;
          while (ProspectiveNode <> nil) do
              begin
                  Den := ProspectiveNode^.Data;
                  { Not already tried, calculate distance }
                  if (not NodeInList(DensTried.First, ProspectiveNode)) then
                      begin
                          Distance := round(sqrt(1.0 *(Target^.X-Den^.X) * (Target^.X-Den^.X) +
                          (Target^.Y-Den^.Y) * (Target^.Y-Den^.Y)));
                          if UseCloseDens then
                              begin
                                  ClosestDen := Den;
                                  ShortestDistance := Distance;
                                  ProspectiveNode := nil; end
                          else
                              begin
                                  if (Distance < ShortestDistance) then
                                      begin
                                          ShortestDistance := Distance;
```

```
                                    ClosestDen := Den;
                                end;
                            ProspectiveNode := ProspectiveNode^.Next;
                        end;
                    end
                else
                    begin
                        ProspectiveNode := ProspectiveNode^.Next;
                        { If end of close den list load in another one }
                        if UseCloseDens and (ProspectiveNode = nil) then
                            begin
                                ProspectiveNode := Target^.LoadNextDen;
                            end
                    end;
                end; { While }
            if ClosestDen = nil then
                begin
                    Found := false;
                    Finished := true;
                end
            else
                begin
                    TotalTravel := TotalTravel + ShortestDistance;
                    { Distance to next den is too far, possum didn't make it, therefore
                    make possum use last den visited whether occupied or not }
                    if TotalTravel > Model.MaxDenTravel then
                        begin
                            Found := true;
                            Den := Target;
                            Finished := true;
                            { Add new date to no den list }
                            New(NewDate);
                            NewDate^ := Time.CurrentDate;
                            NoDenList^.AddNode(New(SNodePtr,Init(NewDate)));
                        end
                    else
                        begin
                            DensTried.AddDen(ClosestDen);
                            { Is den occupied }
                            if (ClosestDen^.Empty)
                            and (DenRejectRandom(DenRejectSeed) > Model.DenRejectProb) then
                                begin
                                    Found := true;
                                    Den := ClosestDen;
                                    Immigrant := false;
                                    Finished := true;
                                end
                            else
                                begin
                                    Target := ClosestDen;
                                    Finished := False;
                                end;
                        end;
                end;
            end;
        if found then Den^.Occupants^.AddPossum(Addr(Self));
    end; { FindEmpty }
begin
    DensTried.Init;
    TotalTravel := 0;
    OldDen := Den;
    { Try last nights den first, or first den in memory if no den }
    if Den = nil then                { No den last night }
    if DenMemory^.First = nil then
        begin
            { Find den at random from all dens}
            Den := Dens.RandomNode^.Data; end
    else
        begin
            {Find a den at random in the memory and then use the closest den to it}
            Den := DenMemory^.RandomNode^.Data;
        end;
```

```
        if (Den^.Empty) and (DenRejectRandom(DenRejectSeed) > Model.DenRejectProb) then
            begin
                Found := true;
                Immigrant := false;
                Den^.Occupants^.AddPossum(Addr(Self));
                DenNode := New(SNodePtr, Init(Den));
                if (DenMemory^.First = nil) or (not NodeInList(DenMemory^.First, DenNode))  then
                    DenMemory^.AddDen(Den);
                Dispose(DenNode, Done);
             end
        else
            begin
                DensTried.AddDen(Den);
                Found := false;
                if Model.UseDenMemory then
                    begin
                        { Work through den memory }
                        DenNode := DenMemory^.First;
                        FindEmpty(DenNode, Den, Found, TotalTravel, false);
                    end;
        if (not Found) then
            begin
                { Starting from the last den tried work thru ALL dens using closest den list}
                DenNode := Dens.First;
                FindEmpty(DenNode, Den, Found, TotalTravel, true);
                { Add den to list, if applicable }
                DenNode := New(SNodePtr, Init(Den));
                if Found and (Model.UseDenMemory or (not NodeInList(DenMemory^.First, DenNode))) then
                    begin
                        DenMemory^.AddDen(Den);
                    end;
                Dispose(DenNode, Done);
            end;
        end;
        if not Found then Den := nil
         else
            if Clinical then
                Den^.DateLastTB := Time.CurrentDate;
  DensTried.Done;
end;

procedure Possum.LeaveDen;
var
  N, N2  : NodePtr;
begin
  if Den <> nil then
        begin
            N := Den^.Occupants^.First;
            while (N <> nil) do
                begin
                    if N^.Data = Addr(Self) then
                        begin
                            Den^.Occupants^.DeleteNode(N);
                            N := nil;
                         end
                     else
                        N := N^.Next;
                end;
        end;
end;

