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A survey of leptospiral infection in dogs and rats in the lower North Island of New Zealand

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Moses Kwabena Kakrada

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ABSTRACT

The increasing number of human cases of leptospirosis due to *Leptospira interrogans* serovars *copenhageni*, *ballum* and *tarassovi* has drawn much attention to the epidemiology of the disease in New Zealand.

Although dogs and humans are commonly known to be infected with *Leptospira interrogans* serovar *copenhageni* in the upper half of the North Island of New Zealand, available scientific data indicate infection with this serovar is not common in other parts of the country.

The brown rat (*Rattus norvegicus*) has been incriminated in the natural maintenance of serovars of the Icterohaemorrhagiae serogroup (*icterohaemorrhagiae*, *copenhageni*) overseas and in certain parts of New Zealand. Some veterinary practitioners in the lower North Island region are actively promoting vaccination of dogs against leptospirosis while others think that this is unnecessary. The canine anti-leptospiral vaccine in use in New Zealand is protective only against serovar *copenhageni*.

In an attempt to investigate the above issues, a one-off serological survey of 355 dogs from selected areas of the lower half of the North Island was undertaken, using the microscopic agglutination test (MAT). A minimum dilution of 1:24 was used in this test. The antigens used in the MAT were serovars *copenhageni*, *pomona*, *hardjo*, *ballum*, *tarassovi* and *canicola*. In addition, 24 captured rats (17 ship and 7 brown) from the study area were investigated serologically and the isolation of leptospires attempted.

Of the 355 sera tested, 50 (14%) showed $\geq 50\%$ agglutination against *copenhageni*, 34 (9.6%) against *ballum*, 60 (16.9%) against *hardjo*, 11 (3.1%) against *pomona*, 107 (30%) against serovar *canicola* and 3 (0.8%) against *tarassovi*. Titres ranged from 24 to 3072 for *copenhageni*, 24 to 384 for *ballum* and *canicola*, 24 to 192 for *pomona* and 24 to 96 for *hardjo*. All reactors to *tarassovi* had a titre of 24.

Twenty one of the 50 sera that reacted to serovar *copenhageni* were collected from recently-vaccinated (7-9 days) dogs. Sera from five other dogs, which were known to be vaccinated, more than 200 days prior to sample collection, did not react to this serovar.

No leptospires were isolated from the rat kidneys after three months of culture in liquid EMJH medium.

Sera from six rats (4 ship and 2 brown) showed reactions of $\geq 50\%$ agglutination against serovar *ballum*. Titres ranged from 24 to 192. Three animals (2 brown rats and 1 ship rat) showed reactions of $\geq 50\%$ agglutination at the 1:24 dilution against serovar *canicola*. Only one ship rat had a titre of 384 against serovar *hardjo* and none of them showed a reaction of $\geq 50\%$ agglutination against serovars *copenhageni*, *pomona* or *tarassovi* at any dilution.

The results of this study provide some evidence that dogs in parts of the lower half of the North Island of New Zealand are being exposed to *Leptospira interrogans* serovar *copenhageni* and that this serovar could be playing an important role in canine leptospirosis. Exposure of dogs to serovars *ballum*, *hardjo* and *pomona* also appears to be common. The significance of the reactions to serovar *canicola* could not be ascertained due to lack of standard serum and ambiguity with respect to the readings of the MAT for this serovar.

The importance of rats as reservoir for serovar *copenhageni* in the study area could not be determined, due to the fact that very few rats were examined. The lack of relevant data on dogs also prevented the identification of risk factors associated with canine leptospirosis.

It is recommended that dog owners be encouraged to vaccinate their dogs against leptospirosis. In addition to serovar *copenhageni*, serovars *ballum*, *pomona* and *hardjo*, should be considered in the differential diagnosis of canine leptospirosis.

It is also recommended that a more detailed longitudinal study of dogs be undertaken to help further understand the infection status as well as risk factors with respect to infection with serovar *copenhageni*, as well as to clarify the situation with serovar *canicola*. It is further recommended that rats and other New Zealand mammals such as mice, hedgehogs and deer be investigated as possible sources of *copenhageni* infection for dogs and humans.

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TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS.....	iv
TABLE OF CONTENTS.....	v
LIST OF FIGURES	vii
LIST OF TABLES	viii
LIST OF APPENDICES.....	ix
1 INTRODUCTION.....	1
2 AIMS	4
3 LITERATURE REVIEW	5
3.1 THE HISTORY OF LEPTOSPIROSIS	5
3.2 CLASSIFICATION OF <i>LEPTOSPIRA</i>	8
3.2.1 <i>Serovar copenhageni and its origin</i>	11
3.3 EPIDEMIOLOGY AND PUBLIC HEALTH.....	12
3.4 LEPTOSPIROSIS IN ANIMALS	15
3.5 LEPTOSPIROSIS IN MAN	16
3.6 SOME ASPECTS OF LEPTOSPIROSIS IN NEW ZEALAND.....	17
3.6.1 <i>Maintenance hosts and serovars in New Zealand</i>	17
3.6.2 <i>Porcine leptospirosis in New Zealand</i>	20
3.6.3 <i>Bovine leptospirosis in New Zealand</i>	20
3.6.4 <i>Ovine Leptospirosis in New Zealand</i>	22
3.6.5 <i>Cervine Leptospirosis in New Zealand</i>	23
3.6.6 <i>Canine Leptospirosis in New Zealand</i>	23
3.6.7 <i>Leptospirosis in New Zealand Rodents</i>	26
4 MATERIALS AND METHODS.....	29
4.1 DOGS - SAMPLE COLLECTION AND QUESTIONNAIRE	29
4.2 RATS - SAMPLE COLLECTION	29
4.3 SEROLOGICAL METHODS	33
4.3.1 <i>Notes on the Microscopic Agglutination Test (MAT)</i>	33
4.3.2 <i>Procedure for the MAT</i>	33
4.4 BACTERIOLOGICAL METHODS	36
4.4.1 <i>Notes on EMJH Culture Medium</i>	36
4.4.2 <i>Procedure for Preparation of EMJH Culture Medium</i>	36
4.4.3 <i>Culture Procedure</i>	38
5 RESULTS.....	39
5.1 DOGS	39
5.2 RATS.....	40
5.2.1 <i>Bacteriological examination</i>	40
5.2.2 <i>Serology</i>	40
6 DISCUSSION	43

6.1 DOGS.....	43
6.1.1 <i>Serovar copenhageni</i>	43
6.1.2 <i>Serovar canicola</i>	44
6.1.3 <i>Other serovars</i>	45
6.2 RATS.....	46
7 CONCLUSIONS AND RECOMMENDATIONS	47
APPENDICES	49
REFERENCES.....	52

LIST OF FIGURES

Figure 1. Regional map of the North Island of New Zealand	31
Figure 2. District map of the lower North Island of New Zealand	32
Figure 3. Schematic diagram of serological procedure (MAT).	35

LIST OF TABLES

Table 1. Some early isolations of leptospire in New Zealand	18
Table 2. Total number of canine sera with MAT titres	39
Table 3. Number of canine sera with MAT titres (vaccinated dogs)	40
Table 4. Trapping data for rats	42
Table 5. Total number of rat sera with MAT titre	42

LIST OF APPENDICES

Appendix I. Individual MAT titres for vaccinated dogs	49
Appendix II. Individual MAT titres for all dogs and rats (Microsoft Access tables on floppy disk)	50
Appendix III. Blood sample collection form for dogs	51

1 INTRODUCTION

Leptospirosis, an infectious bacterial disease caused by various serovars of the genus *Leptospira*, is of economic importance in farm animals as well as an important zoonosis in human beings. The disease is found worldwide due to the organisms' ability to infect most species of warm-blooded animals. A key factor in the epidemiology and control of leptospirosis is the fact that specific animal host populations maintain each serovar in a particular geographical region.

The use of vaccines has substantially reduced the incidence of the disease, particularly in developed countries, but the huge number of leptospiral serovars and the wide range of animals (both domestic and wild) that serve as carriers make it extremely difficult to implement an eradication program in any country. In addition, the appropriate and highly effective treatment of leptospirosis with antibiotics and fluids is rarely implemented because of the difficulties involved in the accurate diagnosis of the disease. The various epidemiological and clinical forms of leptospirosis have resulted in a large number of synonyms being used to describe the disease throughout the years in many parts of the world. The list of synonyms that can be found in textbooks and other scientific publications includes the following: Weil's disease, Stuttgart disease, cane cutter's disease, Fort Bragg fever, fish handler's disease, swamp fever, canicola fever, mud fever, field fever, redwater, infectious jaundice, pretibial fever, marsh fever, harvest fever, Japanese autumnal fever, mouse fever, rice field worker's fever, pea picker's fever, swineherd's disease and haymaker's disease (Torten, 1979).

Generally speaking, the prevalence of leptospirosis has been on a steady decline in most developed countries since the 1970s. This can certainly be attributed to extensive use of vaccines and other control measures. The situation in developing countries is more difficult to quantify because most of these countries lack the necessary equipment to enable them to gather reliable epidemiological data (Torten and Marshall, 1994).

Leptospiral vaccines are serovar-specific and their use is usually limited to the control of infections that result from the serovars that are included in them. These vaccines are widely used in the control of leptospirosis in dogs, pigs and cattle (Faine, 1994). Vaccines for humans have been used in China, Japan and Vietnam (Chen, 1986; Fukumura, 1984; Dang Duc Trach and Trinh Thi Hang Quy, 1975).

In New Zealand, six pathogenic leptospiral serovars are recognised in animals. Serovars

hardjo, *pomona* and *tarassovi* are maintained by domestic animals but *ballum*, *balcanica* and *copenhageni* are maintained in wildlife. Whereas serovars *hardjo*, *pomona*, *tarassovi*, *ballum* and *balcanica* are known to be endemic in most parts of the country, *copenhageni* is considered to be confined to the Northland, Waikato and Auckland regions (Ellison and Hilbink, 1990; Hilbink *et al.*, 1992) (see also Figure 1).

Leptospirosis is of great concern in New Zealand due to the economic loss from death and ill health of farmed animals and the socio-economic implications of pet and human illness. Not surprisingly, the disease in humans has been notifiable since 1952 in this country (Faine and Kirschner, 1953).

The use of vaccines in cattle and pigs may have led to a drop in the incidence of clinical cases of leptospirosis in both animals and humans in New Zealand, but the disease still merits a lot of public health considerations. The annual number of laboratory-reported human cases recorded in 1995, 1996, and 1997 were all less than 100 (Anonymous, 1995; Anonymous, 1996; Anonymous, 1997).

Clinical leptospirosis in dogs in this country is mostly due to serovar *copenhageni* (Ellison and Hilbink, 1990). Dogs and humans may be exposed to *copenhageni* infection from the same source(s) as they share a common habitat.

Of late, there has been an increase in interest in the epidemiology of serovars *copenhageni*, *ballum* and *tarassovi* not only because other serovars (*hardjo* and *pomona*) are somewhat under control but also because there is an increasing number of human cases due to these serovars. Human leptospirosis due to serovar *copenhageni*, as well as due to *ballum*, has been included in the list of emerging diseases in New Zealand (Anonymous, 1998a).

Limited studies have been carried out on canine leptospirosis in New Zealand. Although *L. copenhageni* infection is known to be present in the north of the North Island, available scientific data indicates infection with this serovar is not common in the lower half of the North Island (Brockie, 1977; Hathaway, 1978; Ellison and Hilbink, 1990; Hilbink *et al.*, 1992).

The brown rat (*Rattus norvegicus*) has been incriminated in the natural maintenance of serovars of the Icterohaemorrhagiae (*icterohaemorrhagiae*, *copenhageni*) serogroup overseas and in certain parts of New Zealand.

An increasing number of sick dogs in the lower North Island have been presenting with clinical signs suggestive of leptospirosis (Hill and Thompson, 1997). This has led to a situation whereby some veterinary practitioners in this area are actively promoting vaccination of dogs against leptospirosis while others think that this is unnecessary.

The canine anti-leptospiral vaccine in use in New Zealand is protective against serovar *copenhageni* but not against the other serovars present in the country.

2 AIMS

The aims of this study are threefold:

1. To investigate the prevalence of *Leptospira interrogans* serovar *copenhageni* infection in dogs in the lower half of the North Island of New Zealand.
2. To confirm the reservoir status of the brown rat (*R. norvegicus*) in this region.
3. To identify risk factors associated with exposure of dogs to serovar *copenhageni*.

To achieve these aims, a one-off serological survey of dogs in selected areas of the lower half of the North Island was undertaken. In addition, isolation of leptospires from captured rats and a serological investigation of these rats from the lower North Island were attempted.

3 LITERATURE REVIEW

3.1 The History of Leptospirosis

In 1886, the first official stage of the history of leptospirosis began with a publication of a paper, by Adolf Weil, dealing with "particularities of an acute infectious illness with spleen tumour, jaundice and nephritis" (Weil, 1886). Two years later, Fiedler regarded these jaundice infections as a special entity and called them "Weil's disease" (Fiedler, 1888).

The clinical description of Weil's disease was enough for it to become recognised worldwide despite the fact that the cause was not yet known.

