A STUDY OF THE EPIDEMIOLOGY AND CONTROL OF LEPTOSPIROSIS ON DAIRY FARMS.

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Veterinary Pathology and Public Health at Massey University.

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ABSTRACT

A retrospective analysis of published statistics showed that in the last ten years an average of 488 cases of human leptospirosis was reported annually. Over 90% of these were reported as occupationally associated and the majority were males, 15 to 44 years of age. The geographical distribution of human cases was associated with the distribution of dairy cattle in this country. The majority of cases occurred in October and November which coincided with the seasonal peak of milk production of factory supply dairy farms on which over 90% of N.Z.'s dairy cattle reside. In the Hamilton Health District years of higher than average incidence of reported human leptospirosis were associated with years of higher than average spring rainfall. The rise in reported human incidence over the last 30 years appears to be associated with changes in dairy farm practices over this period which have probably resulted in increased exposure of milkers to infected urine. These changes included transitions from cream supply to whole milk supply and from walkthrough to herringbone milking sheds, and increases in herd size, stocking rates and the number of cows milked per man. These changes appear to have been accompanied by an increased prevalence of *hardjo* and a decreased prevalence of *pomona* infections in dairy cows.

A cross-sectional survey of 213 Manawatu dairy farm residents showed that 34% of the 193 people who milked cows had leptospiral titres of 1:24 or greater, of which approximately two-thirds were to *hardjo* and one-third to *pomona*. Women milkers and farm residents who did not milk were all serologically negative. A third of the seropositive milkers had a history of clinical leptospirosis. A subsequent case-control survey of 25 farms where the milkers had leptospiral titres of 1:96 or greater and 27 farms where the milkers were seronegative showed that leptospiral titres in milkers were associated with the presence of endemic *hardjo* infection in the milking herd

and evidence of *pomona* outbreaks in the herd in the past. Other factors which were significantly correlated with leptospiral titres included the time spent in the dairy shed during milking, the wearing of shorts, the keeping of pigs for sale and the number of years the individual had been working on a dairy farm. The type of milking shed and the size of the herd were interrelated and both showed strong trends towards an association with titres in milkers.

An attempt was made to determine the role of the dog in the epidemiology of leptospirosis in this country. A number of investigations were carried out including a case-study of a clinical outbreak of leptospirosis in a group of hounds, experimental infections of dogs with *tarassovi*, case-studies of *pomona* infections in dogs associated with epidemics of *pomona* infection in cattle and serological surveys of dogs living on dairy farms in the Manawatu and of city dogs which attended the Massey University small animal clinic. It appears that dogs are susceptible to infection with all the serovars present in this country and long term kidney infection may occur. However, dogs are not thought to be maintenance hosts for any of these serovars due to the low intensity of leptospiruria, the poor survival of these leptospires in dog urine and the lack of consistent dog-to-dog transmission. Therefore, dogs are not likely to be significant in the epidemiology of leptospirosis on dairy farms. No definite evidence was found of *canicola* infection in either farm or city dogs.

The results of an experimental infection of cattle and sheep with *balcanica* and an investigation of a natural outbreak of *balcanica* infection of cattle on a dairy farm indicate that, although sporadic infection may occur in cattle and sheep, they are not likely to be maintenance hosts for this serovar and infection is unlikely to become endemic in cattle herds

or sheep flocks. Evidence is presented to suggest that cattle infected with *hardjo* or vaccinated with a *hardjo* bacterin may be resistant to infection with *balcanica*. Therefore, *balcanica* is unlikely to be a significant hazard to dairy farm workers.

An investigation of an epidemic of *pomona* abortions on a dairy farm showed that vaccination with a *pomona* bacterin during the epidemic appeared to prevent approximately 27% of the herd from becoming infected, a third of which may have aborted. It was also found that cattle which aborted had significantly higher titres than infected cattle which did not abort. The outbreak probably originated from infected pig effluent.

The results of vaccination trials showed that two doses of a *hardjo/pomona* bacterin, given four weeks apart, gave cattle significant protection against infection and leptospiruria after natural challenge with *hardjo*. A 30 month trial on a commercial factory supply dairy farm, which entailed the double vaccination of all the calves (9 months or older), yearlings, milking cows and bulls and then annual revaccination of all animals, apparently eliminated *hardjo* infection which had been endemic on the property previously. It is considered that annual revaccination will prevent the introduction of *hardjo* or *pomona* infection into this herd.

It is concluded that the incidence of leptospirosis in dairy farm workers could be significantly reduced by the elimination of *hardjo* and *pomona* infections in the cattle and pigs on dairy farms using an appropriate programme of routine vaccination.

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Occupational exposure. This urine could contain up to a million leptospires per millilitre.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

GENERAL INTRODUCTION

Leptospirosis in man and animals has been diagnosed in most countries of the world since Weils disease was first described clinically in the 1880s and Inada and Ido(1915) first reported the discovery of the infective organism some 30 years later. In this country research by the Department of Agriculture in the 1950s was stimulated primarily by the economic losses associated with "redwater" in calves and bovine abortions. The disease in man caused by *pomona* was recognised as being identical to "swineherds disease" first described in Europe, and the human health aspects were studied by individuals in the Medical Research Council, Dunedin Medical School and the National Health Institute. By the mid 1950s a *pomona* vaccine had been developed and potentially provided a means of reducing the losses in livestock. However, the incidence of human leptospirosis continued to increase through the 1950s and 1960s and its importance as an occupational disease of dairy farmers was acknowledged by the name "dairy farm fever" coined by Kirschner and Maguire (1957). From 1967 to 1972 the Department of Health expended considerable time and effort investigating leptospirosis on dairy farms and this resulted in the isolation of serovar *hardjo* from humans and dairy cattle in 1971 (Christmas *et al*, 1974a). Around this time Jamieson *et al* (1970) wrote "since it is a disease of emerging importance in man there is a need for closer cooperation between the veterinary and medical professions at both research and field levels." Six years later Philip (1976), a medical practitioner in the Hauraki Plains area which has one of the highest incidence rates in New Zealand (N.Z.), wrote a paper entitled "Leptospirosis: New Zealand's No. 1 Dairy Occupational Disease". In it he expressed the frustration felt by many because of the continuing high level of human cases of leptospirosis and he wrote "most occupational diseases in the dairy

industry have been eliminated or prevented, nowadays, with leptospirosis remaining the bogeyman of people working with milking cattle".

The major aims of this study, which has been funded by the Accident Compensation Commission, have been to investigate the epidemiology and control of leptospirosis on dairy farms in the Manawatu, to evaluate a *hardjo/pomona* vaccine under natural conditions and to look at factors which may interfere with a vaccine control programme.

In the last 10 years there have been a number of investigations of the epidemiology of the six serovars known to be endemic in N.Z., especially by my colleagues in the Department of Veterinary Pathology and Public Health at Massey University. During the last two years, three post graduate veterinary students in this department have completed doctoral studies on various aspects of the epidemiology of leptospirosis in N.Z.; J.S.Hellstrom studied leptospirosis in bovines, T.J.Ryan studied the disease in pigs and S.C.Hathaway investigated the epidemiology of leptospirosis in wildlife. I have endeavoured to review their work and that of previous investigators in relation to the epidemiology of these six serovars in domestic livestock. I have also reviewed the literature on the epidemiology of human leptospirosis in this country and the control of infection in both animals and man.

CHAPTER ONE

LEPTOSPIROSIS IN NEW ZEALAND : A REVIEW

LEPTOSPIROSIS IN DOMESTIC ANIMALS AND WILDLIFE

Six serovars belonging to the species *Leptospira interrogans* have been isolated from domestic, feral and wild animals in N.Z. They are *hardjo, balcanica, pomona, copenhageni, ballum* and *tarassovi* (Table 1.1). Recent studies have provided good evidence that under N.Z. conditions *hardjo, pomona* and *tarassovi* are maintained in domestic livestock populations (Hellstrom, 1978; Ryan, 1978) and *balcanica, copenhageni,* and *ballum* are maintained in wildlife reservoirs of infections (Brockie,1976; Brockie, 1977; Brockie and Till, 1977; Hathaway, 1978; Hathaway *et al*,1978; Carter and Cordes, 1980) (see Table 1.2). The following review of the literature will concentrate on the epidemiology of these six serovars and each will be considered separately. The recent isolation of *australis* from a human patient in Northland (Thompson, 1980) indicates that this serovar may also be present in N.Z. livestock and/or wildlife and this will be discussed briefly.