(*********************************************************)
(****************    POSSUMLIST    ********************)
(*********************************************************)

constructor PossumList.Init;
begin
   List.Init;
end;

destructor PossumList.Done;
begin
```

```
  List.Done;
end;

procedure PossumList.AddPossum(NewPossum : PossumPtr);
begin
  AddNode(New(NodePtr, Init(NewPossum)));
end;

(*************************************************************)
(*****************    POPULATION    ********************)
(*************************************************************)

constructor Population.Init;
begin
  PossumList.Init;
  FillChar(AgesHealthy, sizeof(AgesHealthy), char(0));
  FillChar(AgesClinical, sizeof(AgesClinical), char(0));
  FillChar(NewInfected, sizeof(NewInfected), char(0));
  FillChar(NewClinical, sizeof(NewClinical), char(0));
  AveNilDens := 0;
  NoInAve := 0;
end;

destructor Population.Done;
begin
  while First <> nil do DeletePossum(First);
  PossumList.Done;
end;

procedure Population.Fill;
var
  F      : text;
  NodeP  : NodePtr;
  AgeYears : integer;
  AgeMonths: integer;
  Bday, MidYear : Date;
  Ch     : char;
  Sex    : SexType;
  Possum  : PossumPtr;
  D      : integer;
begin
  MidYear := DMYToDate(1, 6, Time.Year);
  if MidYear > Time.CurrentDate then MidYear := MidYear - 365;
  DateDiff(MidYear, Time.CurrentDate, D, AgeMonths, AgeYears);
  Assign(F, PopFile);
  Reset(F);
  while not eof(F) do
      begin
          readln(F, AgeYears, Ch, Ch);
          Bday := IncDateTrunc(Time.CurrentDate, -1*AgeMonths, -1*AgeYears);
          if Ch = 'F' then Sex := Female else Sex := Male;
          AddPossum(New(PossumPtr, Init(Bday, Sex, nil)));
      end;
  Close(F);
  { Infect possums }
  NodeP := First;
  while NodeP <> nil do
      begin
          Possum := NodeP^.Data;
          if (TBPrevRandom(TBPrevSeed) <= Model.InitInfectProb) then
              begin
                  Possum^.DateClinical := Time.CurrentDate;
                  Possum^.DateInfected := Time.CurrentDate;
              end;
          NodeP := NodeP^.Next;
      end;
  Move;
  UpdateAges;
end;


procedure Population.DeletePossum(N : NodePtr);
```

```
var
  DeadPossum : PossumPtr;
begin
  DeadPossum := N^.Data;
  Dispose(DeadPossum, Done);
  DeleteNode(N);
end; { Population.Delete }

procedure Population.Immigration;
{ Calculated daily }
var
  Age, i, Mean : integer;
  Sex : integer;
  Bday : Date;
  Possum : PossumPtr;
  NewPossums : integer;
begin
  for Sex := Female to Male do
      begin
          if Time.Day = 1 then
              begin
              { Initialise monthly Poisson distribution }
                  Mean := Model.Immigration[Time.Month, Sex];
                  if Sex = 0 then DailyImmigrMale := mean / DaysInMonth(Time.Month, Time.Year);
                  if Sex = 1 then DailyImmigrFemale := mean / DaysInMonth(Time.Month, Time.Year);
              end;
          { Determine number of new possums }
          if Sex = 0 then
              begin
                  NewPossums := round(DailyImmigrFemalePoisson(DailyImmigrFemale,DailyImmigrFemaleSeed));
              end;
          if Sex = 1 then
              begin
                   NewPossums := round(DailyImmigrMalePoisson(DailyImmigrMale,DailyImmigrMaleSeed));
              end;
          { Set Birthday for new possums }
          Bday := IncDateTrunc(Time.CurrentDate, -1*(Time.Month+8), 0) + 15;
          for i := 1 to NewPossums do
              begin
                  Possum := New(PossumPtr, Init(Bday, Sex, nil));
                  { Test whether clinical or not }
                  if (TBImmigrRandom(TBImmigrSeed) <= Model.ImmigrantTBProb[Time.Month]) then
                      begin
                          Possum^.DateClinical := Time.CurrentDate;
                          Possum^.DateInfected := Time.CurrentDate;
                      end;
                  AddPossum(Possum);
              end;
      end;
end;