About 30 years after Weil's paper was published, a spirochaete was discovered in the blood of Japanese miners with infectious jaundice (Inada *et al.*, 1916). The organism was named *Spirochaeta icterohaemorrhagiae* (Inada *et al.*, 1916). At about the same time, spirochaetes were isolated in Germany by Uhlenhuth and Fromme who were studying the so called "French disease = Schützengrabenkrankheit" seen among German troops in France (Schonberg, 1991; Uhlenhuth and Fromme, 1916). Uhlenhuth named his isolate *Spirochaeta icterogenes*. A similar spirochaete which Hübener and Reiter isolated from German soldiers by inoculation of infected material into guinea pigs, monkeys and rabbits was named *Spirochaeta nodosa* (Hübener and Reiter, 1915; Hübener and Reiter, 1916). *Spirochaeta icterohaemorrhagiae* became the most popular of these names and in 1917 Ido and co-workers showed that the brown rat (*Rattus norvegicus*) was the carrier of *Spirochaeta icterohaemorrhagiae* that was the causative agent of Weil's disease (Ido *et al.*, 1917).

Noguchi studied the microorganisms that Inada isolated (Noguchi, 1918) and found them morphologically similar to a saprophytic spirochaete isolated at approximately the same time in the United States (Wolbach and Binger, 1914). Wolbach and Binger named the latter, which they isolated from stagnant water, *Spirochaeta biflexa*.

After intensive experimental studies of these isolates, Noguchi found the morphology sufficiently characteristic to justify the creation of a new bacterial genus, which he called *Leptospira* (Noguchi, 1918). He included *Leptospira* in the order *Spirochaetales* along with *Borrelia* and *Treponema*. Based on the knowledge acquired from these investigations, the official classification of *Leptospira* recognised two species:

Leptospira biflexa for all saprophytic leptospires and

Leptospira interrogans for all pathogenic leptospires.

It is of great historical interest to note that until 1720 the brown rat, originating from Asia, had advanced no further than the Volga River in Russia. These rats greatly increased in number during this time and migrated westwards across the Volga. The Baltic harbours were colonised around 1729 and from there the rats spread further (by boat) to England in about 1730 (Gsell, 1984). Assuming that these rats were infected with *Leptospira* in Asia, it is likely that they also spread the disease to Russia, Great Britain and then the rest of the world.

During this century numerous other serovars affecting humans and animals have been discovered throughout the world. *Leptospira autumnalis*, the cause of autumnal fever in Japan, was described in 1925 (Koshima *et al.*, 1925). *Leptospira pyrogenes* (Van Thiel, 1948) and *L. bataviae* (Walch, 1926), causing the anicteric disease, "short-term spirochaetal fever" were described in Indonesia in 1923 and 1926 respectively. In the Netherlands, *L. canicola* was found in 1933 to be carried by the dog (Klarenbeek and Schuffner, 1933). In Russia, *L. grippotyphosa* was isolated from humans and field mice in 1928 (Tarassov, 1928; Koshima *et al.*, 1925). The list of pathogenic leptospiral serovars was destined to grow longer and longer because more people realised the zoonotic potential of leptospirosis and hence there was more research on the disease in most parts of the world. In Australia, *L. australis* (Cotter and Sawers, 1934) and *L. pomona* (Clayton *et al.*, 1937) were isolated. Then in 1948, *L. tarassovi* = *L. hyos* was found as another serovar in pigs with human cases reported in Switzerland, Australia and Argentina. In Denmark, *L. sejroe*, *L. saxkoebing* and *L. ballum* with various murine carriers were isolated from human patients (Borg-Petersen, 1944).

After the 1930s, research on leptospirosis was based on the acceptance of the uniformity of the antigenic properties of particular leptospiral types. Schuffner was instrumental in establishing the opinion that not only was each leptospiral type extraordinarily constant in its antigenic characteristics but also that each particular type was associated with a certain animal species as its natural animal host (Schuffner, 1934). With these discoveries typing of leptospires became epidemiologically and clinically significant.

In the 1950s much attention was drawn to the clinical and epidemiological aspects of leptospirosis. Clinically, so-called severe and mild leptospirosis were distinguished. Epidemiologically, occupation-related epidemics of leptospirosis were recognised, such as cane cutter's disease, swineherd's meningitis, rice field fever, autumnal fever, mud and field fever, and canicola fever (Alston and Broom, 1958).

Since 1960, WHO experts have published lists of serogroups and serotypes (serovars) occurring in various parts of the world. Apart from rats and mice, various mammalian species such as dogs, pigs, cattle, sheep, horses, bats, silver foxes and jackals have been retrospectively associated with a typical leptospiral serovar (Faine, 1994). The 'Leptospiral Serotype Distribution Lists' of the Atlanta Communicable Disease Centre (Alexander *et al.*, 1966), for example, reported 653 leptospiral isolates (260 from humans and the rest from 142 domestic and wild animal species) belonging to 16 serogroups comprising 137 serotypes (serovars) and subtypes worldwide in 1965. A supplement, which covered the years 1966-1973, listed 331 new isolates from humans and a large number (2681) from animals, especially in the USSR and related states and developing countries (Alexander *et al.*, 1973).

The list of animal species from which leptospire have been isolated and properly classified grows with each passing year. Leptospire have been isolated from most wild (Van der Hoeden, 1964) and domestic (Cotter and Sawers, 1934; Ris *et al.*, 1973; Ryan and Marshall, 1976) mammalian species. Pathogenic leptospire have been isolated from toads and frogs (Gravekamp *et al.*, 1991), water birds, reptiles and arthropods (Faine, 1994). Even molluscs and helminths are thought to be able to mechanically carry leptospire. However, so far with few exceptions, these non-mammalian animals have not been shown to be of much importance in the epidemiology of leptospirosis.

More than 240 serovars (Hakan *et al.*, 1996) of pathogenic leptospire have now been identified worldwide and they are grouped into 23 serogroups (Kmetz and Dikken, 1993). The list of classified leptospire belonging to the saprophytic species *L. biflexa* includes a total of 38 serogroups and more than 63 serovars (Kmetz and Dikken, 1988).

The history of leptospirosis in New Zealand began in 1951 when Kirschner and Gray published their findings about murine and canine leptospirosis in this country (Kirschner and Gray, 1951). The disease in humans became notifiable in 1952 after serovar *pomona* was isolated from a dairy farmer whose farm was heavily infested with rats (Kirschner *et al.*, 1952). Kirschner's initiative was later followed by a series of medical and veterinary investigations (Blakelock and Allen, 1956; Dodd and Brackenridge, 1960; Faine, 1958; Russell and Hanson, 1958; Salisbury, 1954; Shortridge, 1960; Te Punga and Bishop, 1953; West and Whitehead, 1953).

It was realised that leptospiral infection was common among mammals (both wild and domestic) in most parts of the country and that it posed a great threat to livestock, pets and human beings. Human infections were noted to be mostly due to serovars *pomona* and

hardjo and associated with pig and dairy farming respectively (Kirschner *et al.*, 1952; Christmas *et al.*, 1974; Christmas *et al.*, 1974). The Women's Division of Federated Farmers (WDF) was instrumental in raising research funds in the 1970s, as there was an urgent need to control leptospirosis in farmers. Since then, many other questions relating to the epidemiology of leptospirosis in New Zealand have been answered through research. For example, the occupational hazards of leptospirosis in the meat industry have been investigated (Blackmore *et al.*, 1979).

The use of a bivalent animal vaccine against *pomona* and *hardjo* in pigs and cattle coincided with a dramatic fall in the number of notified cases of human leptospirosis. The vaccine was introduced at the end of 1979 and notification of human infection dropped from 677 in 1979 to 325 in 1981 (Chereshsky and Baker, 1993). In the following year the number fell to 179 cases and remained at less than 200 for another five years. In the last few years, the number of laboratory-reported cases has remained below 150 per year (Anonymous, 1996) (Anonymous, 1998a).

3.2 Classification of *Leptospira*

The genus *Leptospira* is one of two (the other is *Leptonema*) belonging to the family Leptospiraceae in the order Spirochaetales. An additional genus, *Turneria*, has recently been suggested and is awaiting official approval.

The first attempt to introduce some order into the growing number of serologically different strains of *Leptospira* was undertaken in 1954 (Wolff and Broom, 1954). Wolff and his co-workers formulated the first principles to differentiate serovars, previously designated serotypes, on the basis of their serological characteristics, using rabbit immune sera. Serovar is the contracted form of the term 'serological variety' and denotes the fundamental element in *Leptospira* classification.

The official classification of leptospirae is serologically based and depends on a two-way cross-absorption / agglutination reaction between newly isolated strains and known serovars. By definition, two strains are considered to represent different serovars if, after cross-absorption / agglutination with excess amounts of a heterologous strain, more than 10% of the homologous antibody titre regularly remains in both antisera in repeated tests (Kmety and Dikken, 1993).

For convenience, serovars that are related antigenically are assembled to form a serogroup.

Unfortunately, serological criteria are not very precise, and many discrepancies among identified serovars exist. One of the main problems in leptospiral classification is the presence of strains that differ from one another by a one-way cross-absorption / agglutination reaction and, thus, are deemed unclassifiable by criteria for the two-way cross-absorption / agglutination system (Faine, 1994). Cross-reactions caused by exposure to leptospire with similar antigenic properties also limit the importance of serologic classification techniques. These techniques will not detect critical epidemiological differences such as virulence and the ability to infect some hosts and not others.

The previous official subdivision of the genus *Leptospira* by serologic means named two species: 1) *L. interrogans*, which comprised all pathogenic serovars; and 2) *L. biflexa*, which comprised all saprophytic serovars (Torten, 1979). Unfortunately, the division between the two species is still not absolutely clear, and several of the serovars classified as *L. biflexa* are known to be pathogenic.

Recently, new classification criteria using genomic markers have been developed by various authors (Marshall *et al.*, 1981; Ramadass *et al.*, 1992; Yasuda *et al.*, 1987). By genomic means, the genus *Leptospira* has now been reclassified into the following seven pathogenic species: *L. borgpetersenii*, *L. inadai*, *L. noguchii*, *L. santarosai*, *L. weilii*, *L. kirschneri* and *L. interrogans (sensu stricto)*. The non-pathogenic, free-living leptospire belongs to one species: *L. biflexa*.

Another newly suggested classification method is based on a chemotaxonomic key and proposes the division of the *Leptospira* species by fatty acid methyl ester (FAME) profiles (Cacciapuoti *et al.*, 1991). Monoclonal antibodies (Mabs) against specific leptospiral epitopes in a panel form were found to be useful for identification of leptospire (Korver *et al.*, 1988). The same was partially true for the factorial system analysis developed in rabbit sera (Kmetz, 1967).

Also, characterisation of ribosomal RNA variability has been used in the taxonomic identification of leptospire (Fukunaga *et al.*, 1991). Some comparative studies between the various procedures have been described by a number of workers (Korver *et al.*, 1991; Terpstra *et al.*, 1987; Van Eys *et al.*, 1991). There is a clear indication that these procedures are useful in determining the epidemiological relevance of taxonomic variability. The new taxonomic identification systems are already able to differentiate between serologically identical but epidemiologically different serovars such as *hardjoprajitno* and *hardjobovis* (Marshall *et al.*, 1985).

The genus *Leptospira* comprises thin, flexuous, helical bacteria 0.1-0.2 μm by 6-20 μm ; thus, they pass through filters that retain most other bacteria. They are hooked at one or both ends although under cultural conditions they may become straight. Leptospires are tightly coiled, a physical property that can be easily observed by dark-field microscopy at magnifications of 600X-1000X (Torten and Marshall, 1994). They cannot be seen by ordinary illumination due to their similar refraction of light to that of glass slides.

The common staining procedure for leptospires in tissue is silver impregnation, although their distorted appearance can easily be confused with other tissue elements, especially nerve fibres, cell membranes and strands of collagen fibres (Young, 1969). Leptospires are Gram-positive but stain with great difficulty and therefore are not classified as such.

They are very actively motile. This important characteristic is aided by means of an axistyle that is regarded as a flagellar analogue by its function and chemical composition; it is composed of two axial filaments inserted subterminally at both ends (Torten and Marshall, 1994).

Unlike most other bacteria, leptospires require specific fatty acids as a source of carbon and energy (Faine, 1982); they also utilise glucose to some extent. Ammonium salts can be used as the sole nitrogen source, and there are absolute minimal requirements for vitamin B₁₂, thiamine and various inorganic salts. In addition, leptospires incorporate purine bases but not pyrimidine bases into their nucleic acid. Because of this, they are resistant to the bacteriocidal activity of the pyrimidine analogue, 5-fluorouracil (Johnson and Rogers, 1964a). This compound is used in selective media for the isolation of leptospires from contaminated sources. Leptospires are strictly aerobic microorganisms with a preference for microaerophilic conditions. When grown in semisolid medium they flourish best a few millimetres beneath the top surface. They prefer growing in a liquid or semisolid medium but can form subsurface colonies on solid agar (Kirschner and Graham, 1959). Generation time is about 12 h and is one of the main reasons why contamination of cultures by most other bacteria that have a shorter generation time will result in the death of leptospires (Torten and Marshall, 1994). Optimal pH requirements are 7.2-7.6 (Faine, 1994). Pathogenic leptospires grow best on media that are supplemented with protein, whether in the form of bovine albumin or whole, slightly haemolysed rabbit serum (Faine, 1982). Most serovars will grow on synthetic media. Saprophytic leptospires will grow at 11-13°C, although optimum development occurs at 28-30°C (Johnson and Harris, 1967).