TABLE 1.1 : FIRST REPORTS OF LEPTOSPIRAL SEROVARS ISOLATED FROM NEW ZEALAND ANIMALS

Serogroup	Serovar	Animal	Reference
Hebdomadis	*hardjo*	cattle	Lake, 1973
	"hardjo" *	possum	Brockie, 1975; de Lisle *et al*,1975
	balcanica	possum	Marshall *et al*,1976
	hardjo	sheep	Bahaman *et al*,1980
	balcanica	cattle	Mackintosh *et al*,1980b
	hardjo	goats	Schollum and Blackmore, in press
	balcanica	goats	Schollum and Blackmore, in press
Pomona	*pomona*	sheep	Anon,1951
	pomona	cattle	Anon, 1951
	pomona	dog	Te Punga and Bishop, 1953
	pomona	pig	de Jong and Fowler, unpublished, 1968
	pomona	cat	Harkness *et al*,1970
Icterohaemorrhagiae	*"icterohaemorrhagiae"* *	Norway rat	Kirschner and Gray,1951
	icterohaemorrhagiae AB *(copenhageni)*	cattle	Dodd and Brakenridge, 1960
		ship rat	Carter and Cordes, 1980
Ballum	*ballum*	cattle	Ris *et al*, 1973
	ballum	Norway rat	Brockie, 1976,1977
	ballum	ship rat	Brockie, 1976,1977
	ballum	house mouse	Brockie, 1976,1977
	ballum	hedgehog	Brockie and Till, 1977
Tarassovi	*tarassovi*	pig	Ryan and Marshall,1976
	tarassovi	dog	Mackintosh *et al*.1980a

* not typed

TABLE 1.2 : THE SIX LEPTOSPIRAL SEROVARS KNOWN TO BE ENDEMIC IN NEW ZEALAND LIVESTOCK AND WILDLIFE AND THEIR MAINTENANCE HOSTS.

Serovar	Maintenance Host	Reference
hardjo	cattle	Hellstrom, 1978
pomona	pigs	Ryan, 1978
tarassovi	pigs	Ryan, 1978
balcanica	possum *(Trichosurus vulpecula)*	Hathaway, 1978; Hathaway *et al,* 1978
copenhageni	Norway rat *(Rattus norvegicus)*	Brockie, 1976, 1977; Carter and Cordes, 1980
ballum	ship rat *(R.rattus)*	Brockie, 1976, 1977; Hathaway, 1978; Carter and Cordes, 1980; Blackmore and Hathaway, 1980
	Norway rat	Brockie, 1976, 1977; Hathaway, 1978; Carter and Cordes, 1980; Blackmore and Hathaway, 1980
	house mouse *(Mus musculus)*	Brockie, 1976, 1977; Hathaway, 1978; Blackmore and Hathaway, 1980
	hedgehog *(Erinaceus europaeus)*	Brockie and Till, 1977; Hathaway, 1978

Serovar *hardjo*

The first published reports of Hebdomadis serogroup titres being detected in cattle appeared in the late 1960s (Anon, 1968a; Jamieson *et al*, 1970), although it was not until 1971 that the first confirmed isolation of *hardjo* was made from cattle (Lake, 1973). Since then there have been a number of serological surveys of cattle from the Waikato area (Lake, 1973; Anon, 1974b; Anon, 1974c), the Taranaki area (Brockie, 1976) and the lower North Island areas (Roach, 1973) which showed serological prevalences of Hebdomadis titres ranging from 18 to 57.8%. The most recent survey (Hellstrom, 1978) included 480 bovine sera from throughout N.Z. which were randomly chosen from sera collected during 1973 and 1974 for whole herd brucella tests. The results of serological examinations using the microscope agglutination test (MAT) showed the presence of *hardjo* titres $\geq$ 1:17 in 86% ($\frac{114}{133}$) of herds and 65% of cattle in the North Island and 56% of herds and 39% of cattle in the South Island. This demonstrated the widespread nature of Hebdomadis titres in dairy and beef cattle throughout N.Z. in the mid 1970s.

It is not known how long *hardjo* has been present in N.Z. cattle since Hebdomadis serogroup antigens were not used in routine serological tests until the late 1960s (Hellstrom, 1978). However, Hebdomadis serogroup titres have been detected in humans since the late 1950s (Josland, 1958) and an increasing proportion of clinically affected dairy farmers from the Hauraki Plains area had titres to *medanensis* antigen over the period 1957-66 (Philip and Tennent, 1966). Hebdomadis organisms were isolated from four dairy farmers in the North Island in 1968 and although never definitely identified they were probably all *hardjo* (Till, 1968). It was not until 1971 that confirmed *hardjo* isolations were recorded

from dairy farmers (Christmas *et al*, 1974a). The use of *hardjo* as an antigen in serological tests from the beginning of the 70s has confirmed that *hardjo* is the most commonly occurring serovar in cattle (Hellstrom, 1978) and humans (Brockie, 1976) in this decade. The recognition of *hardjo* infection as a common disease of cattle and dairy farm workers in the 1960s and 1970s has been paralleled by similar increases in the reported incidence of human and cattle *hardjo* infections in Australia (Stallman, 1972; Sullivan, 1974) and the United Kingdom (U.K.) (Coghlan, 1979). Whether these reports reflect true rises in prevalence and incidence or apparent rises due to increased awareness and improved diagnosis and reporting is difficult to assess.

In a study on the epidemiology of *hardjo* in a town supply dairy cattle, Hellstrom (1978) showed that *hardjo* infection continually cycled in the dry-stock herd which included the yearling heifers. A propagating epidemic occurred in these yearlings in the late winter/spring and resulted in a leptospiruria which persisted for up to 14 months, allowing infection to pass from one year to the next.

Serological surveys of Manawatu dairy cattle in the autumn and spring (Hellstrom, 1978) also indicated that infection occurred in the spring. Subsequent serological studies (Anon, 1980b; Bahaman, pers. comm.) of 35 factory supply herds in Taranaki indicated that infection was cycling in the yearlings in less than half of the herds. In most of the others it appeared that infection was cycling in the milking herd, where susceptible first-calving two year old heifers became infected when they entered the herd in spring and were exposed to leptospiruric cows that had been infected the previous year. As factory supply farms account for over 90% of dairy cattle in N.Z. (see Chapter Four) these findings probably represent the two most common endemic cycles of

hardjo infection present in dairy herds in this country. Serological surveys (Ellis and Michna, 1976a; Ellis, 1978) indicate that similar cycles occur in Scottish dairy herds. Forty percent of yearling heifers in these surveys were infected but there was also a peak in the distribution of high titres in cows in their first year of milking.

Hardjo infection in yearling cattle appears to be asymptomatic (Hellstrom,1978; Marshall *et al*,1979b). However, a transient syndrome characterised by pyrexia, agalactia, flaccid udder and yellow milk secretion in all four quarters has been reported in milking cows infected with this serovar in N.Z. (Lake, 1975; Anon,1977b, g, 1980a) and overseas (Sulzer *et al*, 1964; Sullivan and Callan, 1970; Ellis, 1978). Sporadic abortions have been attributed to *hardjo* infection of pregnant cows in N.Z. (Lake, 1975), Australia (Hoare and Claxton, 1972), United States of America (U.S.A.) (Hanson and Brodie, 1967) and the U.K. (Ellis,1978). Various experimental infections of pregnant cows with *hardjo* (Sullivan, 1972; Ellis and Michna, 1977; Hanson and Brodie, 1967) showed that this serovar was capable of causing abortion or premature live birth in the last trimester of pregnancy although the proportion of experimentally infected animals that actually aborted was less than 12%. Little *et al* (1980) conducted a survey of sera from aborted cows and a control group of non-aborting cows in the U.K. and concluded that up to 10% of abortions were associated with Hebdomadis titres. However, it cannot be assumed that these results are applicable to N.Z. as it is possible that there are strain differences between the N.Z. *hardjo* and overseas serovars typed as *hardjo*. In N.Z. abortion investigations conducted under the brucellosis eradication scheme showed that Hebdomadis titres $\geq$1:200 were associated with 11.6% of abortions (Anon,1975a). However, no control group of cattle was tested and because of the high serological prevalence of Hebdomadis titres in the normal cattle population (Hellstrom,1978) it is not possible to interpret these results accurately.

The first report of Hebdomadis titres occurring in sheep was by Ris (1975) who found that 65% of 344 sheep in ten flocks in the lower North Island had Hebdomadis titres of ≥1:100.

Hardjo has subsequently been isolated from sheep (Bahaman *et al*, 1980) and a recent survey of sheep from the lower North Island showed a serological prevalence of 37.8% with Hebdomadis titres of ≥1:24 (Bahaman, 1981). It has not been shown whether these titres reflect endemic infections or sporadic outbreaks. There are no reports of natural outbreaks of clinical disease associated with *hardjo* infection in sheep. Sheep have been experimentally infected with *hardjo* (Hathaway and Marshall, 1979) without showing any clinical signs of infection and shed leptospires in their urine. However, the duration of leptospiruria after natural infection has not been reported, and the ability of sheep to act as maintenance hosts for *hardjo* is not known.