procedure Population.GoClinical;
{ Calculate the date which infected animals become clinical based on monthly probabilities, and distributed randomlly through the month
}
var
  Possum : PossumPtr;
  Node   : NodePtr;
begin
  Node := First;
  while Node <> nil do
      begin
          Possum := Node^.Data;
          if (Possum^.Infected) and not(Possum^.Clinical) and
          (SubToClinRandom(SubToClinSeed) <= Model.GoClinicalProb[Time.Month]) then
              begin
                  Possum^.DateClinical := Time.CurrentDate + trunc(random * DaysInMonth(Time.Month, Time.Year));
                  inc(Pop.NewClinical[Possum^.AgeCode,Possum^.Sex]);
              end;
          Node := Node^.Next;
      end;
end;
```

```
procedure Population.Mating;
{ Mating is based on a buffer zone and a probability of mating. All males in the buffer zone around a female are tested to see if they mate
successfully }
var
  FemaleNode,
  MaleNode     : NodePtr;
  DNode        : SNodePtr;
  NextDen      : DenPtr;
  FemalePossum,
  MalePossum   : PossumPtr;
begin
  FemaleNode := first;
  while FemaleNode <> nil do
      begin
          FemalePossum := FemaleNode^.Data;
          {Select only female possums that are not pregnant or have joeys}
          if (FemalePossum^.Sex = Female) and (FemalePossum^.Joey = nil)
          and FemalePossum^.Mature and (not FemalePossum^.Pregnant) then
              begin
              {Check each den closest to the female for a male possum}
                  DNode := FemalePossum^.Den^.CloseDens^.First;
                  NextDen := DNode^.Data;
                  while (FemalePossum^.Den^.Distance(NextDen^.X,NextDen^.Y) <= Model.MatingBuffer)
                  and (not FemalePossum^.Pregnant) do
                      begin
                      {Search through occupants for a male}
                          MaleNode := NextDen^.Occupants^.First;
                          while (MaleNode <> nil) do
                              begin
                                  MalePossum := MaleNode^.Data;
                                  if (MalePossum^.Sex = Male) and
                                  (MatingRandom(MatingSeed) <= Model.MatingProb[Time.Month]) then
                                      begin
                                          FemalePossum^.Pregnant := true;
                                          if (FemalePossum^.Clinical) and
                                          (TBMatingRandom(TBMatingSeed) <= Model.MatingTBProb) then
                                              begin
                                                  MalePossum^.Infect;
                                              end;
                                          if (MalePossum^.Clinical) and
                                          (TBMatingRandom(TBMatingSeed) <= Model.MatingTBProb) then
                                              FemalePossum^.Infect;
                                      end;
                                  MaleNode := MaleNode^.Next;
                              end;
                          DNode := DNode^.Next;
                          if DNode = nil then DNode := FemalePossum^.Den^.LoadNextDen;
                          NextDen := DNode^.Data;
                      end;
                  end;
              FemaleNode := FemaleNode^.Next;
          end;
end; { Population.Mating }

function Population.Fecundity : real;
var
  PNode     : NodePtr;
  Joeys,
  Total     : integer;
begin
  Joeys := 0;
  Total :=0;
  PNode := First;
  while PNode <> nil do
      begin
          if PossumPtr(PNode^.Data)^.Sex = Female then
              begin
                  if PossumPtr(PNode^.Data)^.Mature then inc(Total);
                  if PossumPtr(PNode^.Data)^.Joey <> nil then inc(Joeys);
              end;
          PNode := PNode^.Next;
      end;
```