The highly sensitive nature of leptospire to drying and acidic environments (pH < 7) ensures their death within a few minutes of being exposed to such conditions. Consequently, infection is normally confined to wet, alkaline environments (Torten and Marshall, 1994).

As a global generalisation, arid lands and deserts should not be considered as potential endemic zones because of their dry conditions under which leptospire cannot survive for more than a few minutes (Torten, 1979).

However, man-made or natural oases, whether formed by water brought in for irrigation or by natural springs, can lead to close contact between humans and wild and domestic animals that are concentrated in such areas. The introduction of a carrier animal into such a place as this can quickly lead to the formation of a well-defined endemic zone. Areas with extreme rainfalls and/or high levels of subterranean water are very conducive for leptospiral survival outside of the host; thus, most of the areas where monsoons are common are natural vast endemic zones.

Leptospire can be stored in liquid nitrogen (Alexander *et al.*, 1972) or by lyophilisation (Annear, 1958; Annear, 1974) and will remain reasonably virulent upon proper thawing.

The difference between parasitic and saprophytic species are based mainly on 1) the ability or inability to parasitise vertebrate hosts, 2) variation in the guanine plus cytosine (G+C) contents of their DNA, and 3) presence of lipase activity (Johnson and Rogers, 1964b).

3.2.1 Serovar *copenhageni* and its origin

Serovar *copenhageni* belongs to the Icterohaemorrhagiae serogroup and cannot be differentiated from serovar *icterohaemorrhagiae*, of the same serogroup, using the microscopic agglutination test (MAT). The reference strain for serovar *copenhageni* is M 20.

The strain M 20 was first isolated by Borg-Petersen (1938) from a patient in Denmark. It was shown by agglutinin-absorption tests to differ serologically from the reference strain of serovar *icterohaemorrhagiae*, strain RGA. Whereas strain M 20 is able to absorb nearly all antibodies from RGA antiserum, strain RGA only removes its homologous antibodies from M 20 antiserum. Strain M 20 was therefore considered to represent a 'subtype' of serovar *icterohaemorrhagiae*.

Gispén and Schüffner (1939) confirmed the serological results but they classified strain M 20 as the 'complete biotype' of *icterohaemorrhagiae*, and named it *icterohaemorrhagiae AB*. The strain was later considered to represent 'a subserotype' of *icterohaemorrhagiae* (WHO, 1965). Kmety (1966), who studied the antigenic structure of serovars of the *Icterohaemorrhagiae* serogroup, suggested that the taxonomy of 'subserotypes' be abandoned and proposed that M 20 should represent a 'serotype' (serovar) named *copenhageni*. This suggestion was accepted by the Subcommittee on the Taxonomy of *Leptospira* (TSC) at their meeting in 1966 (TSC meeting, 1966: Statements and Recommendations, 1971).

3.3 Epidemiology and Public Health.

The epidemiology of leptospirosis as a zoonotic disease concerns the study of all those factors that affect the transmission of the disease from animals to man. Some obvious factors that have to be considered include the genotype of the serovar under study, its major reservoir or animal maintenance host, the size of the animal population and the prevalence of infection within it, the ecosystem in which the animals are found, the degree of contact with humans

and the general environmental factors that affect intra- and inter-species transmission. Thus, the epidemiology of leptospirosis is extremely complex and will vary from country to country and from region to region. The most important host for, say, human infection in a certain area may be quite different from the most significant host for cattle grazing in the same location. Moreover, the relative importance of each of a number of hosts may completely reverse itself with time. Some careful studies of known epidemiological situations have shown that determination of the most important carrier should be followed by constant surveillance of isolated serovars and species from which they were isolated. Only such surveillance can detect changes in serovar virulence and designate relative host importance to any one particular species, be it human or a domestic or wild animal (Torten, 1979).

In nature, many host-parasite relationships develop in such a way that the pathogen is maintained in a particular species of animal with minimal effects. This ensures the continual survival of both host and parasite. In these instances, the prevalence of infection in the population is high, and infected animals excrete leptospires in the urine for long enough to ensure transmission of infection from generation to generation (Blackmore and Hathaway, 1980). Thus it can be said that the serovar is endemic in that population of animals.

If the infection is transmitted to a non-maintenance host, the leptospire are considerably more pathogenic, typical symptoms of disease will occur and usually the duration of excretion of organisms in the urine is short. Under such circumstances, epidemics of disease are of relatively short duration and long-term endemic infection does not occur. As man is not a maintenance host to any serovar, infection usually results in obvious disease but human to human transmission seldom, if ever, occurs. Similarly, if cattle contract *L. pomona* infection from pigs, sporadic outbreaks of abortion may occur in one season but are unlikely to occur in subsequent seasons unless infection is re-introduced.

The control of leptospirosis therefore depends on identifying the maintenance hosts for a particular serovar, and attempting to reduce or eliminate endemic infection in such a population. When such maintenance hosts are free-living wild animals, then this is extremely difficult. If a colony of laboratory rats is found to be infected with *L. icterohaemorrhagiae*, the problem and its concurrent public health hazard can be eliminated by culling the colony and then re-establishing a new one in a building that prohibits access by wild rats (Blackmore and Hathaway, 1980).

Leptospirosis occurs worldwide wherever there is risk of direct or indirect contact with urine or kidneys of infected animals or their infected products of conception. The disease is usually seasonal and each country has its own endemic zones that fit the criteria required for leptospiral survival outside the host (Torten, 1979). In tropical countries, rainy seasons and floods are usually accompanied by an upsurge in the number of new cases of leptospirosis. It must be remembered, however, that sporadic cases do occur all year, especially if the main vectors for human illness are domestic animals. Larger leptospirosis outbreaks may occur at intervals of several years. Although data on this subject is limited, in at least one case a 4-6 year cycle was observed in Israel (Torten *et al.*, 1970).

There are two main methods of transmission of leptospire among farm animals. The first is by congenital or neonatal infection, followed by recovery and a continuing carrier state. The second, which is more important, is spread from the urine of carriers onto farmyard floors, muddy ground, or to sources of drinking water. Human infection arises from contact with the above animals or their urine, and reflects the prevalent serovar (Torten and Marshall, 1994). Rodents, especially rats, may directly infect both farm animals and their own species through contact. This is a common cycle of infection in cattle and pigs, particularly if they are housed indoors (Faine, 1982).

A similar epidemiological pattern is seen where rodent carriers contaminate water or soil, that then become the indirect source of infection for pigs, cattle or sheep, who in turn become carriers and excretors, thus infecting other rodents or more of their own species. An additional problem is contaminated water, as it remains a potential source of infection for humans. This is the common epidemiological pattern in the rice-growing parts of the world (Faine, 1982). The infection cycles confined to feral rodents are self-maintaining, and are related to the territorial limits of individuals, families and species of animals in their natural habitats (Torten and Marshall, 1994).

Domestic animals intruding into uncultivated habitats (such as fringes of the forest) run similar risks and, conversely, the incursions of foraging feral animals into cleared, populated areas pose risks for both people and farm animals. Sometimes the infecting serovars in feral populations and in the geographically-associated farm animals are quite distinct. An example is the presence of *L. hardjo* infection in cattle on farms in areas of New Zealand, while possums (found in neighbouring bushland, and foraging on these farms) are infected with *L. balcanica*, a serovar which is serologically similar to *hardjo* (Mackintosh, 1981). Only through surveillance, based on leptospiral isolation from both the domestic and feral groups, is it possible to elucidate such relationships (Hathaway, 1978). The main source of infection for dogs is urine from other dogs as well as rat urine that has contaminated wet areas frequented by dogs. Hunting dogs that come into direct contact with rats are also at risk. Aerosol transmission may also be important (Faine, 1994)

From the above it is clear that the epidemiology of leptospirosis is centred on the carrier animal which is actively shedding leptospire in its urine. It is generally accepted that most carnivorous animals, which have a low urinary pH, are short-duration carriers and that the leptospire in their urine are usually damaged by this acidity. Herbivores, on the other hand, will support a carrier state for a longer duration and, additionally, the slight alkalinity of their urine will not damage the leptospire so that they therefore remain quite virulent for humans and/or other animals (Torten and Marshall, 1994).

Leptospire gain entrance into a susceptible host through cuts or abrasions in the skin or mucosal surfaces, through the conjunctivae, or by inhalation (into the lungs) of droplets of fluid containing leptospire (Faine, 1982). The disease is not believed to be transmitted by ingestion of food (Faine, 1994), probably due to the deleterious effects of saliva and gastric secretions on leptospire.

The mere presence of large numbers of carriers in any particular area does not necessarily mean that they are the most important hosts for human infections there. Some other host that may be present in small numbers but which is carrying a human-virulent serovar could be the most important. Very little is known about why a serovar maintained in mice, for example, may suddenly gain or lose its virulence for humans. Information is also lacking on the relative importance of host-to-host interaction in causing shifts in leptospiral virulence in humans and domestic animals (Torten, 1979; Gravekamp *et al.*, 1991).

In various endemic zones of the world, a shift of the most important host for human leptospirosis from domestic animals to various field rodents and vice versa is a common phenomenon (Torten and Marshall, 1994). This kind of shift is usually discovered retrospectively via identification of human sickness in various populations. Because specific leptospiral serovars are often considered to be less virulent and, thus, less capable of infecting certain species of animals (*canicola* in rodents and *grippityphosa* in canines are some examples), epidemiologists tend to disregard some species as potential hosts for specific serovars. However, it should be realised that a serovar may adapt itself to a new host and become the most important in the affected area (Torten, 1979).

3.4 Leptospirosis in Animals

Scientists initially considered leptospirosis to be a sporadic infection of rats and dogs, which was caused by a relatively small number of serovars (Alston and Broom, 1958). Later investigations have shown the disease to be common, but generally inapparent, in many species of domestic livestock and wild animals and to be associated with a large number of serovars (Torten and Marshall, 1994). Among domestic animals, cattle and pigs are the most commonly infected. Deer, sheep, goats, horses and dogs are less commonly involved (Wilks and Humble, 1997) with rare cases occurring in cats (Sophet, 1979; Larrison *et al.*, 1984).

The outcome of a leptospiral infection in an animal is variable and depends on attributes of the infecting serovar (pathogenicity, virulence) and host characteristics of the animal (susceptibility, immune status) (Faine, 1994).

The early clinical signs of leptospirosis in animals are non-specific and include pyrexia, malaise, anorexia and conjunctivitis. Later signs, which are more characteristic, include anaemia, icterus, haemoglobinuria, liver and renal failure and central nervous system disorders. Abortion, stillbirth and mastitis in lactating animals are also common occurrences (Faine, 1982).

3.5 Leptospirosis in Man

It is most unlikely that humans act as maintenance hosts for any particular leptospiral serovar (Torten and Marshall, 1994). Most infections with pathogenic leptospires therefore result in clinical disease.

Leptospirosis in humans manifests itself in two main forms: mild and severe. The mild or anicteric form of leptospirosis is usually (but not exclusively) seen in infection with serovars *hardjo*, *grippotyphosa*, or *pomona* (Faine, 1994). Following an incubation period of 3-12 days, the 'septicaemic' phase of infection begins, usually lasting 4-7 days, during which time leptospires are present in the blood. This initial phase is characterised by sudden onset of an 'influenza-like' illness, with fever, headache, photophobia, myalgia, vomiting and abdominal pain. If untreated, the 'tissue' phase of infection follows, which may last for up to one month.

During this phase leptospires disappear from the blood, serum antibody starts rising, and leptospires are excreted in the urine. The patient is by now usually afebrile or may have a low-grade fever for 1-3 days. In 80-90% of patients in the tissue phase of infection, there is lymphocytic meningitis, which is characterised by persisting severe headache and photophobia, and usually lasting only a few days.

Other symptoms include persistent myalgia, nausea, vomiting and abdominal pain. Common physical signs include generalised muscle tenderness and conjunctival suffusion. Splenomegaly and lymphadenopathy may be present in up to 25% of patients, but hepatomegaly is uncommon. A transient macular rash is occasionally seen, usually lasting for 2-3 days (Faine, 1994).

Laboratory findings include proteinuria with granular casts, an elevated blood urea and mild to moderate elevation of serum transaminases. The total leucocyte count is usually normal or only slightly elevated with a relative lymphopenia and the ESR (erythrocyte sedimentation rate) is invariably raised. Cerebrospinal fluid (CSF) findings include a raised leucocyte count of usually $<500 \times 10^6$ cells/L, with a predominantly mononuclear differential, a normal glucose and a normal or raised protein concentration (Wilks and Humble, 1997).

Characteristically, the icteric form of the disease is seen in severe infections with serovars *icterohaemorrhagiae*, *copenhageni*, *bataviae*, *javanica*, and others (Faine, 1994). Illness worsens, usually rapidly after onset, so that renal failure appears within 7 to 10 days,

sometimes accompanied by, or followed by, skin and mucosal haemorrhages, jaundice, haemoptysis, myocarditis or liver failure, and even death if untreated. The patient's conscious state may range from disorientation to delirium or coma. Cardiogenic shock may also occur. Leptospirosis in pregnancy carries the risk of intrauterine infection and foetal death.