Although there have been no confirmed cases of abortions in mares associated with *hardjo*, Hebdomadis titres ≥1:200 have been found in horses suffering mild clinical signs of pyrexia and inappetance (Anon, 1975b). A serological survey of horses in the Manawatu area showed a low prevalence of Hebdomadis titres but these were not associated with a history of clinical disease (Doe, 1979).

Despite extensive surveys conducted in N.Z. there is no cultural or serological evidence of *hardjo* infection in pigs or rodents despite their having been exposed to infected cattle on farms (Blackmore *et al*, 1976; Brockie, 1977; Hathaway *et al*, 1978; Ryan, 1978). There have been few serological surveys of leptospirosis in dogs in N.Z. (Salisbury, 1954; Anon, 1968b) and none of these have reported Hebdomadis titres. There has been one report of Hebdomadis titres occurring in two dogs living

on a dairy farm where the farmer had contracted leptospirosis (Anon,1972).

In the mid 1970s it was postulated that the possum *(Trichosurus vulpecula)* was the maintenance host for *hardjo* due to a high serological prevalence of Hebdomadis titres and the isolation of a leptospire that was provisionally typed as *"hardjo"* (Brockie, 1975; de Lisle *et al*,1975). Subsequently, it has been shown that *balcanica* infection is widespread in the possum population (Marshall *et al*, 1976; Hathaway *et al*, 1978) and it appears that earlier workers were misled by the serological similarity of *balcanica* and *hardjo*.

Thus it appears that cattle are the maintenance hosts for *hardjo* but other species may become infected and act as accidental or short term hosts.

Serovar *pomona*

Serovar *pomona* was first isolated in N.Z. from cattle and sheep in 1950 (Anon, 1951) although a clinical syndrome of haemoglobinuria and death in young calves had been recognised for some years prior to this (Salisbury, 1954; Jamieson *et al*, 1970). The first human cases were diagnosed in 1951 (Kirschner *et al*,1952) and these authors recognised the significance of pigs as a source of *pomona* for cattle and humans on dairy farms, referring to it as "swineherd's disease" a term popularised by Gsell (1952). Up until the early 1960s the majority of factory supply dairy farms supplied cream to the factory and kept pigs to consume the skim milk. Thus dairy farmers were also "swineherders". With the transition from cream supply to whole milk supply in the 1960s and a consequent decline in the number of pigs kept on dairy farms there has been a decline in the incidence of redwater in calves (Hellstrom,1978). However, the total number of pigs in N.Z. has declined only slightly over

the last 30 years (see Chapter Four) and today the majority of pigs are kept on farms where more than 50% of the farm income is derived from pigkeeping. A recent survey (Ryan, 1978) of 234 adult sows from throughout N.Z. showed 65% had *pomona* titres ≥1:12, with a higher prevalence in the North Island than the South Island. A similar survey in 1958 (Russell and Hansen, 1958) showed a serological prevalence of 43% with titres ≥1:10 to *pomona* and they also found a higher prevalence in the North Island. This apparent increase in prevalence over the last 20 years may reflect either a greater sensitivity of the test used in the recent survey or it may reflect a true increase in the prevalence of *pomona* due to the larger sow herds and more intensive pig farming practiced in the 1970s compared with the more widespread keeping of small numbers of pigs on dairy farms in the 1950s ..

A survey of abattoir pigs from the lower North Island showed that 87% of 84 young pigs and 86% of 65 adult pigs had *pomona* titres ≥1:12 (Ryan, 1978). However, 45% of these young pigs' kidneys yielded *pomona* isolates compared with only 2% of the adult pigs' kidneys. Ryan concluded that in the endemic state infection cycles in young pigs, 6 to 12 months old, and although leptospiruria can last for 12 to 24 months (Mitchell *et al*,1966) it is not lifelong. Infection in young pigs is usually asymptomatic (Ryan, 1978) but if pregnant sows become infected, especially in the last three weeks of gestation, abortion, stillbirths and/or weak piglets may result (Powers *et al*, 1956). Ryan (1978) concluded that in N.Z. the pig is the maintenance host for *pomona* and is the source of infection for other animals directly or indirectly.

A number of syndromes associated with *pomona* infection in cattle have been recorded : haemoglobinuria or "redwater" and sudden death in calves (Anon, 1951; Ensor and McClure, 1953; Salisbury,1954),

abortions in cattle (Te Punga and Bishop, 1953) and agalactia in milking cows (Lake, 1975). Adult cattle and calves can also be asymptomatic carriers (Salisbury, 1954). In a review of the literature on *pomona* infections of cattle, Hellstrom (1978) found that the reported shedding times for cattle infected with *pomona* varied from one to four months. Thus, although transmission readily occurs between cattle (Doherty, 1967a; Blackmore *et al*, 1976; Hellstrom, 1978) they are relatively short term hosts. Consequently outbreaks of *pomona* infection are likely to be self-limiting in a closed herd (Hellstrom,1978). Some authors (Tennent and Philip, 1964; Jamieson *et al*. 1970; Christmas *et al*, 1974a) largely discounted pigs as a source of *pomona* infections in cattle and there have been a number of reports of outbreaks occurring where there has been no apparent pig contact (Webster, 1957; Blackmore *et al*,1976). However, a number of such outbreaks may have been initiated by the introduction of infected cattle (Blackmore *et al*, 1976), or by access to infected water (Shield, 1974) or pig effluent (Anon, 1974a). Hellstrom(1978) isolated *pomona* from a waterway on a dairy farm downstream from a piggery in which *pomona* infection was endemic, and a number of authors (Kirschner and Maguire, 1957; Okazaki and Ringen, 1957; Hellstrom,1978) have demonstrated the survival of *pomona* in soil and water under various conditions for up to 183 days. Swimming in contaminated water has frequently been implicated in human cases of *pomona* in the USA (Anon, 1978b). Hellstrom (1978) concluded from reviewing the literature and from the results of his studies that cattle are not long term maintenance hosts for *pomona* and that bovine and human *pomona* infections result from either direct or indirect contact with pigs or from cattle which are experiencing epidemics following direct or indirect contact with pigs.

Sheep are susceptible to infection with *pomona* which may cause sudden death in lambs but is usually asymptomatic in adult sheep (Hartley, 1952; Salisbury, 1954). Experimental infections have been readily

established in lambs resulting in leptospiruria lasting three months, although leptospiruria lasting nine months has been observed in a naturally infected ewe (Webster and Reynolds, 1955). In recent years the incidence of *pomona* outbreaks in N.Z. sheep has declined and it is now an insignificant cause of lamb mortality (Anon, 1979a), and it is unlikely that sheep are important hosts for *pomona* in this country. A recent survey of abattoir sera from the lower North Island showed that 7.5% of sheep had *pomona* titres $\geq$1:24 (Bahaman, 1981).

Abortions in horses in N.Z. due to *pomona* have been reported (Anon, 1977d) but are probably uncommon and sporadic.

Pomona has been isolated from a dog on a dairy farm which was experiencing a *pomona* abortion storm in the herd (Te Punga and Bishop,1953). Two other dogs on that property had titres to *pomona* although none of these dogs showed any clinical signs of disease. This and another similar case (Anon, 1975 c, d) demonstrate that dogs become infected on farms where *pomona* is prevalent. A limited survey reported by Salisbury (1954) showed that of 63 canine sera received by Wallaceville Animal Research Centre for diagnostic purposes, seven were "positive" for *pomona*. In a survey of 67 police dogs from throughout N.Z., all were serologically negative to a" variety of leptospiral antigens" (Anon,1968b; Jamieson *et al*,1970). However, there are no reports of the serological prevalence of leptospirosis in farm dogs which appear to be at risk. The degree and duration of leptospiruria and the importance of dogs as hosts for *pomona* are not known.

Pomona has also been isolated from a cat living on a dairy farm (Harkness *et al*,1970) and these authors suggested that it may have been a "carrier" for six months or more although its titre of 1:3000

indicated a more recent infection. Hathaway (1978), in a survey of wildlife in the lower North Island, found a *pomona* titre of 1:96 in one of 11 feral cats, but failed to isolate leptospires from any of their kidneys.

Despite extensive surveys of wildlife, including rats, mice, hedgehogs, mustelids, rabbits, possums, deer, feral goats and pigs (Daniel, 1966, 1967; Blackmore *et al*, 1976; Brockie, 1976; Brockie and Till, 1977; Hathaway *et al*, 1978), no serological or cultural evidence of a reservoir of *pomona* infection was found. This is in contrast to the situation reported in North America (Roth *et al*, 1963; Mitchell *et al*, 1966), where some wildlife species are suspected of acting as reservoirs of *pomona*. It thus appears that only domestic animals are involved in the epidemiology of *pomona* in N.Z.