```
  if Total = 0 then Fecundity := 0 else Fecundity := Joeys/Total;
end;

procedure Population.TB;
{ Four mechanisms of TB infection are included here: a) Denning in a clinical den (clinical den), b) Denning in a buffer zone around an
clinical possum, c) Mother (clinical) to joey}
var
  N, PN : NodePtr;
  Possum, P : PossumPtr;
  Count, Offset : integer;
  DenNode : SNodePtr;
  Den : DenPtr;
begin
  N := First;
  while N <> nil do
      begin
          Possum := N^.Data;
          { Denning in infected den }
          if (not Possum^.Clinical) then
              begin
                  if (Possum^.Den <> nil) and (Possum^.Den^.Clinical) and
                  (TBDenRandom(TBDenSeed) <= Model.DenTBProb) then
                      Possum^.Infect;
               end
           else
              begin
              { Mother to joey transfer}
                  if (Possum^.Joey <> nil) then Possum^.Joey^.Infect;
                  {Denning in buffer zone}
                  if Possum^.Den <> nil then
                      DenNode := Possum^.Den^.CloseDens^.First
                   else
                      DenNode := nil;
                  while (DenNode <> nil) do
                      begin
                          Den := DenNode^.Data;
                          { Is Den in buffer zone }
                          if (Den^.Distance(Possum^.Den^.X,Possum^.Den^.Y) < Model.DenTBBuffer) then
                              begin
                              { Infect all possums in this Den, even if current den }
                                  PN := Den^.Occupants^.First;
                                  while PN <> nil do
                                      begin
                                          P := PN^.Data;
                                          if (TBBufferRandom(TBBufferSeed) <= Model.BufferTBProb) then P^.Infect;
                                          PN := PN^.Next;
                                  end; { while }
                                  if (DenNode^.Next = nil) then
                                      begin
                                          DenNode := Possum^.Den^.LoadNextDen;
                                       end
                                   else
                                      DenNode := DenNode^.Next;
                               end
                           else
                              DenNode := nil;
                      end; {While}
              end; {Clinical Possum}
      N := N^.Next;
  end;
end; { Population.TB }

procedure Population.UpdateAges;
var
  N  : NodePtr;
  Age : integer;
  Sex : integer;
  Possum : PossumPtr;
begin
  for Age := 0 to MaxAge do
      for Sex := Female to Male do
          begin
```

```
                AgesHealthy[Age, Sex] := 0;
                AgesInfected[Age, Sex] := 0;
                AgesClinical[Age, Sex] := 0;
            end;
        N := First;
        while N <> nil do
            begin
                Possum := N^.Data;
                Age := Possum^.Age div 365;
                if Age > MaxAge then Age := MaxAge;
                if Possum^.Clinical then
                    inc(AgesClinical[Age, Possum^.Sex])
                else if Possum^.Infected then
                        inc(AgesInfected[Age, Possum^.Sex])
                     else
                        inc(AgesHealthy[Age, Possum^.Sex]);
                if Possum^.Joey <> nil then
                    begin
                        Age := 0;
                        Sex := Possum^.Joey^.Sex;
                        if Possum^.Joey^.Clinical then
                            inc(AgesClinical[Age, Sex])
                        else if Possum^.Joey^.Infected then
                                inc(AgesInfected[Age, Sex])
                             else
                                inc(AgesHealthy[Age, Sex]);
                    end;
                N := N^.Next;
            end;
end; { Population.UpdateAges }

procedure Population.UpdateAveNilDens(NewValue : integer);
begin
  inc(NoInAve);
  if NoInAve = 1 then AveNilDens := NewValue
       else AveNilDens := AveNilDens + (NewValue - AveNilDens)/NoInAve;
end;