3.6 Some aspects of leptospirosis in New Zealand

3.6.1 Maintenance hosts and serovars in New Zealand

Although more than 200 serovars of the seven pathogenic *Leptospira* species are recognised worldwide, only six serovars (*hardjo*, *copenhageni*, *ballum*, *pomona*, *tarassovi* and *balcanica*) are officially considered to be endemic in New Zealand. The fact that at least three others (*canicola*, *australis* and *bratislava*) have been either isolated or diagnosed serologically in animals and man does not seem to change this official ruling. Arguments for the non-endemicity of the latter serovars, include the fact that these cases have been very sporadic and that no natural maintenance hosts have been identified for these serovars (Ellison and Hilbink, 1990; Hathaway and Blackmore, 1981b).

Table 1 shows the six recognised leptospiral serovars and the mammalian species from which they have been isolated in New Zealand.

Serovar *hardjo* is maintained in cattle and leptospiruria can persist for two years (Hellstrom, 1978). In unvaccinated herds, the majority of first calving heifers will be infected, and will be a potential source of infection for cohorts.

Pigs maintain serovars *pomona* and *tarassovi*. Endemic infection of pigs with *pomona* is more common than *tarassovi*, and the majority of pigs examined by Ryan (1978) were found to have infected kidneys at the time of slaughter.

SEROVAR	HOST	REFERENCE
<i>L. pomona</i>	Bovine	(Anonymous, 1951)
	Ovine	(Anonymous, 1951)
	Porcine	(Ryan, 1978)
	Feline	(Harkness <i>et al.</i> , 1970)
	Human	(Kirschner <i>et al.</i> , 1952)
	Canine	(Te Punga and Bishop, 1953)
<i>L. hardjo</i>	Bovine	(Lake, 1973)
	Human	(Christmas <i>et al.</i> , 1974)
<i>L. balcanica</i>	Possum (<i>Trichosurus vulpecula</i>)	(Marshall <i>et al.</i> , 1976)
<i>L. ballum</i>	Bovine	(Ris <i>et al.</i> , 1973)
	Human	(Anonymous, 1967)
	Hedgehog (<i>Erinaceus europaeus</i>)	(Brockie and Till, 1977)
	Rat (<i>R. norvegicus</i> and <i>R. rattus</i>)	(Brockie, 1977)
	Mouse (<i>Mus musculus</i>)	(Brockie, 1977)
<i>L. copenhageni</i>	Bovine	(Dodd and Brackenridge, 1960)
	Rat (<i>R. norvegicus</i>)	(Kirschner and Gray, 1951)
<i>L. tarassovi</i>	Porcine	(Ryan and Marshall, 1976)

Table 1. Some early isolations of leptospires in New Zealand

In New Zealand, as in most other parts of the world, the wildlife (especially rodent) population plays a significant role in the epidemiology of leptospirosis. Serovar *ballum* is maintained in ship rats (*R. rattus*), house mice (*Mus musculus*) and hedgehogs (*Erinaceus europaeus*) (Hathaway, 1978; Brockie, 1974). As with most other serovars maintained in wildlife, infection usually occurs in sexually mature animals only. The brown rat (*R. norvegicus*) does not appear to be a natural maintenance host for *ballum* but can maintain infection in very high population densities (Hathaway, 1978). The Polynesian rat or kiore (*R. exulans*) probably does not play much of a role in the epidemiology of leptospirosis in New Zealand. This is because these rats are confined to certain offshore islands and national parks and so do not have much contact with domestic animals or humans, but could also be because they have not been subjected to much investigation.

In New Zealand, the earliest isolate of a strain of *Leptospira* of the Icterohaemorrhagiae serogroup from brown rats was identified as serovar *icterohaemorrhagiae* (Kirschner and Gray, 1951), at the time when serovar *copenhageni* had not yet been discovered. Subsequently, Dodd and Brakenridge (1960) isolated a strain of *Leptospira* from calves, which was identified to be serovar *icterohaemorrhagiae AB*.

Whereas Kirschner and Gray (1951) identified their isolate by using the MAT, Dodd and Brakenridge (1960) sent their isolate to a reference laboratory to be analysed by agglutinin-absorption tests. This, therefore, is why serovar *icterohaemorrhagiae AB* (renamed *copenhageni* (Kmety, 1966)) is known to occur in New Zealand, while the status of serovar *icterohaemorrhagiae* is still unclear.

Serovar *copenhageni* is maintained in brown rats (*R. norvegicus*) (Brockie, 1977), but the distribution of such infected populations is limited. Infection has been detected in brown rats in the northern parts of both the North and South Islands (Carter and Cordes, 1980; Kirschner and Gray, 1951). Sporadic, inapparent *copenhageni* infections in domestic stock are not uncommon. Serious outbreaks of *copenhageni* infection can occur, however, especially in dogs and calves in areas heavily populated with infected rats (Dodd and Brackenridge, 1960; Mackintosh, 1981).

Sexually mature possums (*Trichosurus vulpecula*) maintain serovar *balcanica* and up to 60% of such animals may be infected (Hathaway, 1978). Transmission of *balcanica* to other mammalian species appears to be uncommon.

The isolation of serovar *australis* has occurred only once from a farmer in Northland (Thompson, 1980). Although this serovar is maintained in rodents overseas, an animal reservoir in New Zealand has not yet been identified.

Evidence for the presence of serovar *canicola* in New Zealand is very limited and weak. Serological evidence of its occurrence has been reported in dogs (Kirschner and Gray, 1951; Hilbink *et al.*, 1992) and in humans (Anonymous, 1987; West and Whitehead, 1953; Anonymous, 1998a). Veterinary authorities generally claim that the country is free (Anonymous, 1987) from this serovar and limited serological testing at the Central Animal Health Laboratory supports this (Ellison and Hilbink, 1990).

3.6.2 Porcine leptospirosis in New Zealand

Pigs have been known to be associated with leptospirosis in New Zealand since 1952 (Kirschner *et al.*, 1952). They are considered the maintenance host for serovar *pomona* and thus are the primary source of infection for animals and man (Ryan, 1978)

Ryan (1978) did extensive work on porcine leptospirosis in New Zealand. A sample of 234 adult sows from throughout New Zealand showed that 150 (64%) had antibodies to *pomona* (Ryan, 1978). An earlier serological survey of 1125 pigs showed 484 (44%) had antibody titres to this serovar (Russell and Hanson, 1958). In a serological and cultural survey at abattoirs in the lower North Island, it was found that 72/84 (86%) of 5 -10 month old pigs and 57/65 (88%) of pigs older than 10 months had antibody titres ≥ 12 to serovar *pomona* (Ryan, 1978). In the same survey, serovar *pomona* was isolated from 38/84 (45%) of the kidneys from the 5-10 month old pigs and from 1/65 (2%) of the kidneys of pigs older than 10 months (Ryan, 1978). Young pigs infected with serovar *pomona* show no clinical signs but abortions have been recorded in pregnant sows (Powers *et al.*, 1956; Ryan, 1978).

The earliest serological evidence of serovar *tarassovi* in New Zealand was produced when six percent of 100 adult pigs surveyed in 1954 had antibody titres ≥ 150 (Kirschner, 1954). In another survey, Russell and Hanson showed that 431/1125 (38%) of pigs had antibody titres ≥ 12 to the same serovar (Russell and Hanson, 1958). The presence of serovar *tarassovi* infection in New Zealand pigs was later confirmed when the organism was isolated from a kidney culture (Ryan and Marshall, 1976). Follow-up investigations revealed that both serovars *tarassovi* and *pomona* were endemic in the pig herd from which the isolate originated. Unlike the situation in Europe, however, serovar *tarassovi* is not recognised as a major problem on pig farms in New Zealand.

Pigs in New Zealand do not seem to become infected with *hardjo* but 2/234 (1%) and 9/234 (4%) animals have been found in a survey to have antibody titres ≥ 24 to *ballum* and *copenhageni* respectively (Ryan, 1978). It was concluded that serovars *ballum* and *copenhageni* play a minor role in leptospirosis in New Zealand pigs.

3.6.3 Bovine leptospirosis in New Zealand

The clinical importance of serovar *pomona* in cattle in New Zealand has long been recognised with reports of death in young calves as well as haemoglobinuria (Jamieson *et al.*, 1970; Salisbury, 1954) and abortion in cows (Te Punga and Bishop, 1953). This serovar was also the first to be isolated from cattle in New Zealand (Filmer, 1951).

In a national survey of cattle, Hellstrom (1978) found 85/480 (18%) had antibody titres ≥ 24 to serovar *pomona*. Hellstrom (1978) also found that cattle infected with *pomona* shed the organism in their urine for one to four months. Pigs probably act as the source of *pomona* infection for cattle (Mackintosh, 1981).

Lake (1973) reported the first isolation of serovar *hardjo* from cattle. A number of serological surveys indicate that this serovar is endemic in New Zealand cattle (Lake, 1973; Brockie, 1976; Hellstrom, 1978).

Hardjo infection in yearling cattle is generally asymptomatic (Marshall *et al.*, 1979), but there are some reports of pyrexia, agalactia, flaccid udders and discoloured milk secretions in lactating cows (Lake, 1975). Leptospire of the serovar *hardjo* have been reported to cause abortion, infertility and mastitis overseas (Handon and Brodie, 1967; Higgins *et al.*, 1980; Little *et al.*, 1980; Sullivan and Callan, 1970) but *hardjo* does not appear to produce such disease in New Zealand (Hellstrom, 1978). This is believed to be due to strain differences (Mackintosh, 1981).

Most calves will be protected from infection by maternally-derived passive immunity and, with the use of vaccines, it is now possible to eradicate *hardjo* infection from individual dairy herds (Marshall *et al.*, 1979).

By using the microscopic agglutination test, however, misclassification of cattle infected with either *balcanica* or *hardjo* is unavoidable, as it is impossible to distinguish between these two serovars with this technique (Hellstrom, 1978). The results of an experimental infection of cattle with *balcanica* and an investigation of infection on a dairy farm due to this serovar indicate that, although sporadic infection may occur, cattle are not likely to be maintenance hosts for this serovar (Mackintosh, 1981).

The importance of *ballum* infection in cattle is unclear. *Ballum* has been isolated from two three-month old healthy calves (Ris *et al.*, 1973). A subsequent survey indicated that 4% of New Zealand cattle had antibody titres to *ballum* (Hellstrom, 1978).

A report on the isolation of serovar *copenhageni* from cattle was published in 1960 (Dodd and Brackenridge, 1960). Infected cattle had severe clinical signs including weakness, laboured breathing and a pendulous abdomen. A serological investigation of cattle found 11/480 (2%) with antibody titres to *copenhageni* and that all positive sera originated from

the North Island (Hellstrom, 1978). *Copenhageni* has also been isolated from clinically healthy calves (Ris *et al.*, 1973) and it has been suggested that calves may acquire infection through contact with infected rat urine.

There is no evidence to suggest that serovar *tarassovi* is important clinically, or that cattle act as maintenance hosts for this serovar (Ryan and Marshall, 1976).

3.6.4 Ovine Leptospirosis in New Zealand

The question of whether the sheep is a maintenance host to any of the leptospiral serovars present in New Zealand has often been asked. This is partly due to the large sheep population of this country (Anonymous, 1998b) and partly because sheep are often kept in close contact with other farm animals, such as cattle and pigs, which play an important role in the epidemiology of leptospirosis.

The examination of 928 ovine serum samples for evidence of leptospiral agglutinins showed that 20% had titres ≥ 48 to *hardjo*, 3.8% to *pomona*, 2.6% to *tarassovi*, 2.3% to *copenhageni*, and 2.7% to *ballum* (Blackmore *et al.*, 1982).

Serovar *pomona* was the first to be isolated from sheep in New Zealand (Anonymous, 1951). This serovar was subsequently shown to be associated with sporadic epidemics in sheep causing high morbidity and mortality in lambs and variable clinical disease in mature animals (Hartley, 1952; Webster, 1955). A comprehensive table of *pomona* outbreaks in sheep from 1958 to 1980 was published in 1981 (Bahaman, 1981).

Sheep are unlikely to be maintenance hosts to *L. pomona* and infection with this serovar is probably due to close contact with pigs. Recent serological, cultural and DNA restriction profile studies showed that *L. pomona* infection in sheep is uncommon (Cortes, 1996).

Serovar *hardjo* is usually found in sheep one year and older. Ewes infected with this serovar develop antibodies that are passed on to their offspring but show no clinical signs of infection (Bahaman, 1981). There is some overseas evidence that sheep can be maintenance hosts for *L. hardjo* (Cousins *et al.*, 1989) but this situation has not been found with *hardjo* in New Zealand (Blackmore *et al.*, 1982).

The low prevalence of infection with serovars *copenhageni*, *tarassovi* and *ballum* in sheep is

thought to be due to sporadic contact with the maintenance hosts of these serovars (Blackmore *et al.*, 1982).

3.6.5 Cervine Leptospirosis in New Zealand

Leptospirosis in wild deer has once been reported to be of low prevalence in New Zealand (Daniel, 1966).

The publication of the first serological evidence of leptospirosis in farmed deer in New Zealand (Anonymous, 1980) was followed by a series of reports (Fairley *et al.*, 1984; Flint *et al.*, 1986; Wilson and Walker, 1988; Wilson and McGhie, 1993), which confirmed the presence of clinical disease in this country. The most common serovars associated with disease were *hardjo* and *pomona*, whereas infections with *copenhageni* and *tarassovi* were less common.