Serovar *tarassovi*

The first evidence that *tarassovi* was present in N.Z. was provided by Kirschner (1954) in a serological survey of 100 adult pigs and 100 cattle at the Dunedin abattoir. He found that 6% of pigs and 3% of cattle had titres $\geq$1:150 to *mitis (tarassovi)*. In the same paper the author reported the serological diagnosis of *tarassovi* infection in three men who had pig and cattle contact. The first extensive survey of pigs in this country (Russell and Hansen, 1958) showed that 38% of pigs had *tarassovi* titres $\geq$1:10 which was lower than the 45% prevalence of titres to *pomona*. However, like *pomona*, the prevalence was greater in the North Island than the South Island. Ryan (1978) obtained similar results in a survey using 234 adult pig sera collected between 1975 and 1977 from throughout the country. Ryan and Marshall (1976) reported the first isolation of *tarassovi* from pigs in N.Z. during a survey based on kidney culture of 80 pigs from the lower North Island. In that survey they obtained one

tarassovi isolate and 38 *pomona* isolates, which probably reflects the relative importance of these two serovars in this area. A trace-back revealed the origin of the pig from which *tarassovi* was isolated. On the farm of origin, *tarassovi* was endemic in the pig herd together with *pomona*, and both infections cycled in the young pigs. Unlike the situation in Europe, *tarassovi* has not been recognised as a major source of problems on pig farms in N.Z. (Ryan, 1978) and there are few reports of abortions attributed to this serovar (Anon,1976c; Anon, 1977a).

Since the finding of a 3% serological prevalence of titres ≥1:150 to *tarassovi* in cattle in the early 1950s (Kirschner, 1954) there have been three other published serological surveys of cattle which tested for this serovar. Brockie (1976) found a zero prevalence of titres ≥1:100 in 100 sera from 20 herds in the Taranaki area. Ryan and Marshall (1976) found a 6% prevalence of titres ≥1:50 from 300 cattle sera from throughout N.Z. and Hellstrom (1978) found that 9% of 480 cattle sera from throughout N.Z. had *tarassovi* titres ≥1:17. In this latter survey there were distinct regional differences, with Taranaki, South Auckland and Wellington districts having the lowest prevalence (0-5%) and Central Auckland, Bay of Plenty and Nelson-Marlborough districts having the highest (17-21%). The prevalences for the North Island and South Island were the same (9%). Hellstrom (1978) presented some evidence to support the view that some of the low *tarassovi* titres represented crossreactions with high *hardjo* titres.

There are no reports of clinical disease resulting from bovine infection with *tarassovi* in N.Z. and,after reviewing the overseas literature, Ryan and Marshall (1976) concluded that this serovar does not normally cause severe clinical disease in cattle. There is no evidence to suggest that cattle are significant maintenance hosts for *tarassovi* in N.Z.

In a recent survey of sera from abattoir sheep from the lower North Island 7% had titres ≥1:24 to *tarassovi* (Bahaman, 1981). Again there are no reports of clinical disease in sheep attributable to this serovar and no evidence to suggest that sheep are maintenance hosts.

In 1978 *tarassovi* was isolated from four hounds in kennels in the South Auckland district (Mackintosh *et al*, 1980a)and this outbreak together with an experimental infection with *tarassovi* in dogs will be discussed in Chapter Six.

Despite extensive surveys (Blackmore *et al*,1976; Brockie,1977; Brockie and Till, 1977; Hathaway, 1978) there is no evidence of any reservoir of *tarassovi* infection in wildlife in N.Z.

Serovar *balcanica*

Prior to 1975 limited numbers of possum sera were examined serologically for evidence of leptospiral infections with negative results. Salisbury (1954) used only *pomona* antigen and Jamieson *et al* (1970) gave no details of antigens used. Evidence of Hebdomadis serogroup infections in possums was provided by de Lisle *et al* (1975) and Brockie (1975) who found positive titres to a *hardjo* antigen in two separate surveys. In both investigations leptospires were isolated from possum kidneys and they were provisionally typed as *"hardjo"*, although none of these isolates were subjected to cross-agglutination absorption tests. Based on these findings these authors suggested that the possum could act as a reservoir of *hardjo* infection for bovines in N.Z. Also in 1975, Hathaway (1978) commenced a serological survey of possums in the lower North Island and found 55% of 600 possums with Hebdomadis serogroup titres ≥1:24. In the course of these investigations leptospires were isolated from possums and subsequently typed as serovar *balcanica* (Marshall *et al*,1976). All subsequent isolates

from N.Z. possums which have been typed have been shown to be serovar *balcanica* and epidemiological studies have shown that the possum is the maintenance host for this serovar (Hathaway, 1978; Hathaway *et al*, 1978). It appears from this work that earlier isolates were probably also *balcanica* but were mistaken for *hardjo* due to the high degree of serological cross-reactivity between these two serovars. Studies by Australian workers (Durfee and Presidente, 1977, 1979 a,b,c) have also shown a high serological and cultural prevalence of *balcanica* in possums in Australia from where the N.Z. possum originated. No other wild animals in N.Z. have been identified as hosts for *balcanica* despite a number of surveys (Daniel, 1967; Brockie, 1977; Hathaway, 1978; Carter and Cordes, 1980), and there have been no reports of the isolation of *balcanica* from or clinical disease associated with this serovar in cats and dogs in this country.

The high serological prevalence of Hebdomadis serogroup titres in cattle and sheep have been assumed to be due largely, if not entirely, to *hardjo* infection (Ris, 1975; Hellstrom, 1978) although Hellstrom (1978) and Hathaway (1978) both postulated that some of these titres may be due to sporadic bovine infection with *balcanica*. Both these authors conducted limited experimental infections in small numbers of sheep and cattle, but failed to demonstrate significant kidney infection or leptospiruria. Because these results were inconclusive, further experimental infections of cattle and sheep were conducted to investigate the infectivity of *balcanica* in sheep and cattle (Mackintosh *et al*, 1981) and these are fully described in Chapter Seven. It was not known if cattle could become infected with *balcanica* under natural pasture conditions until this serovar was isolated from cattle in 1980 (Mackintosh *et al*, 1980b). The details and implications of this isolation are discussed in Chapter Eight.

There have been no reported isolations of *balcanica* from sheep.

However, *balcanica* has been isolated from a goat from the Waikato district (Schollum and Blackmore, in press) although the significance of infections with this serovar in goats is not known.

Serovar *ballum*

Some surveys in the last five years (Brockie, 1976, 1977; Hathaway, 1978; Blackmore and Hathaway,1980; Carter and Cordes, 1980) have demonstrated a high serological and cultural prevalence of *ballum* infection in the house mouse (*Mus musculus*), the ship rat (*Rattus rattus*) and the Norway rat *(R.norvegicus)* and the widespread distribution of this serovar throughout the North Island. Epidemiological studies and experimental infections conducted by Hathaway (1978) provide good evidence that under normal conditions the house mouse and the ship rat are maintenance hosts for *ballum* and under conditions of high population density Norway rats can also maintain endemic infection in their biotope. Such conditions commonly exist in rubbish tips throughout N.Z. (Hathaway, 1978; Blackmore and Hathaway, 1980). *Ballum* has also been isolated from the hedgehog *(Erinaceus europaeus)* (Brockie and Till,1977; Hathaway, 1978; Blackmore and Hathaway,1980) and the relatively high serological and cultural prevalence of *ballum* in this species indicate that it may also be a maintenance host for this serovar (Hathaway, 1978). Hathaway (1978) also found that nine of 600 possum sera had titres $\geq$1:24 to *ballum* and he suggested that this low serological prevalence (1.5%) represented sporadic infection in an accidental host.

Serovar *ballum* has been isolated from two healthy three-month old calves in the Hauraki Plains district (Ris *et al*, 1973). However, these authors did not determine the duration of leptospiruria, and the significance of cattle as carriers of *ballum* is unknown. Recently, *ballum* infection

has been associated with a calf showing signs of photosensitisation (Anon,1976b) and with the death of a calf which had haematuria and nephritis (Anon, 1977e). In a serological survey of N.Z. cattle, Hellstrom (1978) found that 4% had *ballum* titres ⩾1:17 and in a serological survey of N.Z. pigs, Ryan (1978) found that 1% had *ballum* titres ⩾1:24. Both authors considered that some of these low titres represented cross reactions with high levels of *hardjo* and *pomona* antibodies and concluded that *ballum* was not a significant cause of leptospiral infection in cattle or pigs. There have been no reports of clinical disease in pigs referable to this serovar in N.Z. However, the presence of this serovar in the dairy farm environment is emphasised by the isolation of *ballum* from two North Island dairy farmers (Anon, 1967; Till, 1968, 1971).