procedure Population.Grow;
{ Perform life functions on each possum. Note that reproduction occurs before death and joeys are subject to death, ie each loop starts a
new breeding season}
var
  N, N2 : NodePtr;
  Joey, Adult : PossumPtr;
  JoeyPop : Population; { Temporary object to hold newly independent joeys }
begin
  JoeyPop.Init;
  N := First;
  while N <> nil do
       begin
            Adult := N^.Data;
            if Adult^.Joey <> nil then
                 Adult^.Joey^.Independence;
            Adult^.Reproduce;
              N2 := N^.Next;       { Copy N in case possum dies, but only after  any new possums are added }
            if not Adult^.Live then DeletePossum(N);
            N := N2;
       end;
  { Insert independent joeys into the population }
  while JoeyPop.First <> nil do
       begin
            Adult := JoeyPop.First^.Data;
            if Adult^.Live then AddPossum(Adult);
            JoeyPop.DeleteNode(JoeyPop.First);
       end;
  JoeyPop.Done;
  UpdateAges;
end; { Population.Grow }


procedure Population.Move;
```

```
function DensEmpty : boolean;
var
  DenNode : SNodePtr;
  found   : boolean;
begin
  found := false;
  DenNode := Dens.First;
  while DenNode <> nil do
      begin
          if DenPtr(DenNode^.Data)^.Occupants^.Length <> 0 then
              found := true;
          DenNode := DenNode^.Next;
      end;
  if found then DensEmpty := false else DensEmpty := true;
end; { Dens Empty }
var
  N : NodePtr;
  Possum : PossumPtr;
  Count, Offset : integer;
begin
  N := First;
  while N <> nil do
      begin
          Possum := N^.Data;
          Possum^.LeaveDen;
          N := N^.Next;
      end;
{ Reallocate dens, start with random possum }
  Offset := Random(Length);
  N := First;
  while Offset > 0 do
      begin
          N := N^.Next;
          Offset := Offset - 1;
      end;
  Count := 0;
  repeat
      if N = nil then N := First;
      Possum := N^.Data;
      Possum^.FindDen;
      N := N^.Next;
      inc(Count);
  until Count = Length;
end;

procedure Population.DensityDynamics;
{ This procedure simulates the emmigration and/or death of possums due to competition for den sites }
var
  Possum    : PossumPtr;
  PNode,
  PNodeNext : NodePtr;
  FirstDate : ^Date;
  PossumGoes : boolean;
  Count     : integer;
begin
  PNode := First;
  Count := 0;
  while PNode <> nil do
      begin
          PossumGoes := false;
          Possum    := PNode^.Data;
          PNodeNext := PNode^.Next;
          if Possum^.NoDenList^.First <> nil then
              begin
                  { Test dying or naffing off due to lack of dens }
                  if (Possum^.NoDenList^.Length > Model.ResidentDenThreshold) and
                   (PopDensEmigrRandom(PopDensEmigrSeed) <=
                  Model.NoDenMortality[Time.Month]/DaysInMonth(Time.Month,Time.Year)) then
                      begin
                          PossumGoes := true;
                      end
                  else
```

```
                    begin
                        FirstDate := Possum^.NoDenList^.First^.Data;
                        if (Time.CurrentDate - FirstDate^) = Model.ResidentDenWindow then
                            begin
                                Dispose(FirstDate);
                                Possum^.NoDenList^.DeleteNode(Possum^.NoDenList^.First);
                            end;
                    end;
                end;
            if Possum^.Immigrant then
                begin
                    if Possum^.NoDenList^.Length > Model.ImmigrantDenThreshold then
                        begin
                            PossumGoes := true;
                        end;
                end;
            if PossumGoes then
                begin
                    DeletePossum(PNode);
                end;
            PNode := PNodeNext;
        end; { While }
end; {Population.DensityDynamics}

function Population.Size : integer;
var
  Age, Sex, i, tmp : integer;
begin
  tmp := 0;
  for Age := 0 to MaxAge do
      for Sex := Female to Male do
          tmp := tmp + AgesHealthy[Age, Sex] + AgesInfected[Age,Sex] +AgesClinical[Age, Sex];
      Size := tmp;
end ;

function Population.NoMales(Status : byte) : integer;
var
  Age, Sex, tmp : integer;
begin
  tmp := 0;
  for Age := 0 to MaxAge do
      case Status of
          pHealthy  : tmp := tmp + AgesHealthy[Age, Male];
          pInfected : tmp := tmp + AgesInfected[Age, Male];
          pClinical : tmp := tmp + AgesClinical[Age, Male];
      end;
  NoMales := tmp;
end;

function Population.NoFemales(Status : byte) : integer;
var
  Age, Sex, tmp : integer;
begin
  tmp := 0;
  for Age := 0 to MaxAge do
      case Status of
          pHealthy  : tmp := tmp + AgesHealthy[Age, Female];
          pInfected : tmp := tmp + AgesInfected[Age, Female];
          pClinical : tmp := tmp + AgesClinical[Age, Female];
      end;
  NoFemales := tmp;
end;

function Population.NilDens : integer;
var
  tmp : integer;
  Node : NodePtr;
  Possum : PossumPtr;
begin
  Tmp := 0;
  Node := First;
  while Node <> nil do
```