A recent serological slaughterhouse survey of farmed deer in the lower North Island (Wilson *et al.*, 1998) showed titres of ≥ 96 against serovar *hardjo* (73.6%), *pomona* (41.5%), *copenhageni* (11.3%) and *tarassovi* (15.1%) of the 53 farms surveyed. Kidneys from selected serologically-positive animals were found to have histological lesions. The lesions included extensive focal interstitial infiltration of lymphocytes and plasma cells in the cortex and the accumulation of leucocytes in tubules typical of leptospirosis in other species. Some animals also had titres to serovars *australis*, *balcanica* and *ballum* but it was concluded that *hardjo* and *pomona* are the most important in leptospirosis of farmed deer.

3.6.6 Canine Leptospirosis in New Zealand

Leptospirosis is known to affect dogs worldwide. Overseas, dogs are believed to be maintenance hosts for *Leptospira interrogans* serovar *canicola* (Klarenbeek and Schuffner, 1933) but can become accidental hosts to a range of other serovars, such as *copenhageni*, *icterohaemorrhagiae*, *grippotyphosa*, *autumnalis*, *australis*, *tarassovi*, *ballum*, *pomona*, *bataviae* and *bratislava* (Ellis, 1986; Hanson, 1982; Mackintosh *et al.*, 1980; Michna, 1970). Like leptospirosis in other domestic and wild animals, the incidence and prevalence of an infective serovar in dogs in any given geographical area depends on the presence of a corresponding maintenance (primary) host, the activity of the animal and vaccination programs. The worldwide prevalence of serovars of the Icterohaemorrhagiae serogroup is associated with the distribution of the brown rat as a maintenance host.

The first isolate of a leptospire from a dog in New Zealand was serovar *pomona*. The sick dog was a resident on a dairy farm in the Waikato district, which was experiencing an abortion storm in the herd of cattle (Te Punga and Bishop, 1953). Two other dogs on the farm, which had antibody titres to *pomona*, were clinically healthy. Seven dogs, from three dairy farms that had concurrent epidemics of *pomona* infection in their cows or calves, were found to have antibody titres ≥ 192 to *pomona* in another investigation (Mackintosh, 1981).

Antibody titres to *hardjo* in New Zealand dogs were first reported in two dogs on a dairy farm in the Waikato (Anonymous, 1972). Mackintosh in 1981 studied 64 cattle dogs from 37 dairy farms in the Manawatu and showed that 28 of the dogs (44%) had antibody titres ≥ 24 to at least one serovar. Twenty eight per cent of the dogs had antibodies to serovar *hardjo* whilst 9% and 6% had antibodies to *ballum* and *pomona* respectively (Mackintosh, 1981).

In a similar survey of 47 city dogs only two (4%), one (2%) and one (2%) had antibody titres ≥ 24 to *australis*, *hardjo* and *pomona*, respectively (Mackintosh, 1981). These results reflect the greater exposure of rural dogs to cattle and pig populations, which respectively maintain *hardjo* and *pomona*.

An investigation of an outbreak of leptospirosis due to serovar *copenhageni* in a pack of 38 hounds in the Auckland region revealed that 26 (68%) had antibody titres ≥ 24 to *copenhageni* (Mackintosh, 1981). Four of the affected dogs showed clinical signs and two died. Rats were presumed to be the source of infection for these dogs. Some (9/34) of the hounds were also found to have low (≤ 24) antibody to *tarassovi* and this serovar was isolated from four hounds. This infection may have resulted from contact with pig urine during hunting.

An experimental infection of five dogs with *tarassovi* caused subclinical infection and leptospiruria in all animals for up to ten months (Mackintosh, 1981). It appears unlikely, however, that dogs act as maintenance hosts for *tarassovi* in New Zealand due to the low intensity of leptospiruria and the lack of evidence for dog-to-dog transmission in the study of the pack of hounds (Mackintosh, 1981).

Previous suggestions concerning the prevalence of *L. canicola* in New Zealand have been a matter of dispute. In a small investigation carried out in Dunedin on 10 dogs, three tested seropositive for *canicola* (Kirschner and Gray, 1951). A strong titre against *canicola* in a human with severe symptoms of leptospirosis has been reported in the Hutt Valley

(West and Whitehead, 1953) and only one weak reaction to this serovar was found in a nationwide serological survey of 6375 dogs (Hilbink *et al.*, 1992).

Until the early 1990s, canine leptospirosis due to serovar *copenhageni* was considered to be geographically confined to the Waikato and Auckland regions of New Zealand. This observation is strongly supported by information from central and regional animal health laboratories. Laboratories in Auckland and Ruakura regularly diagnose cases of *copenhageni* infection while laboratories at Palmerston North, Lincoln and Invermay do not. Further, leptospiral microscopic agglutination testing (MAT) of dogs from throughout New Zealand has found evidence of infection only in the Auckland-Waikato regions (Ellison and Hilbink, 1990). Data from dog export health certification is also in keeping with this observation. Also, in a nationwide serological survey carried out during 1990-91, 78/8730 (0.9%) of plasma samples from dogs tested for serovar *copenhageni* showed reactions of $\geq 50\%$ agglutination at the 1:100 dilution (Hilbink *et al.*, 1992). Those that reacted were also from around Auckland and the Waikato. The test results of more recent submissions to Animal Health Laboratories are in agreement with this finding (Hill and Thompson, 1997).

In a study carried out to determine the role that dogs play in the epidemiology of leptospirosis in New Zealand, it was concluded that dogs appear to be susceptible to all the serovars present in this country (Mackintosh, 1981). However, they are not believed to be maintenance hosts for any of these serovars, due to the low intensity of leptospiuria, the poor survival of these leptospires in dog urine and the lack of consistent dog-to-dog transmission. Knowledge about the source of infection for dogs may, however, be used in the control of the disease in both dogs and humans.

Both acute and chronic forms of canine leptospirosis have been reported. Overseas, serovar *icterohaemorrhagiae* has been associated with three clinical syndromes in the dog: 1) acute haemorrhagic disease characterised by high fever, prostration and death; 2) a less acute hepatic syndrome with severe icterus, depression, fever and haemorrhages; and 3) uraemia, haemorrhagic enteritis and death (Timoney *et al.*, 1988). Similar signs have been seen with serovar *copenhageni* infection in dogs in New Zealand (Ellison and Hilbink, 1990). The outcome of infection depends on the infecting serovar of *Leptospira*, the age of the dog and its vaccination status (Wohl, 1996).

Current canine leptospiral vaccines used in most parts of the world only protect against clinical disease caused by serovars *canicola* and *icterohaemorrhagiae* (*copenhageni*). It has been observed in the USA that the use of such vaccines has seen a decrease in disease

attributable to serovars *canicola* and *icterohaemorrhagiae*, whereas there has been an increase in the incidence of other serovars such as *grippotyphosa*, *pomona*, and *bratislava* (Rentko *et al.*, 1992).

A vaccine ("Leptoguard", Pfizer Animal Health) which protects against infection with serovar *copenhageni* has been approved for use in New Zealand dogs since 1992 (Hilbink *et al.*, 1992).

3.6.7 Leptospirosis in New Zealand Rodents

There are four species of rodents in New Zealand:

1. The brown rat (*Rattus norvegicus*),
2. The ship rat (*Rattus rattus*),
3. The polynesian rat (*Rattus exulans*) and
4. The house mouse (*Mus musculus*).

The ship rat is widely distributed throughout New Zealand in both urban and rural areas and is commonly found inhabiting the walls and roofs of houses and farm buildings (Best, 1968; Watson, 1959). In forested areas, the ship rat prefers mature forest to scrub and bush-edge areas (Innes, 1977). The brown rat typically lives in close association with man and therefore has a patchy distribution compared to the widespread distribution of the ship rat (Wodzicki, 1950). The Polynesian rat is confined to limited parts of the southwest coast of the South Island and some offshore Islands, whereas the house mouse can be found practically anywhere in the country (Daniel, 1967; Wodzicki, 1950).

A few investigations have been carried out on the occurrence of leptospiral infection in rodents within New Zealand. In all instances, the Polynesian rat was considered unimportant in the epidemiology of leptospirosis because of the remoteness of its habitat from domestic animals and man.

Kirschner and Gray (1951) conducted the earliest leptospiral studies of rats in New Zealand. They studied the sera from 53 brown and 47 ship rats which were collected from Auckland, Dunedin and Christchurch. All sera were tested for antibodies to *copenhageni*, *canicola*, *bataviae* and *australis*. They found that 8/53 (15 %) brown rats had serological evidence of *copenhageni* (at the time referred to as *icterohaemorrhagiae*) infection and this serovar was isolated from 2/53 (4 %) kidneys. The minimum serum dilution used was not given but the titres for the positive rats varied between 1:80 and 1:320. Unfortunately, serovar *ballum* was not included in the panel of antigens used. The 47 ship rats showed no serological or

cultural evidence of leptospirosis.

A survey of 62 brown and 121 ship rats in the Wellington district, found no evidence of antibodies to *australis*, *canicola*, *hyos* and *pomona* antigens at a minimum dilution of 1:10 (Blakelock and Allen, 1956). Serovars *ballum* and *copenhageni*, which are now known to be endemic in rat populations, were not used as antigens.

On the other hand, Shortridge (1960) reported eight 'positive or suspicious' antibody titres to *copenhageni* out of 25 sera from rats (species not specified) in the Pukekohe district.

An important study of rodents from dairy farms has been carried out in Taranaki and the Waikato (Brockie, 1977). The combined cultural and serological evidence established that 10/79 (12.7 %) brown rats, 4/14 (28.6 %) ship rats and 10/73 (14 %) mice tested were renal carriers or had suffered a recent infection of *ballum*. Eight of the 79 brown rats showed cultural and/or serological evidence of infection with *copenhageni*. All the *copenhageni*-positive rats were from the Waikato. A minimum dilution of 1:100 was used and no evidence of *copenhageni* infection was demonstrated in the ship rats or mice in this study.

In an attempt to describe some epidemiological aspects of leptospiral infection in rodents inhabiting both natural and synanthropic foci in the southern half of the North Island of New Zealand, 61 ship rats, 243 brown rats and 12 house mice were examined (Hathaway and Blackmore, 1981a). At a minimum serum dilution of 1:12, antibody titres to serovar *ballum* were found in 8/30 (27%) ship rats and 6/168 (4%) brown rats. Titres ranged from 24-192 and 12-48 in ship rats and brown rats respectively. A few ship rats also had low titres (24 or less) to serovars *pyrogenes* (2), *copenhageni* (1), *tarassovi* (1) and *canicola* (1) but similar titres were detected only to serovars *pyrogenes* (3) and *tarassovi* (2) in brown rats. Leptospire of the serovar *ballum* were isolated from 21/61 (34%) ship rats, 63/243 (26%) brown rats and 2/12 (17 %) mice.

An earlier survey to determine the leptospiral status of rats in New Zealand was conducted on 160 rats from urban and rural Waikato (Carter and Cordes, 1980). The rats belonged to either the species *R. rattus* or *R. norvegicus*. Only two (*ballum* and *copenhageni*) of the six officially recognised serovars were isolated from them. Serovar *copenhageni* was isolated from 33/132 brown rats and 3/28 ship rats. Serovar *ballum*, on the other hand, was isolated from 12/132 brown rats and 3/28 ship rats. One hundred and thirty four rats were subjected to serological examination, using serovars *pomona*, *hardjo*, *copenhageni*, *tarassovi* and *ballum* as antigens, at a minimum dilution of 1:20. The twelve animals in which leptospiral

antibodies were detected were all brown rats. Eight had antibodies to *copenhageni*, one to *pomona*, one to *hardjo*, one to *ballum* and one to *pomona* and *hardjo*.

4 MATERIALS AND METHODS

4.1 Dogs - Sample Collection and Questionnaire

A letter requesting clotted blood samples or sera from dogs was sent to twenty private veterinary practitioners whose addresses were selected from telephone directories. The study area covered the lower half of the North Island of New Zealand, but the practitioners practised mostly in the Manawatu, Horowhenua and Rangitikei districts (see Figure 2) and each of them was provided with copies of a standard questionnaire form (see Appendix III), to be filled in for each dog from which a blood sample was collected. Blood samples (2-5ml) were taken from healthy dogs presented for corrective and/or cosmetic surgery or for consultation on minor problems. A total of 105 samples from dogs, which had not been vaccinated against leptospirosis, were received from practitioners.

In addition, 221 frozen canine sera were collected from the Batchelar Animal Health Laboratory (46 samples) and the Clinical Pathology Laboratory of the Institute of Veterinary, Animal and Biomedical Sciences at Massey University (175 samples). These sera were from dog samples submitted for a variety of investigations. With the exception of 5, the vaccination status of these dogs could not be determined.

A further 29 samples came from dogs belonging to friends and acquaintances in the Manawatu district (8 samples, from 7 unvaccinated dogs and 1 vaccinated dog) and the Animal Health Services Centre of Massey University (21 samples, all from dogs 7 days post-vaccination).

Each clotted blood sample was first centrifuged at 2500 r.p.m. for 20 minutes before the serum was separated. All sera were then stored at -20°C.