There are no reports of serological or cultural diagnoses of *ballum* infection in dogs in New Zealand. In a serological survey of 225 domestic and feral cats from around the North Island, Shophet (1979) found 25 (11.1%)with titres ⩾1:12 to one or more leptospiral serovars. Eight cats (3.5%) had titres to *ballum* and this was the most common serological reaction detected. Experimental infections by this author confirmed that cats could be infected by feeding them mice infected with *ballum* and one cat was found to be leptospiruric for 122 days after infection. This result lead to the suggestion that prey-predator transmission might occur in the wild. However, the low serological prevalence seen in the survey indicates that *ballum* infection in cats is uncommon and/or the decay rate of titres after natural infection is rapid, as was seen in experimental infection. In a limited survey of 11 feral cats from the lower North Island, Hathaway (1978) found one with a titre of 1:24 to *ballum* and one with a titre of 1:96 to *pomona*, although no isolations were made from kidney cultures. In the same survey no serological or cultural evidence of leptospiral infection was detected in 20

ferrets, stoats and weasels. It was concluded that wild carnivores were not significant reservoirs of *ballum* and did not appear to be involved in a predator-prey chain of transmission.

Serovar *copenhageni*

The first isolation of leptospires belonging to the Icterohaemorrhagiae serogroup was made by Kirschner and Gray (1951) from the kidneys of Norway rats trapped in Dunedin. In the same survey, 53 Norway rat and 47 ship rat sera from Auckland, Christchurch and Dunedin were tested for leptospiral antibodies. Eight Norway rat sera from Auckland and Dunedin had Icterohaemorrhagiae serogroup titres while all the Christchurch sera and all the ship rat sera were negative. Subsequent surveys of rats in the North Island (Brockie, 1977; Hathaway, 1978; Blackmore and Hathaway, 1980; Carter and Cordes, 1980) have shown *copenhageni* to be present in the South Auckland and Waikato districts but not in Taranaki or Manawatu districts. Infections with this serovar were found predominantly in Norway rats, whereas *ballum* infection was found in all four districts, in both Norway and ship rats. Blakelock and Allen (1956) failed to find any serological evidence of leptospirosis in rats trapped in the Wellington district. Thus it appears likely that *copenhageni* is not evenly distributed through N.Z. and may not occur in the Manawatu district.

No other maintenance host has been identified for *copenhageni* in N.Z. However, this serovar has been isolated from calves in the South Auckland and Hamilton districts (Dodd and Brakenridge, 1960; Shortridge,1960; Anon,1977[f]) where it is reported to have caused a severe clinical syndrome in young calves characterised by weakness, laboured breathing and a pendulous abdomen. Gross pathological findings included hydrothorax, hydroperitoneum and perirenal oedema. Photo-

sensitivity has also been recorded as a sign of *copenhageni* infection in calves (Anon, 1974d; Lake,1975; Anon,1976b, 1977f). Asymptomatic *copenhageni* infection in calves from the Hauraki Plains County has also been reported (Ris *et al*,1973) and it has been suggested (Dodd and Brakenridge ,1960; Ris *et al*, 1973) that calves become infected sporadically through contact with infected rat urine. A survey of N.Z. cattle sera by Hellstrom (1978) showed that the overall serological prevalence of *copenhageni* titres ≥1:17 was 2% and all the positive sera originated in the North Island, the greatest number being from the Waikato district. He also suggested that some of the low titres were due to cross reactions from sera with high *pomona* titres. In a survey of pig sera from throughout N.Z. Ryan (1978) found 4% with *copenhageni* titres ≥1:24. He also considered that many of these represented cross reactions with high *pomona* titre sera, and concluded that *copenhageni* is not a significant cause of leptospirosis in N.Z. pigs.

Unlike the situation in the U.K. (Michna and Ellis, 1973) and Australia (Watson *et al*, 1976b) where surveys have shown that approximately 5% of dogs have Icterohaemorrhagiae serogroup titres, the prevalence of infection with serovars from this serogroup appears to be very low in N.Z.. In the Wellington area, Salisbury (1954) and Hartley (1956) examined 63 and 50 dog sera respectively and found no titres to *icterohaemorrhagiae* or *canicola*. Sixty-seven police dogs from throughout N.Z. in the mid 1960s were all serologically negative (Anon,1968b) although the antigens used were not stated. Recently, however, Icterohaemorrhagiae serogroup organisms have been responsible for the deaths of dogs in the Whangarei Animal Health District (Anon,1978a)and in the South Auckland area (Mackintosh *et al*,1980a)and in both cases it was considered likely that these outbreaks originated from rat contact.

Serovar *australis*

Despite the isolation of *australis* from a man in Northland (Thompson, 1980) there have been no reports of serological or cultural diagnoses of leptospirosis due to this serovar in animals in N.Z., although diagnostic laboratories do not use *australis* routinely as an antigen. In a survey of 234 pig sera from around N.Z., Ryan (1978) found 5% with titres ≥1:24 to *australis*, but he concluded that these were cross reactions with high *pomona* titres. Hathaway (1978) found no evidence of *australis* infection in a survey of wildlife from the lower North Island which included mice, rats, hedgehogs, mustelids, feral cats and possums. Hellstrom (1978) in his serological survey of 480 cattle sera from around N.Z. did not use *australis* as an antigen. As the sera were still available they were recently retested against serovar *australis* (Mackintosh, unpublished). One sample had a titre of 1:48 to *australis*, but this was probably a cross reaction, as the sample also had titres of 1:600 to *pomona* and 1:96 to *hardjo*.

In Australia, where serovar *australis* was first isolated (Lumley, 1937) the native rat, *Rattus conatus*, is considered to be the principal carrier (Johnson, 1950). This serovar is one of two responsible for "cane cutter's disease" in men working in the canefields in Queensland.

LEPTOSPIROSIS IN HUMANS

In the last 30 years, five serovars, "*icterohaemorrhagiae*", *pomona*, *hardjo*, *ballum* and *australis* have been isolated from humans, and diagnoses of leptospirosis due to organisms in the Icterohaemorrhagiae, Canicola, Tarassovi and Hebdomadis serogroups have been made based on serological grounds (see Table 1.3). The isolation of *balcanica* from man has not been reported in N.Z. but it has been suggested that some Hebdomadis serogroup infections in man may in fact be due to this serovar (Hathaway, 1978; Blackmore, 1979).

The first serologically confirmed case of human leptospirosis in N.Z. occurred in a dairy farmer in 1949 (Kirschner and Gray, 1951). It was described as a typical case of "Weils disease" and the patient had a high Icterohaemorrhagiae serogroup titre. A visit to the patient's farm in the Auckland area showed a heavy infestation of "large brown rats", presumably *Rattus norvegicus*. In 1950, acting on the reported isolation of *L.pomona* from dairy farmers in Australia (Clayton *et al*, 1937), Kirschner informed hospital superintendents of the diagnostic facilities offered by the Leptospirosis Reference Laboratory in Dunedin, and drew their attention to the existence of atypical, mild forms of leptospirosis without jaundice. This resulted in the first isolations of *pomona* from 12 patients, all of whom were associated with dairy farms (Kirschner *et al*, 1952). Six of these patients were associated with one outbreak of "redwater" in calves on a farm in Westland (Bruere,1952). One of the co-authors also reported having seen 20 similar but unconfirmed cases in the Whangarei district prior to the availability of serological testing facilities in Dunedin, and again all these cases had occurred in dairy farm workers. In 1952, leptospirosis was made a notifiable disease and in 12 months, November 1951 to November 1952, sera from 315 patients were tested.

TABLE 1.3 : FIRST REPORTS OF SEROLOGICALLY OR CULTURALLY CONFIRMED CASES OF HUMAN LEPTOSPIROSIS IN NEW ZEALAND.

Serogroup	Serovar	Reference
Icterohaemorrhagiae	S	Kirschner & Gray,1951
Icterohaemorrhagiae	"*icterohaemorrhagiae*"*	Kirschner (unpublished) cited Anon, 1966.
Pomona	*pomona*	Kirschner *et al*, 1952
Canicola	S	West & Whitehead, 1953
Tarassovi	S	Kirschner, 1954
Hebdomadis	S	Josland *et al*, 1957
Hebdomadis	*hardjo*	Christmas *et al*, 1974b
Ballum	*ballum*	Anon, 1967
Australis	*australis*	Thompson, 1980