```
        begin
            Possum := Node^.Data;
            if Possum^.Den = nil then inc(Tmp);
            Node := Node^.Next;
        end;
    NilDens := Tmp;
end;

procedure Population.PrintAges;
var
    Age, Sex : integer;
begin
    writeln('        ', '0':5,'1':5, '2':5, '3':5, '4':5, '5':5);
    writeln;
    for Sex := Female to Male do
        begin
            if Sex = Female then
                write('Female':10)
             else
                write('Male':10);
    for Age := 0 to 5 do
        write(AgesHealthy[Age, Sex]:5);
        writeln;
    end;
    readln;
end;

function Population.AgeSummary : string;
const
    asJoey      = 0;
    asImmature  = 1;
    asMature    = 2;
    asClear     = 0;
    asInfected  = 1;
    asClinical  = 2;
    asNewInfected = 3;
    asNewClinical = 4;
var
    Data    : array [Female..Male, asJoey..asMature, asClear..asNewClinical] of integer;
    Node    : NodePtr;
    Possum  : PossumPtr;
    Age, Status, Sex    : integer;
    Result  : string;
begin
    Node := First;
    FillChar(Data, sizeof(Data), char(0));
    while Node <> nil do
        begin
            Possum := Node^.Data;
            if Possum^.Mature then Age := asMature else Age := asImmature;
            if Possum^.Clinical then Status := asClinical
            else if Possum^.Infected then Status := asInfected
                else Status := asClear;
            inc(Data[Possum^.Sex, Age, Status]);
            if Possum^.Joey <> nil then
                begin
                    if Possum^.Joey^.Clinical then Status := asClinical
                    else if Possum^.Joey^.Infected then Status := asInfected
                        else Status := asClear;
                    inc(Data[Possum^.Joey^.Sex, asJoey, Status]);
                end;
            Node := Node^.Next;
        end;
    Result := '';
    for Status := asClear to asClinical do
        for Age := asMature downto asJoey do
            for Sex := Male downto Female do
                begin
                    Result := Result + Long2Str(Data[Sex,Age,Status]) + ',';
                    if Status = asInfected then
                        Result := Result + Long2Str(NewInfected[Age,Sex]) + ','
                     else
```

```
                    if Status = asClinical then
                        Result := Result + Long2Str(NewClinical[Age,Sex]) + ',';
                end;
                Result[0] := chr(ord(Result[0]) - 1);
                AgeSummary := Result;
                FillChar(NewInfected, sizeof(NewInfected), char(0));
                FillChar(NewClinical, sizeof(NewClinical), char(0));
            end;

procedure Population.PrintList;  { Print population to screen }
var
  N : NodePtr;
  Possum : PossumPtr;
begin
  N := First;
  while N <> nil do
      begin
          Possum := N^.Data;
          write(Possum^.Age:2, Possum^.Sex);
          if Possum^.Joey <> nil then
              write(Possum^.Joey^.Age:2, Possum^.Joey^.Sex, ' ')
           else
              write('    ');
              N := N^.Next;
          end;
  writeln;
end;

procedure Population.PrintDenSummary;
{ Summary of the number of number of possum with den memories of a  certain size }
const
  DenLimit = 14;
var
  DenPrintSummary : array [0..DenLimit] of integer;
  N : NodePtr;
  P : PossumPtr;
  i : integer;
  Sum : longint;
begin
  Sum := 0;
  for i := 0 to DenLimit do DenPrintSummary[i] := 0;
  N := First;
  while N <> nil do
      begin
          P := N^.Data;
          i := P^.DenMemory^.Length;
          Sum := Sum + i;
          if i > DenLimit then i := DenLimit;
          inc(DenPrintSummary[i]);
          N := N^.Next;
      end;
  for i := 0 to DenLimit do
      write(DenPrintSummary[i]:4);
  writeln(Sum/Length:6:2);
end;