4.2 Rats - Sample Collection

Night visits to three rubbish tips (Palmerston North, Feilding and Levin) (see Figure 2 for these locations) between November 1997 and April 1998, with the intention of shooting rats under spotlight, were unfruitful. Live trapping, using wire mesh cage traps, was therefore chosen for rat capture. The traps, which measured 30 x 15 x 12 cm, had a spring-hinged door at one end and were baited with bread, apple and bacon.

Trapping was carried out on farms, at grain storage plants and around human dwellings in

the Manawatu and Horowhenua districts (Figure 2), between April 1998 and October 1998. In all, 24 rats (17 ship and 7 brown) were captured.

Rats were killed by transferring them from the cage trap into a plastic bag in which was placed a cotton wool swab or tissue paper soaked in halothane or chloroform. The air was squeezed from the bag and the top twisted and held in place for 3-5 minutes during which time the rat became asphyxiated.

After the species of each rat was determined using the criteria of Daniel (1974), the animal was weighed, sexed and classified as sexually mature or immature.

Sexual maturity is reached when the ship rats weigh approximately 80 g (Bentley and Taylor, 1965; Innes, 1977) and when brown rats weigh 115 g to 240 g (Calhoun, 1962; Leslie *et al.*, 1946).

A simple, partial necropsy, which involved opening the chest and abdomen, was carried out on each carcass. A 1-2 ml blood sample without anticoagulant was collected by cardiac puncture and both kidneys were removed from each rat. Other thoracic and abdominal organs were examined for macroscopic lesions and samples of abnormal tissues were fixed in formalin.

Once clotted, blood samples were centrifuged at 2500 r.p.m. for 20 minutes to collect the serum for storage. The kidneys were processed in a stomacher for culture.

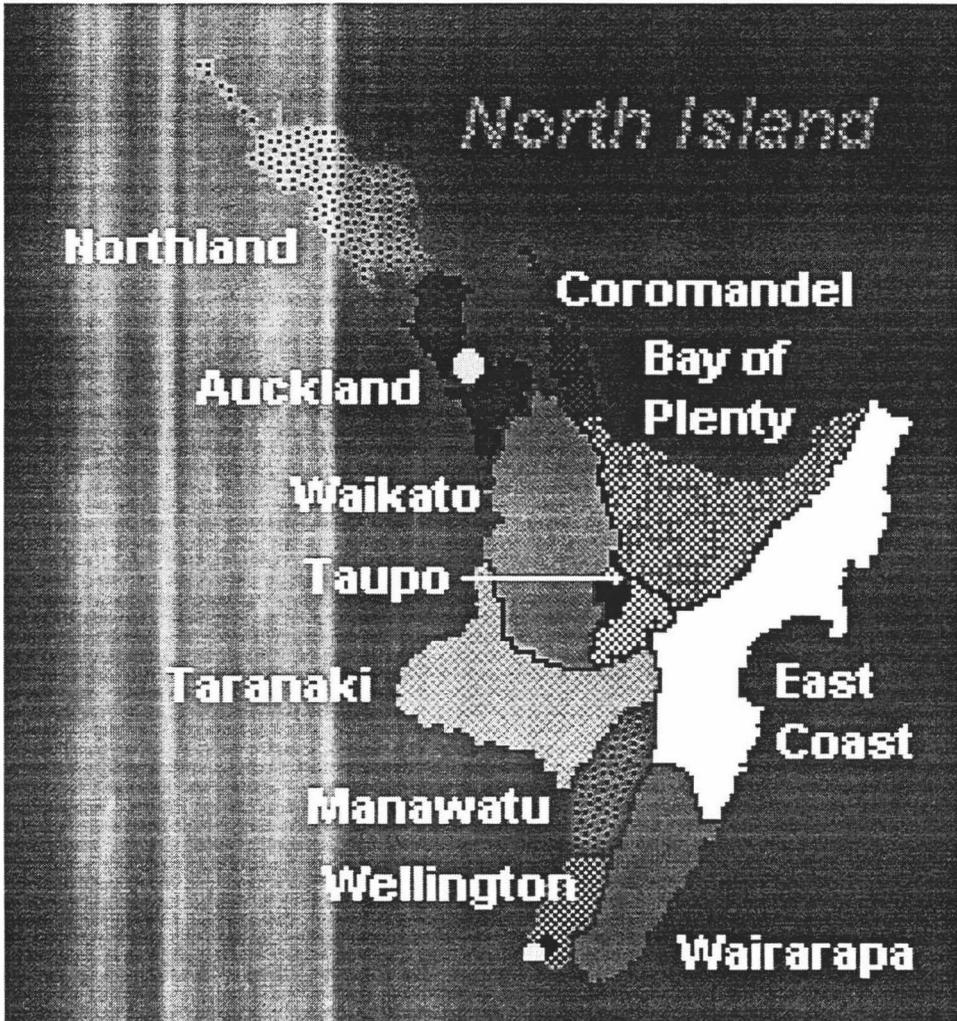


Figure 1. Regional map of the North Island of New Zealand

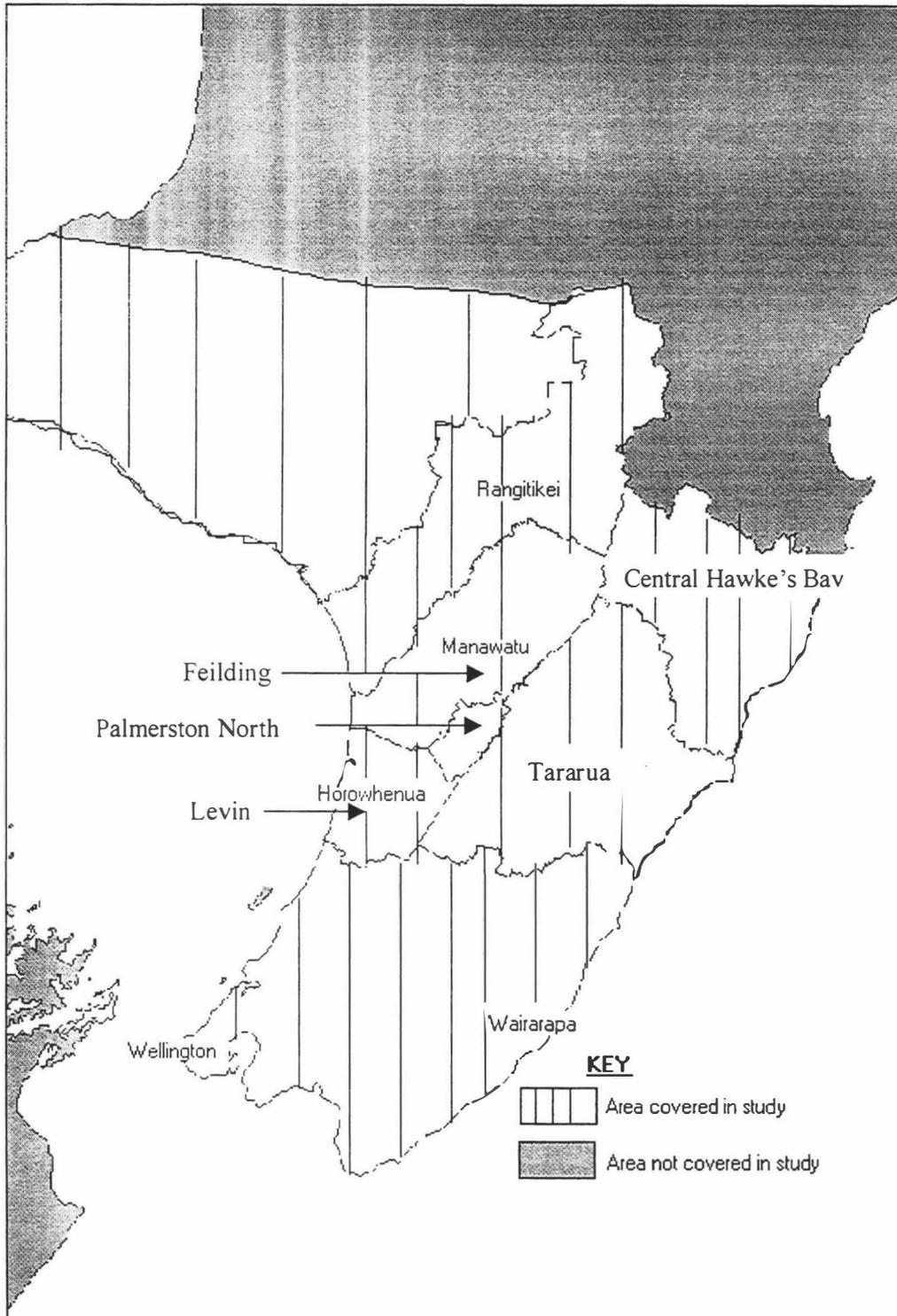


Figure 2. District map of the lower North Island of New Zealand

4.3 Serological Methods

4.3.1 Notes on the Microscopic Agglutination Test (MAT)

The discoverers of leptospires included in their initial publications a description of immune agglutination by convalescent or immune sera (Ido *et al.*, 1917). Development of diagnostic serology for leptospirosis quickly followed (Jacobsthal, 1917). This agglutination reaction, which is maximal when a pure culture of *Leptospira* is mixed with homologous antiserum, is the basis for serological diagnosis of leptospiral infection in animals and humans. Modified agglutination tests which used killed cultures and macroscopic rather than microscopic endpoints, were developed, but all had disadvantages, so the microscopic agglutination test (MAT) remains the standard for most purposes (Faine, 1994).

The MAT was originally developed in 1926 (Schüffner and Mochter, 1926). Of all the variations of this test, the most widely accepted is the one developed by Galton *et al.*, (1965) and later modified by Cole *et al.*, (1973).

The MAT involves the reaction of any class of antibodies and is specific for the infecting serovar or closely antigenically related serovars (Faine, 1994). However, cross-reactivity may occur due to sharing of common antigens or having similar antigenic determinants to two or more antigens. Cross-reactions to heterologous antigens are common during the early stages of leptospiral infection (Worthington, 1982).

Cross-reactivity between different serovars belonging to different serogroups is a rare phenomenon compared to cross-reactivity between serovars of the same serogroup (Faine, 1994). Each of the serovars being tested for in this study belongs to a different serogroup.

4.3.2 Procedure for the MAT

All sera (from dogs and rats) were examined for a serological reaction to the pathogenic leptospiral serovars *copenhageni*, *pomona*, *ballum*, *hardjo*, *tarassovi* and *canicola*.

Masterplates containing 1:6 dilutions of sera were prepared by adding 150 μ l of sterile standard saline solution to 30 μ l of a serum sample in each well of a flat-bottomed microtitre plate (see Figure 1). The masterplate was covered with parafilm and carefully sealed so that serum would not pass between wells and then stored at -20°C for later use.

The wells of round-bottomed serology plates were prepared by filling them with 25 μ l of

sterile standard saline solution using a multipipetter (see Figure 3 for diagrammatic illustration).

A row of serum samples from each thawed masterplate was then serially diluted by placing the combs of a multidiluter in the wells, mixing, then carrying across 25 µl samples of sera. The combs were then placed in the top row of a prepared serology plate (test plate) and the resulting solution mixed for 5 seconds. Doubling dilutions of each sample were made by repeating this process through successive rows of the serology plate. At the end of each serology plate, the combs were washed by agitating them in distilled water in a row of wells in a serology wash plate and then blotted with a paper towel. A standard serology plate was prepared in a similar manner using standard sera produced in rabbits against serovars *hardjo*, *pomona*, *ballum*, *copenhageni*, and *tarassovi*. These antisera were all produced at the Central Animal Health Laboratory (CAHL), Wallaceville from New Zealand isolates. No standard antiserum was available for serovar *canicola*, as this serovar has not been isolated from animals in this country.

Finally, 25-µl aliquot of freshly grown live antigen, at a density of approximately $1-2 \times 10^8$ organisms/ml was added to each well using a multipipetter. The density was established by comparison with standard solutions of sulphuric acid plus barium chloride. These solutions are equivalent to standard densities of cultures of leptospire in liquid media (McFarland nephelometer scale).

All antigens that were required were grown from standard stocks maintained in the Massey University Leptospirosis research laboratory. The identity of these antigens were verified (DNA typed) by restriction enzyme analysis (REA) in the Massey laboratory and are as follows:

- Serovar *hardjo* (strain hardjoprajitno) – originally from Brisbane WHO laboratory;
- serovar *pomona* (strain pomona) - originally from Brisbane WHO laboratory;
- serovar *ballum* (strain Mus 127) - originally from Brisbane WHO laboratory;
- serovar *tarassovi* (strain tarassovi) - original source unknown;
- serovar *copenhageni* (strain M 20) - original source unknown;
- serovar *canicola* (strain Hond Utrecht) - originally from Brisbane WHO laboratory.