S = serology only

* = not definitively typed

Sixty-eight were diagnosed as having leptospirosis, and the majority of these cases were dairy farmers or meat workers (Faine and Kirschner,1953). Of these 68 diagnosed cases, 59 had titres to *pomona*, 9 to *"icterohaemorrhagiae"* and one, in the Wellington district, to *canicola*. This latter patient is the only human case of "canicola fever" to have been reported in N.Z. (West and Whitehead,1953). The clinical symptoms were typical of leptospirosis. A serum sample gave an agglutination titre of 1:9600 to *canicola* but it was negative to *pomona* and *icterohaemorrhagiae* antigens. The source of infection was never identified and the patient denied having direct dog contact. *Canicola* has never been isolated from N.Z. from man or animal. The following year, Kirschner (1954) noted that the sera from a number of patients with clinical symptoms of leptospirosis and working in occupations at risk, did not react with *icterohaemorrhagiae*, *canicola* or *pomona* antigens in the agglutination test. This lead to the additional use of *mitis (tarassovi)* antigen. Johnson (1942) had isolated this organism in Queensland from patients with symptoms similar to those seen in *pomona* infections. Kirschner (1954) subsequently reported three patients who were serologically positive to *mitis (tarassovi)* with titres up to 1:1200 but they were seronegative (<1:30) to the other three serovars. If a Hebdomadis serogroup antigen had been used at this time it is possible that some of the clinical leptospirosis cases negative to the other four antigens may have had Hebdomadis titres. In September, 1954, the National Health Institute, Wellington, started a leptospirosis diagnostic service and in the following two years 774 human sera from patients suffering from "pyrexia of unknown origin" or clinically diagnosed leptospirosis cases were tested (Josland *et al*,1957). Serological titres of ≥1:300 were found to *pomona* (39 patients), *icterohaemorrhagiae* (8 patients) and *hyos (tarassovi)* (6 patients). However, titres were also found to *medanensis* (8 patients), *sentoti* (6 patients), *australis B* (1 patient) and *autumnalis* (4 patients), "suggesting the possibility of the presence of an additional four types

in N.Z." (Josland *et al*, 1957). This was the first serological evidence in this country of the presence of Hebdomadis serogroup infections represented by *medanensis* titres. Subsequently, there has been no further evidence of the existence of infection to the last three serovars and those titres may represent cross reactions with other serovars.

In 1967 *ballum* was isolated from two dairy farmers in the Waikato area (Anon,1967), during a survey of clinical cases in the Whangarei, Hamilton, New Plymouth and Rotorua Health Districts. Altogether, leptospires were isolated from 19 patients but due to contamination three cultures were lost and difficulty was experienced in serotyping the others(Till, 1968). Subsequently (Till, 1971) these 16 isolates were identified as ten *pomona*, two *ballum*, and four Hebdomadis serogroup organisms. Convalescent sera from these 19 patients showed that seven cases had titres $\geq$1:300; four to *medanensis*, two to *pomona* and one to *autumnalis* antigens. Five of the other sera had titres <1:300 and four were serologically "negative" at the time of testing (Till, 1968). This demonstrates some of the inaccuracies of diagnosis on serological grounds using an agglutination titre of $\geq$1:300 as a criterion of infection. In retrospect it seems likely that the four Hebdomadis isolates were *hardjo*. The first typed isolation of *hardjo* was made during another survey of clinical cases in the Hauraki Plains in 1971 (Christmas *et al*, 1974b). The only leptospiral isolate from humans for which a maintenance host has not yet been identified is serovar *australis*. This organism was isolated in January 1977 (Thompson,1980) from a dry-stock farmer in Northland suffering from clinical leptospirosis.

EPIDEMIOLOGY OF HUMAN LEPTOSPIROSIS

Leptospirosis throughout the world shows a natural nidality (Galuzo,1975; Blackmore and Hathaway, 1980) where each serovar has a

specific range of maintenance host populations which act as continuous reservoirs of infection in their specific ecosystems or biotopes. Man is an accidental host and usually becomes infected with a particular serovar by entering the biotope of the maintenance population specific for that serovar (Blackmore and Hathaway, 1980) although it is also possible for man to become infected from a temporary or short term carrier (Turner, 1967). Infection may be by direct contact with the urine or kidneys of infected animals, or by indirect contact via an environment contaminated with infected urine (Turner, 1967; Willcox, 1976). Entry into the host is through skin abrasions or cuts or via mucous membranes (Turner, 1967). Examples of human leptospirosis acquired by indirect contact are well documented in overseas and N.Z. literature; "rice planters disease" in Italians exposed to mud and water contaminated by *grippotyphosa* carried by field mice (Babudieri, 1958); "cane cutters disease" in Australia caused by *australis* carried by rodents (Johnson, 1950); recreational leptospirosis in the U.S.A. from swimming in infected waterways especially in rural environments where contamination by livestock as well as rodents occurs (Nelson *et al*, 1973; Anon, 1978b); Weils disease in sewer workers from occupational exposure to waterways contaminated by rat urine (Alston and Broom , 1958; Heath *et al*, 1965).

Examples of human leptospirosis acquired by direct contact include : *canicola* infection in children who played with infected dogs in a wet environment (Barkin and Glosser, 1973); *pomona*, *tarassovi* and *hardjo* infections in abattoir workers, especially those involved in killing and dressing animals (Forbes and Wannan, 1964; Blackmore *et al*, 1979), "swineherds disease" in farmers keeping pigs infected with *pomona* (Gsell, 1952), *hardjo* and *pomona* infections in dairy farmers in Israel (Shenberg *et al*, 1978), U.K. (Sakula and Moore, 1969; Coghlan, 1979), U.S.A. (Andrew and Marrocco, 1977), Australia (Johnson, 1950) and N.Z. (Christmas *et al*, 1974a).

It appears that, generally, man is infected indirectly from wildlife and directly from livestock and pets, although the converse may still occur.

In N.Z. it has been shown (see page 3) that the serovars *hardjo, pomona* and *tarassovi* are maintained in domestic livestock populations, while *ballum, copenhageni* and *balcanica* are maintained in wildlife populations. In the 1970s, *hardjo* and *pomona* were diagnosed in over 99% of human cases reported to the Health Department (Brockie, 1976). The majority of people affected were dairy farmers and their families (Robinson, 1975). Approximately 90% of notified cases were reported as occupationally associated (Department of Health, Annual Reports, 1970-1979). Other occupational groups in which leptospirosis has been reported frequently over the last 30 years are pig farmers, stock truck drivers, abattoir workers, butchers and veterinarians (Kirschner and Maguire, 1957; Robinson, 1975; Penniket, 1977)(see Tables 1.4 & 1.5). All these occupations have close contact with livestock, and infection is likely to have been acquired by direct rather than indirect contact.

There have been a number of epidemiological investigations of human leptospirosis carried out in N.Z. over the last 30 years (Table 1.4 & 1.5) and the majority of these have been clinical case-studies. From these the following associations with leptospirosis have been reported : occupation, first noted by Kirschner *et al* (1952); the distribution of livestock (Faine and Kirschner, 1953; Brockie, 1976); season, with a peak in spring and a low in winter (Kirschner, 1954; Brockie, 1976); unusually heavy spring rainfall (Kirschner, 1954; Philip and Tennent, 1966; Brockie, 1976); male workers (Faine and Kirschner, 1953; Kirschner, 1954; Brockie, 1976; Penniket, 1977); age, with people between 15 and 45 years most affected (Kirschner *et al*, 1952; Faine and Kirschner, 1953; Christmas *et al*, 1974a; Robinson, 1975; Brockie, 1976); milking in herringbone sheds and

increased herd size (Anon,1969); and the time spent in the milking shed (Brockie, 1976).

Recently, there have been a number of serological surveys of different human populations in N.Z. (Table 1.5). These included healthy blood donors, divided into urban and rural dwellers and meat company employers (Thompson,1979), veterinarians (Robinson and Metcalfe,1976; Blackmore, pers.comm.), meat inspectors (Blackmore *et al*,1979) and meat workers (Blackmore and Schollum,1980). The first of these surveys confirmed that urban dwellers are at negligible risk while 2 to 4% of rural dwellers, had leptospiral titres $\geq$1:100. In the second survey (Robinson and Metcalfe, 1976) only one veterinarian out of 80 had a leptospiral titre $\geq$1:100 and this was to *hardjo* while another survey of meat works veterinarians (Blackmore, pers.comm.) showed a 6.6% prevalence of titres $\geq$1:24 (3 titres to *pomona* and one to *copenhageni*). Meat inspectors (Blackmore *et al*,1979) had an overall serological prevalence of 10.2% with *pomona* and *tarassovi* responsible for 85% of these titres, while 6.3% of meat workers had leptospiral titres $\geq$1:24 with *pomona* the most common serovar (Blackmore and Schollum,1980). In these last two studies, associations were found between the presence of *pomona* and *tarassovi* titres in abattoir workers and their contact with pigs, and between the presence of leptospiral titres in abattoir workers and their specific job of killing and dressing carcases.

Prior to the publication of Mackintosh *et al*(1980d) there were no reports of cross-sectional serological surveys of dairy farmers in N.Z. to determine the prevalence of leptospirosis in this occupational group. The results of this survey will be fully discussed in Chapter Five.