function Population.DenSummary : string;
{ Summary of the number of number of possum with den memories of a certain size written to file}
const
  DenLimit = 14;
var
  DenFileSummary : array [0..DenLimit] of integer;
  N : NodePtr;
  P : PossumPtr;
  i : integer;
  Sum : longint;
  DenSummaryString : string;
begin
  DenSummaryString := ' ';
  Sum := 0;
  for i := 0 to DenLimit do DenFileSummary[i] := 0;
  N := First;
```

```
  while N <> nil do
      begin
          P := N^.Data;
          i := P^.DenMemory^.Length;
          Sum := Sum + i;
          if i > DenLimit then i := DenLimit;
          inc(DenFileSummary[i]);
          N := N^.Next;
      end;
  for i := 0 to DenLimit do
      begin
          DenSummaryString := DenSummaryString + Long2Str(DenFileSummary[i]) + ',';
      end;
  DenSummary := DenSummaryString;
end;

procedure Population.PrintNonDens;
{ Summary of the frequency of non denning possums }
const
  Limit = 14;
var
  Summary : array [0..Limit] of integer;
  N : NodePtr;
  P : PossumPtr;
  i : integer;
  Sum : longint;
begin
  Sum := 0;
  for i := 0 to Limit do Summary[i] := 0;
  N := First;
  while N <> nil do
      begin
          P := N^.Data;
          i := P^.NoDenList^.Length;
          Sum := Sum + i;
          if i > Limit then i := Limit;
          inc(Summary[i]);
          N := N^.Next;
      end;
  for i := 0 to Limit do
      write(Summary[i]:4);
  writeln(Sum/Self.Length:6:2);
end;

function Population.NonDenSummary : string;
{ Summary of the frequency of non denning possums during a month}
const
  Limit = 14;
var
  NoDSummary : array [0..Limit] of integer;
  N : NodePtr;
  P : PossumPtr;
  i : integer;
  Sum : longint;
  NonDenSummaryString : string;
begin
  NonDenSummaryString := ' ';
  Sum := 0;
  for i := 0 to Limit do NoDSummary[i] := 0;
  N := First;
  while N <> nil do
      begin
          P := N^.Data;
          i := P^.NoDenList^.Length;
          Sum := Sum + i;
          if i > Limit then i := Limit;
          inc(NoDSummary[i]);
          N := N^.Next;
      end;
  for i := 0 to Limit do
      begin
          NonDenSummaryString := NonDenSummaryString + Long2Str(NoDSummary[i]) + ',';
```

```
        end;
  NonDenSummary := NonDenSummaryString;
end;

procedure UpdateSummary (S : SummaryPtr; P : Population);
var
  Age, Sex : integer;
  X : longint;
begin
  for Age := 0 to MaxAge do
      for Sex := Female to Male do
          begin
              X := P.AgesHealthy[Age,Sex];
              S^.Sum[Age,Sex]   := S^.Sum[Age,Sex]   + X;
              S^.Sumsq[Age,Sex] := S^.Sumsq[Age,Sex] + X*X;
          end;
end;

procedure TMyApplication.InitMainWindow;
begin
  MainWindow := New(PMyWindow, Init(nil, 'Welcome to PossPop'));
end;

procedure TMyWindow.About(var Msg: TMessage);
var
  AboutWnd: TDialog;
begin
  AboutWnd.Init(@Self, 'About');
  AboutWnd.Execute;
  AboutWnd.Done;
 end;