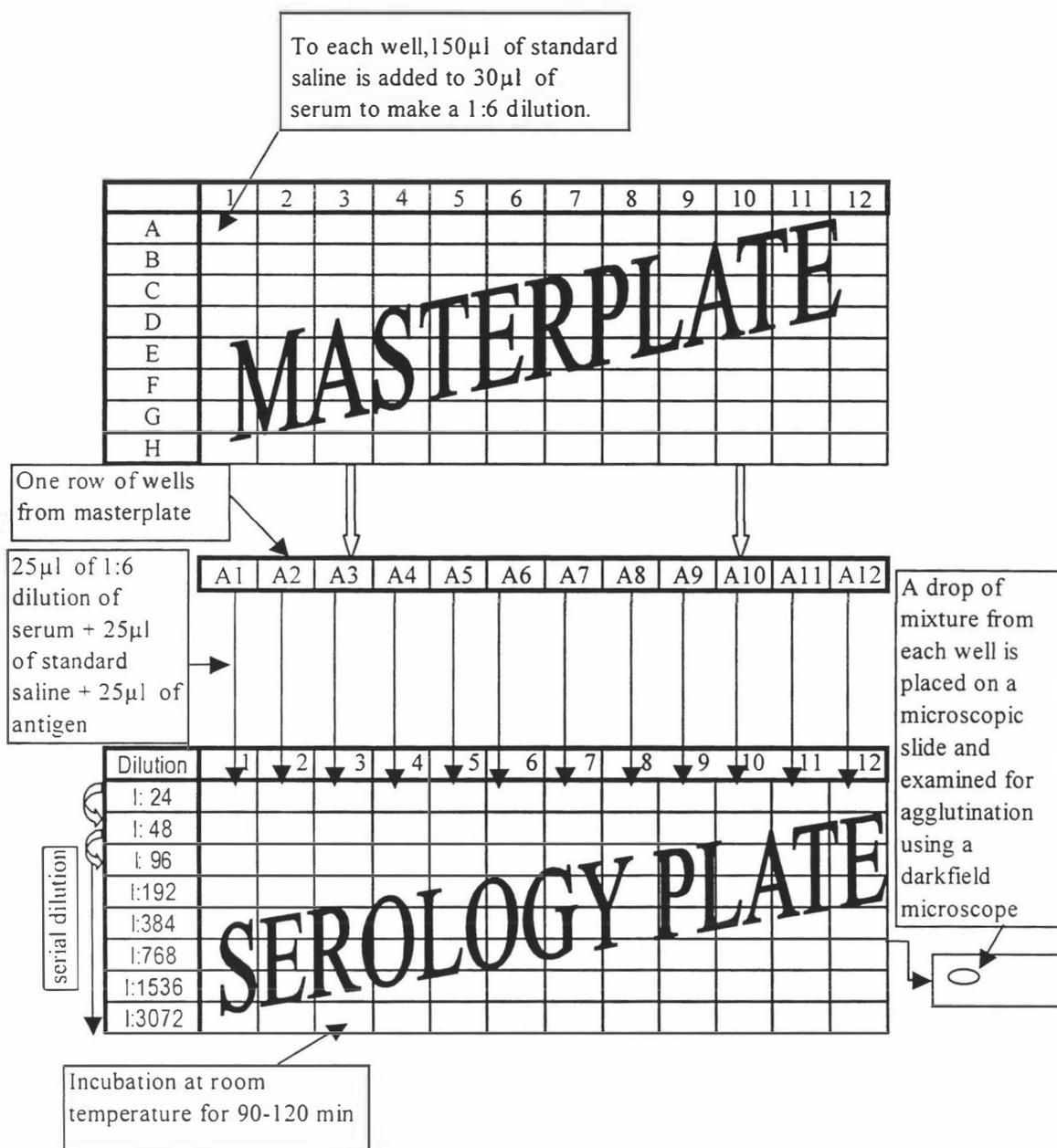


Figure 3. Schematic diagram of serological procedure (MAT).

In the end, eight, two-fold dilutions, inclusive of the added antigen, were made. The minimum serum dilution was 1:24 and the maximum was 1:3072. Plates were then sealed and left at room temperature for 90 to 120 minutes. A sample from each well, starting with the standard serology plates and then the test plates, was transferred to a microscope slide and examined using a darkfield microscope at 120 times magnification. The microscopic readings of the samples were decided by comparing with the readings of the standard plates. This comparison could not be made for *canicola* because there was no standard serum for this serovar. The end-point of an agglutination reaction was considered to be the highest dilution at which about 50% of the organisms had been agglutinated (Faine, 1982). A positive test was considered to be 50% agglutination at the lowest serum dilution of 1:24 (titres are read as the reciprocal of the dilution). Titres ≥ 96 in the case of nonvaccinated animals were considered to be equivalent to ≥ 100 and interpreted as recent or current infection (Smith *et al.*, 1994).

4.4 Bacteriological Methods

4.4.1 Notes on EMJH Culture Medium

Building on studies of mycobacterial and treponemal nutrition (Oyama *et al.*, 1953; Johnson and Gary, 1962; Johnson and Gary, 1963; Vogel and Hutner, 1961), it was demonstrated that leptospire will grow in a medium containing long-chain fatty acids as a nutritional source provided serum albumin is present as a detoxicant. This oleic acid-albumin medium, which was later modified (Johnson and Harris, 1967), is well known and widely used as Ellinghausen-McCullough-Johnson-Harris, or EMJH medium (Faine, 1994). The description below is a laboratory-adapted version of EMJH medium.

4.4.2 Procedure for Preparation of EMJH Culture Medium

Initially, stock solutions were prepared as follows:

- A: 1.5 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ plus 1.5 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in distilled H_2O to make 100 ml;
- B: 0.03 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in distilled H_2O to make 100 ml;
- C: 0.4 g of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled H_2O to make 100 ml;
- D: 0.02 g of Cyanocobalamin (vitamin B_{12}) in distilled H_2O to make 100 ml; and
- E: 10 g of Tween 80 in distilled H_2O to make 100 ml.

These solutions were sterilised by autoclaving at 121°C for 20 min at 15lbs / sq. inch.

In addition, stock solutions of FeSO_4 and 5'-fluorouracil were prepared. The stock solution

of FeSO_4 had to be freshly made, when needed, by dissolving 0.1g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 20 ml of distilled H_2O (Solution F).

The solution of 5'-fluorouracil was prepared by adding 1g of 5'-fluorouracil and 1-2 ml 2M NaOH to 50 ml of distilled H_2O in a beaker. The beaker was placed on a magnetic stirrer and heated gently to dissolve the 5'-fluorouracil. The pH was adjusted to 7.6 using HCl or NaOH and the volume was taken up to 100 ml with distilled H_2O . The resulting solution was then filter-sterilised in a biohazard cabinet and stored at 4°C.

An albumin supplement was then prepared by dissolving 10 g of bovine serum albumin (BSA fraction V) powder in 50 ml of distilled H_2O and, while stirring, the following respective volumes of stock solutions were added:

One ml of each of A, B, C and D plus 10.0 ml of F and 12.5 ml of E. The pH of the resulting solution was adjusted to 7.4 and the final volume was brought to 100 ml by adding distilled H_2O .

Next, a basal medium was prepared by dissolving 2.3 g of Difco basal powder (Difco Laboratories, Detroit, Michigan USA) in 900 ml of distilled H_2O and adjusting the pH of the resulting solution to 7.4. The basal medium was then sterilised at 121°C for 20 min at 15lbs / sq. inch.

Finally, the liquid EMJH culture medium was prepared by adding 100 ml of the albumin supplement to 900 ml of basal medium. The resulting solution was placed in a pressure chamber, and then filter sterilised in a biohazard cabinet into 500 ml bottles. To reduce bacterial contamination of the medium, 5'-fluorouracil, an antibiotic which selectively spares leptospire but inhibits the growth of most other bacteria (Johnson and Rogers, 1964a), was added at a ratio of 1 ml to 100 ml of EMJH.

The EMJH medium bottles were kept for 2 days at 37°C and then at 30°C for another 2 days to check for possible bacterial contamination. They were then stored at room temperature or at 4°C.

Each new batch of EMJH culture medium was tested before use. As *Leptospira hardjo* tends to be the most sensitive of the strains to adverse conditions, it was used as the test organism (Faine, 1982). A 5ml sample of EMJH in a 10ml narrow-necked bottle was seeded aseptically with 100 μl of a stock culture of this organism and observed for growth 3-7 days thereafter.

4.4.3 Culture Procedure

Paired kidneys from each rat were processed for culture in liquid EMJH medium within one hour of the rat being killed.

The external surface of both kidneys was sterilised by dousing in 70% ethanol and then quickly flaming over a bunsen burner. The sterilised kidneys were then placed in a sterile stomacher bag and 15-20 ml of sterile standard saline solution was added aseptically. The bag was then placed in a stomacher (Colworth Stomacher 400) and pummelled until the kidneys were mashed into a pulp. The bag was left to stand for a few minutes. Working in a biohazard cabinet, 100 μ l of liquid from the resulting suspension from the homogenised kidneys was then transferred to a 10 ml narrow-necked culture bottle containing 5 ml of liquid EMJH medium. After shaking gently to mix the contents, 100 μ l of the mixture was transferred to another culture bottle containing 5 ml of liquid EMJH medium. The process was repeated to obtain three serial dilutions of kidney suspension in liquid EMJH medium.

The cultures were checked fortnightly for growth of leptospire by using a flamed platinum loop to place a drop of culture medium onto a microscope slide and examining them using a darkfield microscope.

5 RESULTS

5.1 Dogs

In all, 355 canine sera were tested, and 201 (56.6 %) had antibody titres to one or more serovars, while the remaining 154 (43.4 %) did not react to any of the serovars tested. One hundred and forty eight (41.7 %) sera reacted exclusively to one serovar, 43 (12.1 %) had antibody titres to two serovars, 9 (2.5 %) had antibody titres to three serovars and one reacted to antigens of four serovars.

	Titre								Total
	24	48	96	192	384	768	1536	3072	
<i>copenhageni</i>	8	7	6	7	15	5	0	2	50
<i>ballum</i>	17	8	4	4	1	0	0	0	34
<i>pomona</i>	4	2	2	2	0	0	1	0	11
<i>tarassovi</i>	3	0	0	0	0	0	0	0	3
<i>hardjo</i>	26	18	13	3	0	0	0	0	60
<i>canicola</i>	78	21	6	1	1	0	0	0	107

Table 2. Total number of canine sera with MAT titres

Of the 355 sera tested, 50 (14%) showed $\geq 50\%$ agglutination against serovar *copenhageni*, 34 (9.6%) against *ballum*, 11 (3.1%) against *pomona*, 60 (16.9%) against *hardjo*, 3 (0.8%) against *tarassovi* and 107 (30%) against *canicola*. Titres ranged from 24 to 3072 for *copenhageni*, 24 to 384 for *ballum* and *canicola*, 24 to 192 for *pomona* and 24 to 96 for *hardjo*. All reactors to *tarassovi* had a titre of 24. See Table 2 for MAT results.

Twenty one of the 50 sera that reacted to serovar *copenhageni* were collected from recently-vaccinated (7-9 days) dogs. Table 3 and Appendix I show the individual titres in these vaccinated dogs. The canine leptospiral vaccine contains bacterins of the *Icterohaemorrhagiae* serogroup and therefore vaccinated dogs would be expected to have titres against serovar *copenhageni* (Hilbink *et al.*, 1992).

Sera from five other dogs, which were known to be vaccinated more than 200 days prior to sample collection, did not react to serovar *copenhageni*.

The blood sample collection form accompanying each blood sample lacked useful information on the living conditions, history of travel and clinical history for the majority of the dogs from which samples were collected.

	Titre								Total
	24	48	96	192	384	768	1536	3072	
<i>copenhageni</i>	0	0	0	3	13	5	0	0	21
<i>ballum</i>	3	1	1	1	0	0	0	0	6
<i>pomona</i>	1	0	1	2	0	0	0	0	4
<i>tarasasovi</i>	0	0	0	0	0	0	0	0	0
<i>hardjo</i>	2	1	1	1	0	0	0	0	5
<i>canicola</i>	2	1	0	0	0	0	0	0	3

Table 3. Number of canine sera with MAT titres (vaccinated dogs)

5.2 Rats

No rats were collected by night shooting as none were seen under spotlight.

In all, twenty four rats (17 ship and 7 brown) were captured by trapping. Five ship rats were sexually immature but the remainder of both species were mature. The sexually immature animals were all identified as ship rats. Table 4 shows the trapping data.

5.2.1 Bacteriological examination

No leptospire were isolated from the rat kidneys after three months of culture in liquid EMJH medium.

5.2.2 Serology

The agglutinating titres for the rats are shown in Table 5. At a minimum serum dilution of 1:24, eight rats had agglutinating titres against at least one of three serovars (*ballum*, *hardjo* and *canicola*). Two of these had a dual reaction against serovars *ballum* and *canicola*.

Sera from six rats (4 ship and 2 brown) showed reactions of $\geq 50\%$ agglutination against serovar *ballum*. Titres ranged from 24 to 192. Two of these rats had titres against *canicola* as

well, at a dilution of 1:24.

Three animals (2 brown rats and 1 ship rat) showed reactions of $\geq 50\%$ agglutination at the 1:24 dilution against serovar *canicola*. Only one ship rat had a titre of 384 against serovar *hardjo* and no rat showed a reaction of $\geq 50\%$ agglutination against serovars *copenhageni*, *pomona* or *tarassovi* at any dilution.