TABLE 1.4 : PUBLISHED REPORTS OF OCCUPATIONS AND SEROVARS ASSOCIATED WITH HUMAN CLINICAL INFECTIONS IN NEW ZEALAND

Year	No. of confirmed cases	Occupation (%)	Serovars implicated (%)	Reference
1951	12	All dairy farmers or residents	12 *pomona** (100)	Kirschner *et al*,1952
Nov.51 - Nov.52	68	Farmers,meatworkers, and farm residents	58 *pomona** (85.3) 9 *icterohaemorrhagiae* (13.2) 1 *canicola* (1.5)	Faine & Kirschner,1953
1952-53	130	87 dairy farm workers (67) 20 wives of farmers (15.4) 12 pig breeders (9.2) 9 butchers, meat inspectors,vets (6.9) 2 children of farmers (1.5)	Mostly *pomona**	Kirschner, 1954
1951-57	502	Dairy farmers & families (83) Pig breeders (9.2) Vets,slaughtermen, livestock carriers (6.0) Unknown (1.8)	Mostly *pomona**	Kirschner & Maguire,1957
Sept.54 - May 55	5	Not reported	2 *pomona** (40) 2 *hyos* (40) 1 *icterohaemorrhagiae* AB (20)	Josland *et al*,1957
June 55 - Mar. 56	23	Not reported	9 *pomona** (40) 4 *hyos* (17) 3 *icterohaemorrhagiae* AB (13) 5 *sentoti* (22) 1 *australis* B (4) 1 *autumnalis* AB (4)	
April 56 - Oct. 56	44	Not reported	28 *pomona** (64) 2 *hyos* (5) 2 *icterohaemorrhagiae* AB (5) 1 *sentoti* (2) 3 *autumnalis* AB (7) 8 *medanensis* (18)	
1957-65	68	Majority dairy farmers and families,2 infected slaughtering animals, 1 infected recovering dead stock from a drain	22 *medanensis** (35) 14 *pomona* (22) 14 *andaman* A (22) 6 *ballum* (10) 4 *ictero* AB (6) 2 *hyos* (3) 1 *sentoti* (2) (5 serologically indistinguishable)	Philip and Tennent,1966
1968	212	191 dairy farmers (90) 21 not reported (10)	Not reported	Jamieson *et al*,1970
Sept.71 - Jan. 72	35	Dairy farmers or farm residents	Isolates: 22 *hardjo* (63) 13 *pomona* (37)	Christmas *et al*, 1974(a)
1973/74	22	Dairy farm workers	Isolates: 15 *hardjo* (68) 6 *pomona* (27) 1 *ballum* (5)	Robinson, 1975
Jan. 71 - June 71	80	64 dairy farmers (80) 7 freezing workers (9) 1 stock dealer (1) 1 meat inspector (1) 2 school boys (3) 1 clerical worker on a dairy farm vacation (1) 4 dairy farmers wives (5)	Not reported	Christmas, 1976
1970/71	280	Majority dairy farmers	*hardjo* (59.6) *pomona* (40.1)**	Brockie, 1976
1971/72	592		*hardjo* (64.5) *pomona* (35.5)	
1972/73	319		*hardjo* (56.6) *pomona* (43.4)	
1073/74	335		*hardjo* (67.0) *pomona* (33.0)	
1974/75	113(incomplete)		*hardjo* (65.1) *pomona* (34.9) (small numbers of *ballum*, *copenhageni*, and *tarassovi*<1%)	

* MAT ≥1:300 Diagnostic titre
** MAT ≥1:200 Diagnostic titre

TABLE 1.5 : SEROLOGICAL SURVEYS OF OCCUPATIONAL GROUPS

Type of Survey	Year	No. of confirmed cases	Occupation		Serovars implicated	(%)	Reference
Survey of N.Z.Veterinarians at a conference	1974	86	Veterinarians (10% of N.Z. veterinarian population)	*	1 *hardjo*	(1)	Robinson & Metcalfe,1976
Blood Donor Survey Wellington	1974	196		*	0		Thompson, 1979
Blood Donor Survey Christchurch	1975	340	Urban dwellers		0		
Blood Donor Survey Wellington	1976	100					
Blood Donor Survey Taranaki	1977	577			24	(4)	
Blood Donor Survey Hamilton	1978	379	Rural dwellers		8	(2)	
Blood Donor Survey Wellington	1976	136			2	(1)	
Blood Donor Survey Dunedin	1976	51	Meat company employees		1	(2)	
Blood Donor Survey Invercargill	1976	47			0	(0)	
Blood Donor Survey Napier	1977	106			0	(0)	
Blood Donor Survey N.Z.	1978	886			38	(4)	
Blood Donor Survey N.Z.	1979	65			0	(0)	
Survey of Meat Inspectors New Zealand	1978	1003	Meat inspectors from 44 meat works. 76% of total N.Z. meat inspectors	+	78 *pomona*	(7.8)	Blackmore *et al*, 1979
					19 *tarassovi*	(1.9)	
					12 *hardjo*	(1.2)	
					4 *copenhageni*	(0.4)	
					1 *ballum*	(0.1)	
					103 Total (11 with dual titres)		
					Overall serological prevalence 10.2%		
Survey of Meat Workers Six abattoirs	1978/79	1250	Meat workers (= 4% N.Z.meat workers)	+	42 *pomona*	(3.4)	Blackmore & Schollum, 1980
					17 *hardjo*	(1.4)	
					2 *tarassovi*	(0.2)	
					21 *copenhageni*	(1.7)	
					7 *ballum*	(0.6)	
					79 Total (10 with dual titres		
					Overall serological prevalence 6.3%		
Survey of meat works veterinarians	1978/79	61	Meat Division Veterinarians	+	3 *pomona*		Blackmore (pers.comm.) 1980
					1 *copenhageni*		
					4 Total		
					Overall serological prevalence 6.6%		

* MAT ≥1:100 survey titre - recent infections
+ MAT ≥1:24 survey titre

CONTROL

The control of leptospirosis in man is dependant on a knowledge of the epizootiology of the serovars involved (Stoenner,1976). Therefore once the causative serovar has been identified the following factors must be considered; the ecology of the carrier host, the mode of transmission and the population at risk (Alston and Broom,1958). Although the epidemiology of each serovar should be considered separately, for control purposes serovars generally fall into two categories : those maintained by rodents and largely transmitted indirectly via the environment, and those maintained by domestic animals and usually transmitted by direct contact with infected urine. The majority of human cases of leptospirosis reported worldwide are caused by serovars in the first category (Alston and Broom, 1958). Therefore most control programmes have concentrated on rodent control, personal hygiene, avoidance of urine-contaminated water or objects, wearing of protective clothing, the disinfection of surfaces and infected areas,and the vaccination of people in occupations at risk (Babudieri, 1957; Alston and Broom, 1958; Turner, 1967, 1969; Stoenner, 1976). Serovars *ballum* and *copenhageni* fall into this first category and the number of cases of leptospirosis in man in N.Z. due to these serovars is very low, accounting for less than 1% of cases reported annually in the 1970s (Brockie, 1976). Therefore control measures directed at these serovars are of minor importance in this country. In the last 30 years in Europe, Australia and the U.S.A. there has been an increasing incidence of "anicteric" or "benign" leptospirosis acquired by close contact with domestic animals, and the control of human leptospirosis caused by these serovars has been primarily aimed at controlling the infection in livestock by vaccination, isolation, treatment or destruction of infected animals and herd management (Alston and Broom, 1958; Michna, 1970; Stoenner,1976). Frequently, these measures have been initiated more for economic reasons,

to reduce the animal wastage, than for public health reasons (Michna, 1970) and this has tended to be the case in N.Z.(Jamieson *et al*, 1970). The first reported case of leptospirosis in N.Z. was due to *icterohaemorrhagiae* and was associated with rodent contact (Kirschner and Gray, 1951). However, it soon became apparent that *pomona* was the most important serovar recognised at that time and its association with pigs was well known (Kirschner, et al, 1952). These authors recommended that pigs, which are carriers of *pomona*, should be kept completely separate from cattle, and suggested that the use of antibiotics for the elimination of the carrier state be investigated. The following year Ensor and McClure (1953) stated that the eradication of *pomona* from cattle by test and slaughter or test and treatment was impractical and too expensive, and they thought the best hope for control lay in the development of an effective vaccine to increase herd immunity and prevent infection. Encouraged by the development of vaccines overseas, Webster and Reynolds (1955) and McDonald and Rudge (1957) developed *pomona* vaccines that were shown to be effective in sheep and cattle. The former authors emphasised the importance of a vaccine preventing the development of leptospiruria, and the latter authors showed that vaccination of pregnant cows late in gestation could provide colostral immunity for newborn calves which lasted for six to eight weeks. A commercial *pomona* vaccine* came on the market in the mid 1950s for the prevention of abortion in cows and pigs and the prevention of "redwater" in calves. However, in spite of the use of the vaccine in domestic animals the annual reported incidence of human cases continued to increase. In the early 1960s, alarmed by the rise in the number of cases in their district, Philip and Tennent (1966) tested a human leptospiral vaccine, which included *pomona*, *icterohaemorrhagiae* and *tarassovi*, in people at risk in their area, notably dairy farmers. The results appeared disappointing but in retrospect it seems likely that some of the apparent "failures" of the vaccine were due

* Leptovax, manufactured by Tasman Vaccine Laboratories, New Zealand.

to infections with *hardjo*, a serovar not included in the vaccine. However, this was not appreciated at the time (Philip, 1976).