procedure TMyWindow.RunModel(var Msg: TMessage);  { Procedure to run model in window}
var
  choice : integer;
  RunQuit : string;
begin
  StrCopy(WindowTitle,'PossPop');
  InitWinCrt;
  writeln('  Welcome to the Computer Simulation Model');
  writeln('              "PossPop"');
  writeln;
  writeln('developed by D.U.Pfeiffer, M.Stern and R.S.Morris');
  writeln('         programmed by M.Stern');
  writeln('   Dept. Vet. Clin. Sci., Massey University');
  writeln('      Palmerston North, New Zealand');
  writeln;
  writeln;
  Model.Init;
  writeln(MemAvail);
  Dens.Init;
  Dens.SetFromFile(DenMapFile);
  writeln(MemAvail);
  writeln(Dens.Length,' dens loaded.'); {press return to continue');readln;}
  Dens.LoadGeography;
  repeat
      write('Enter R to run model, D to calculate den distances or Q to exit (R=default): ');
      readln(RunQuit);
      RunQuit := UpCase(RunQuit[1]);
      Choice := 0;
      if RunQuit = 'R' then Choice := 0;
      if RunQuit = 'D' then Choice := 7;
      if RunQuit = 'Q' then Choice := 2;
      case Choice of
              0  : Model.Go;
              7  : Dens.CalculateGeography;
              2  : DoneWinCrt;
      end; {Case}
  until Choice = 2;
end;

procedure TMyWindow.MakeFont;
```

```
var
  MyLogFont : TLogFont;
begin
  with MyLogFont do
      begin
          lfHeight := 30;
          lfWidth := 0;
          lfEscapement := 0;
          lfOrientation := 0;
          lfWeight := fw_Bold;
          lfItalic := 0;
          lfUnderline := 0;
          lfStrikeOut := 0;
          lfCharSet := ANSI_CharSet;
          lfOutPrecision := Out_Default_Precis;
          lfClipPrecision := Clip_Default_Precis;
          lfQuality := Default_Quality;
          lfPitchAndFamily := Variable_Pitch or ff_Swiss;
          StrCopy(@lfFaceName, 'Helv');
      end;
  MainFontRec := MyLogFont;
end;

procedure TMyWindow.Paint(PaintDC : HDC; var PaintInfo: TPaintStruct);
var
  DC, MemDC: HDC;
  OldBitMap, BitMap: HBitMap;
  BM: TBitMap;
  TextString1, TextString2, TextString3 : array[0..20] of Char;
  MyBitMap : HBitMap;
begin
  BitMap := LoadBitMap(HInstance, 'Possum2');
  MemDC := CreateCompatibleDC(PaintDC);
  OldBitMap := SelectObject(MemDC, BitMap);
  GetObject(BitMap, SizeOf(BM), @BM);
  with SplashRect do
      begin
          Left := 50;
          Top := 70;
          Right := Left + BM.bmWidth;
          Bottom := Top + BM.bmHeight;
          BitBlt(PaintDC, Left, Top, BM.bmWidth, BM.bmHeight, MemDC, 0, 0, SRCCopy);
      end;
  DeleteObject(SelectObject(MemDC, OldBitMap));
  DeleteDC(MemDC);
  DeleteDC(PaintDC);
  StrCopy(TextString1, 'PossPop');
  TextOut(PaintDC, 200, 10, TextString1, StrLen(TextString1));
  StrCopy(TextString2, 'a Computer Simulation Model');
  TextOut(PaintDC, 125, 30, TextString2, StrLen(TextString2));
  StrCopy(TextString3, 'of Bovine Tuberculosis Infection in Feral Possum Populations');
  TextOut(PaintDC, 25, 50, TextString3, StrLen(TextString3));
end;

constructor TMyWindow.Init(AParent: PWindowsObject; ATitle: PChar);
begin
  TWindow.Init(AParent, ATitle);
  with attr do
      begin
          w:=550;{ Force window size }
          h:=650;
      end;
end;

procedure TMYWindow.GetWindowClass(var WndClass: TWndClass);
begin
  TWindow.GetWindowClass(WndClass);
  WndClass.hIcon := LoadIcon(HInstance, 'PosIco');
  WndClass.Style := CS_DBLCLKS;   { Respond to double click }
  WndClass.lpszMenuName := 'MainMenu';
end;
```

```
(*************************************************************)
(*****************      MAIN      ********************)
(*************************************************************)

var
  MyApp: TMyApplication;
begin
  MyApp.Init('Posspo10');
  MyApp.Run;
  MyApp.Done;
  InvalidateRect(0, @SplashRect, True);
end.
```