Date	Species	Sex	Weight (g)	Age	Location
25-Apr-98	Brown	F	281	Mature	Aokautere
06-May-98	Brown	M	423	Mature	Aokautere
06-May-98	Brown	M	416	Mature	Ashhurst
06-May-98	Brown	M	270	Mature	Massey
06-May-98	Ship	F	45	Immature	Linton
06-May-98	Brown	M	480	Mature	Levin
07-May-98	Ship	F	156	Mature	Levin
08-May-98	Ship	M	161	Mature	Massey
09-May-98	Ship	F	143	Mature	Massey
18-May-98	Brown	M	348	Mature	Otaki
18-May-98	Ship	M	130	Mature	Palmerston North city
23-May-98	Ship	M	120	Mature	Pohangina
25-May-98	Ship	M	151	Mature	Pohangina
26-May-98	Ship	M	150	Mature	Pohangina
27-May-98	Ship	M	163	Mature	Pohangina
02-Jun-98	Ship	F	98	Mature	Shannon
03-Jun-98	Brown	F	130	Mature	Sanson
05-Jun-98	Ship	M	59	Immature	Shannon
07-Jun-98	Ship	F	79	Immature	Shannon
24-Jun-98	Ship	F	78	Immature	Shannon
26-Jun-98	Ship	F	79	Immature	Shannon
29-Jun-98	Ship	F	84	Mature	Shannon
18-Aug-98	Ship	M	100	Mature	Shannon
05-Oct-98	Ship	M	110	Mature	Shannon

Table 4. Trapping data for rats

	Titre								Total
	24	48	96	192	384	768	1536	3072	
<i>copenhageni</i>	0	0	0	0	0	0	0	0	0
<i>ballum</i>	3	1	1	1	0	0	0	0	6
<i>pomona</i>	0	0	0	0	0	0	0	0	0
<i>hardjo</i>	0	0	0	0	1	0	0	0	1
<i>tarassovi</i>	0	0	0	0	0	0	0	0	0
<i>canicola</i>	3	0	0	0	0	0	0	0	3

Table 5. Total number of rat sera with MAT titre

6 DISCUSSION

In interpreting the results of this study, MAT titre levels and the vaccination status of dogs (if known) were taken into consideration. As mentioned in Materials and Methods, titres ≥ 96 were considered to be equivalent to ≥ 100 in nonvaccinated animals and were interpreted as recent or current infection (Smith *et al.*, 1994). The phenomenon of cross-reactivity was also borne in mind.

6.1 Dogs

6.1.1 Serovar *copenhageni*

The *copenhageni* titres in 21 sera from recently vaccinated dogs were most probably due to vaccination rather than natural infection as the vaccines used contained bacterins of the Icterohaemorrhagiae serogroup. Titres of the other 29 (Table 3) of the 50 sera (8%) that had ≥ 50 % agglutination against *copenhageni* ranged from 24-3072. Fourteen of these titres were ≥ 96 and were considered to have probably resulted from natural infection (Smith *et al.*, 1994). The remaining 15 lower titres (< 96) could indicate waning titres from previous exposure (natural or vaccination), cross-reactivity or a nonspecific reaction.

In this study, the prevalence of *L. copenhageni* infection as estimated by the MAT was 14/355 or 3.9%. These results strengthen the supposition that clinical canine leptospirosis can occur in dogs in the Manawatu / Horowhenua / Rangitikei districts of New Zealand. The estimated prevalence of infection in these districts is higher than those of Hilbink *et al.*, (1992), who found the prevalence to be between zero and 1.7%. It is worth noting that these workers carried out their survey on a more representative sample size compared to the present study. It should also be borne in mind that some of the dogs in the present study could have visited the Auckland/Waikato regions, where serovar *copenhageni* is known to be endemic (Hilbink *et al.*, 1992).

The fact that 5 dogs, which were known to have been vaccinated more than 200 days before sample collection, had no reaction against serovar *copenhageni* is interesting. It could indicate that the vaccination titres had waned beyond detection by the MAT or that these vaccinated dogs were not protected at the time of blood sample collection. An experimental challenge with leptospires could be carried out to find out if these dogs were still protected.

The vaccination status as well as other relevant information (including living conditions, history of travel and clinical history) could not be established for most dogs and therefore possible risk factors associated with MAT reactions in dogs could not be identified.

6.1.2 Serovar *canicola*

The *canicola* titres as indicated in Table 2 need to be interpreted cautiously for a number of reasons:

1. The readings of the MAT with respect to serovar *canicola* were equivocal compared to other serovars since the laboratory had little experience in dealing with this serovar.
2. No standard anti-*canicola* serum was used. No anti-serum was available because this serovar has not been isolated from animals in New Zealand.
3. Thirty five of the 107 sera that reacted to serovar *canicola* reacted to other serovars as well. In most cases, the reaction was weakest against serovar *canicola*.
4. The MAT titres against serovar *canicola* were generally low (99/107 had titres less than or equal to 48).

In the light of the above circumstances, it will be reasonable to consider the *canicola* reactions to be most probably due to nonspecific reaction or cross-reactivity.

On the other hand, it could be argued that these *L. canicola* results are a true reflection of the current situation in New Zealand, considering the fact that the dog is known to be the maintenance host for serovar *canicola*. This implies a more efficient host-parasite relationship, features of which include:

1. A high susceptibility of the dog (host) to infection – as demonstrated by 30.1% of dogs reacting to this serovar.
2. Disease generally occurring in a mild or subclinical form – probably reflected by the low titres.

Of the above two suppositions, the former seems more plausible in the light of the fact that clinical canine leptospirosis as reported by clinicians throughout New Zealand presents with severe jaundice (Ellison and Hilbink, 1990). This is a prominent feature of *L. copenhageni* infection rather than the varying severity of interstitial nephritis reported overseas to be associated with *L. canicola* infection (Baldwin *et al.*, 1987).

Also, from January 1987 to April 1990 (Ellison and Hilbink, 1990), the Central Animal Health Laboratory (CAHL) tested by MAT 116 diagnostic samples from dogs with a history

of leptospirosis and 34 samples from healthy dogs for export certification. Thirty three of the healthy dogs were completely negative (no reaction at 1:50 final serum dilution). One reacted against several serovars including *L. canicola* but this last reaction was weak with titres lower than those found against the other serovars. Of the diagnostic samples, only four reacted against *L. canicola* and the titres were always lower than the corresponding *L. copenhageni* titres. In addition, Hilbink *et al.* (1992) found only one weak reactor to serovar *canicola* in a nationwide survey of 5800 dogs.

It is important to note that all reports to date, relating to the occurrence of serovar *canicola* in New Zealand animals are serologically-based. Laboratory isolation of the organism will be more confirmatory and convincing. The rarity of reported human cases of *canicola* infection is strongly suggestive of the importation of the disease through overseas travel by New Zealanders and immigration.

6.1.3 Other serovars

Since the canine antileptospiral vaccine licensed for use in New Zealand only contains bacterins of the Icterohaemorrhagiae serogroup, it is likely that the serological reactions against serovars *ballum*, *hardjo*, *pomona* and *tarassovi* resulted from natural infection. Cross-reactivity between serovars belonging to different serogroups is a rare phenomenon compared to cross-reactivity between serovars of the same serogroup (Faine, 1994). Each of the above serovars belongs to a different serogroup.

In this survey 34, 11 and 60 of the 355 dogs reacted to serovars *ballum*, *pomona* and *hardjo* respectively. There were also 3/355 weak reactions against serovar *tarassovi*. The lack of data on the origins of the dogs precludes any comment; however, these serovars are known to be endemic in New Zealand (Brockie, 1974; Hellstrom, 1978; Hathaway, 1978; Ryan, 1978). It is possible that the sera that reacted to these serovars may have originated from dogs that had come into contact with the urine of maintenance hosts.

At a dilution of ≥ 100 , Hilbink *et al.* (1992) found a much lower prevalence (0.3- 1%) of serovar *ballum* among dogs in the lower half of the North Island of New Zealand. This and earlier reports relating to infection of dogs with serovars *pomona*, *tarassovi* and *hardjo* (Mackintosh *et al.*, 1980; Te Punga & Bishop, 1953) have not been conclusively linked to clinical disease.

Even though these serovars have not been known to significantly contribute to canine

leptospirosis in this country, they could be zoonotically spread to human beings in whom these serovars cause serious illness.

6.2 Rats

As only a small number of rats were captured, any interpretation of the above results must be made with extreme caution. The fact that no rats were seen at rubbish tips may have been due to rat control programs instituted by local city and town councils.

Even though there was no isolation of, or reaction to, serovar *copenhageni*, the small number of rats precludes one from arriving at the conclusion that this serovar is absent in the rat population of the Manawatu / Horowhenua / Rangitikei districts of New Zealand.

The agglutination reactions to serovars *ballum* and *hardjo* are in agreement with the findings of Hathaway (1978) who also failed to find any *copenhageni* infection among rats from these districts. Both ship and brown rats have been demonstrated to be maintenance hosts for serovar *ballum* in New Zealand (Hathaway, 1978).

The reactions against serovar *canicola* are probably non-specific or due to cross-reactivity with some other serovar as the titres are very low. Also, as with the case of the dog results, the serological test readings of serovar *canicola* were not as clear-cut as those of other serovars. This was because the readings were not compared with any standard readings.

7 CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn from this study:

1. The results of this study provide some evidence that dogs in the Manawatu, Horowhenua and Rangitikei districts of New Zealand are being exposed to *Leptospira interrogans* serovar *copenhageni*. Serovar *copenhageni* may be an important cause of canine leptospirosis in these regions.
2. A possible source of *copenhageni* infection in dogs could not be established in this study as too few rats were examined. Possibilities include rats, mice, hedgehogs and deer.
3. Exposure of dogs to serovars *ballum*, *hardjo* and *pomona* appears to be common in the study area. The result of these exposures could be sporadic clinical canine leptospirosis due to these serovars. Exposure to serovar *tarassovi* appears to be less common.
4. The source of serovar *ballum* infection for dogs in the study area may be rats. Other possibilities include mice and hedgehogs.
5. It is not clear whether serovar *canicola* occurs or does not occur in the study area, however, all studies to date support the absence of this serovar in New Zealand.
6. Trapping was a more successful method for capturing rats in the study area compared to night shooting.

It is recommended that:

1. Concerned dog owners (especially those with farm dogs) are encouraged to vaccinate their dogs against leptospirosis.
2. In addition to serovar *copenhageni*, serovars *ballum*, *pomona* and *hardjo* should be considered in the differential diagnosis of canine leptospirosis.
3. A more detailed study (longitudinal) of dogs is undertaken to further investigate the infection status as well as risk factors with respect to serovar *copenhageni*. Serovar *canicola* also requires urgent investigation since overseas, dogs are known not only to manifest clinical leptospirosis due to this serovar, but also to serve as maintenance hosts.
4. In addition to rats, other New Zealand mammals such as mice, hedgehogs and deer should be investigated as possible sources of *copenhageni* infection for dogs and humans.
5. In order to reduce ambiguity and subjectivity in the MAT results, tests that could be more sensitive and less cumbersome than the MAT, such as enzyme-linked immunosorbent assay (ELISA) (Smith *et al.*, 1994) or micro capsule agglutination tests (MCAT) (Sehgal *et al.*, 1997), should be developed and used for leptospiral investigations.

6. In order to capture rats, for the purpose of studying leptospirosis, live trapping should be used in conjunction with other methods.

APPENDICES

Appendix I. Individual MAT titres for vaccinated dogs

Sample ID	No of Days Postvaccination	Titres				
		<i>ballum</i>	<i>copenhageni</i>	<i>pomona</i>	<i>hardjo</i>	<i>canicola</i>
AHS1	7		768			
AHS10	7	48	192			24
AHS11	7		384			
AHS12	7	24	384			
AHS13	7	24	384			
AHS14	7		384			
AHS15	7		768		48	
AHS16	7	192	384			
AHS17	7		192			
AHS18	7	96	384		24	24
AHS19	7		384	192		
AHS2	7		768	96		
AHS20	7		768			
AHS6	7					
AHS21	7		384			
AHS3	7	24	384	192		
AHS4	7		768	24		
AHS5	7		384			
AHS7	7		192			
AHS8	7		384			
AHS9	7		384		24	
HMS5	825					
J1	9		384			48
MMS16	211					
SRV17	367					
SRV13	367				192	
SRV18	436				96	

Appendix II. Individual MAT titres for all dogs and rats (Microsoft Access tables on floppy disk)

Appendix III. Blood sample collection form for dogs

SAMPLE NUMBER:.....

DATE OF BLOOD SAMPLING:.....

SUBMITTER:.....ADDRESS:.....

.....PHONE / FAX:.....

ANIMAL ID. NUMBER:.....

AGE:.....BREED:.....

SEX: FEMALE MALE

HOME GEOGRAPHIC LOCATION (e.g. Levin, Feilding).....

.....

LIVING CONDITIONS:

• Farm dog? Yes No • Confined to home or yard? Yes No

HISTORY OF TRAVEL:

• Has the dog been to/at Auckland/Waikato region in the last 12 months? Yes No

• Where else outside of the lower half of the North Island has the dog been in the last 12 months?.....

CLINICAL HISTORY:

• What is the main reason for visit to vet (e.g. surgery, vaccination etc.)?.....

.....

• Has the dog shown clinical signs suggestive of leptospirosis in the last 12 months?

Yes No

LEPTOSPIROSIS VACCINATION HISTORY:

• Has the dog been vaccinated against leptospirosis? Yes No

• Last date of vaccination (leptospirosis).....

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