As stated by Jamieson *et al* (1970), *pomona* in cattle can theoretically be controlled by a continuous vaccination policy. However, this does not happen in practice because farmers stop vaccinating in the absence of disease and only start when clinical disease in their stock reappears. These authors suggest that the value of vaccination should not only be judged in terms of animal health, but also in human health terms. Jamieson *et al* (1970) also noted that a serovar in the Hebdomadis serogroup appeared to be a significant cause of leptospirosis in dairy farmers and was probably maintained by cattle. Although it did not appear to be a problem in cattle they suggested that the identification and inclusion of this serovar in a cattle vaccine should help to control the disease in man. In 1971 (Christmas *et al*,1974a) this organism was isolated and identified as *hardjo* and these workers suggested that a human vaccine containing all the local strains, including *hardjo*, should be developed. But they also admitted that "measures directed to the protection of the individual ..... are unlikely to succeed until the source of the infection can be controlled in the animal reservoir". Hellstrom (1978), Ryan (1978) and others (Blackmore *et al*,1976; Brockie, 1976) have studied the epidemiology of *hardjo* in cattle and *pomona* in pigs, and have provided much of the knowledge necessary for the development and implementation of control programmes for these serovars. A dual *hardjo/pomona* vaccine* has been developed and trials in N.Z. (Marshall *et al*, 1979 a, b); Mackintosh *et al*, 1980c) have shown that it is effective in cattle and sheep and it will be discussed fully in Chapter Ten.

In addition to vaccination, other measures for the control of

*Leptavoid, Wellcome,Cooper New Zealand Ltd.

leptospirosis on dairy farms have been recommended and these also require a knowledge of the epidemiology of the serovar. They include : the isolation of cattle from pigs (Kirschner and Gray, 1951); the isolation and treatment of infected stock and the disinfection of premises and working surfaces (Alston and Broom, 1958; Michna, 1970); the isolation of calves and recently introduced animals from the rest of the herd until vaccinated or until infection has died out after an outbreak (Alston and Broom, 1958); the maintenance of clean and uncontaminated water supplies (Alston and Broom, 1958; Stoenner, 1976) and the isolation of contaminated mud or water (Philip, 1976; Stoenner, 1976). Buddle and Hodges (1977) demonstrated that *pomona* could be controlled in a piggery by improving effluent disposal and attention to building design and management in order to prevent exposure of susceptible pigs to infected pigs and their effluent. The avoidance of urine and the wearing of protective clothing, including gloves, aprons and waterproof boots, are however the most obvious steps farmers can take to reduce the risk of becoming infected (Alston and Broom, 1958; Robinson,1975; Brockie, 1976; Philip, 1976; Penniket,1976). These factors are stressed in the N.Z. Department of Health pamphlet No. 179 which also emphasises the importance of covering cuts and scratches with waterproof dressings. Kirschner and Maguire (1957) advocated the use of seawater or brine to disinfect farm soil and muddy areas and this was echoed by Tennent and Philip (1964). The Department of Health carried out a study of milking shed design in 1967 and concluded that working in a herringbone shed was associated with an increased risk of contracting leptospirosis (Anon,1969). They also found that there were optimum widths and depths for the pit in these sheds associated with a reduced risk of exposure to urine splash. The optimum dimensions reported were 25 to 30 inches in width and 49 to 54 inches in depth.

The importance of knowledge of the epidemiology of a particular serovar is emphasised by the pessimistic predictions of some earlier authors (Philip and Tennent, 1966; Brockie, 1976) who assumed that there were reservoirs of *pomona* and *hardjo* in wildlife which would make it impossible to eradicate these serovars from domestic herds. Subsequently it has been shown (see pages 3-14) that *pomona* and *hardjo* are maintained only by domestic animals in N.Z. and therefore are potentially controllable.

CHAPTER TWO

THE SELECTION AND DEVELOPMENT OF SUITABLE LABORATORY TECHNIQUES

INTRODUCTION

As there is little emphasis on the development of laboratory techniques in this work the literature review in the preceeding chapter did not include this subject. Therefore this chapter will discuss some of the general laboratory techniques and methods available for the study of leptospirosis. Investigations of the precision of equipment used in the serological tests and the most suitable anticoagulants for the collection of blood for culture are recorded. The development of a technique for the rapid detection of leptospiraemia will also be described.

DISCUSSION OF GENERAL TECHNIQUES

The confirmation of past and present leptospiral infections has to be based on the detection of specific antibodies using serological tests, the isolation of leptospires by culture or animal inoculation or the demonstration of the organism in tissues and body fluids (Shotts,1976).

Serology : The most commonly used serological test for detecting specific antileptospiral antibodies is the microscopic agglutination test (MAT) performed with either live or formalised leptospiral cultures (Alston and Broom, 1958; Galton *et al*, 1965; Turner, 1968; Shotts, 1976). It is regarded as the most sensitive and accurate test in routine use (Torten, 1979). Turner (1968) recommended that a "battery of antigens" including at least one "serotype" from each serogroup should be used for such tests. However, most laboratories use the range of serovars expected in their geographic locations (Torten, 1979). It is important to note that an MAT titre is only "potentially serogroup indicative" (Turner,1968) and not specific for the serovar used as the antigen. This is particularly relevant

where two or more serovars belonging to the same serogroup are present in the area being studied. In N.Z. serovars *hardjo* and *balcanica*, are both members of the Hebdomadis serogroup and a high degree of cross reaction occurs between them (Hathaway and Marshall, 1980).

It is also important to decide on the lowest titre which can be considered specific. Turner (1968) regarded an MAT reaction of $\geqslant$ 75% agglutination at 1:10 as indicative of past infection. He contended that "the onus is on those who regard such reactions as ' non-specific' to demonstrate the alternative cause". Hathaway (1978) and Hellstrom (1978) both used 1:24 as the lowest dilution in their MAT tests in recent surveys of wildlife and cattle and for monitoring experimental and natural infections. They regarded this level of significance as sensitive and specific, with only a low prevalence of cross-reactions. Ryan (1978), on the other hand, used 1:12 as the lowest dilution in a serological survey of pig sera and found a high degree of cross-reactivity which he regarded as non significant and was largely eliminated by using a minimum titre of 1:24.

In surveys of meat workers and meat inspectors, a minimum titre of $\geqslant$1:24 has also been used by Blackmore *et al*(1979) and Blackmore and Schollum(1980) and these workers found a significant correlation between people with leptospiral titres $\geqslant$1:24 and a history of clinical leptospirosis. For diagnostic purposes the National Health Institute, Wellington, regard a titre of $\geqslant$1:200 as indicative of recent infection (Thompson, 1979). However, this is not suitable for survey work where evidence of past infection is being sought. Hellstom (1978) and Blackmore *et al*(1979) have provided evidence that bovine and human titres $\geqslant$ 1:24 respectively persist for ten years or more. Therefore, for the purposes of this study, which includes surveys of present and past infections in humans, cattle and dogs, 1:24 was used as the lowest dilution in the MAT which could be considered specific.

Other serological tests : A semi-automated complement fixation test (CFT) using a polyvalent antigen containing serovars *hardjo, pomona, copenhageni,* and *ballum*, has been developed for use in N.Z. as a screening test (Hodges, 1974; Hodges and Weddell,1977; Hodges *et al*, 1979a) . These workers have shown that this is a useful diagnostic test for recent infection because it detects IgM antibodies which predominate over IgG antibodies for the first few weeks after infection (Hellstrom, 1978) and correlates well with the MAT up to 90 days after infection. However, for the purposes of this study a test was needed which would identify the serovar involved and detect both IgM and IgG antibodies. The CFT was considered to be less useful than the MAT and it was therefore not used.

Other tests for the serological diagnosis of leptospirosis reviewed by Turner (1968) include the haemagglutination test, direct and indirect fluorescent antibody tests and the sensitised erythrocyte lysis test. None of these tests offered greater sensitivity or specificity than the MAT for use in this study, which required the detection of both recent and past infections, as well as indicating the serogroup of the infecting organism.

Culture : Valuable epidemiological information on leptospiral infections of animals can be gained by the isolation and precise identification of the infecting serovar, and the detection of leptospiraemia, kidney infection and leptospiruria. Thus an efficient system for the culture of leptospires is essential. Turner (1970) stated that there was no standard medium which would provide optimum requirements for all "strains" due to their different nutrient requirements. Therefore, either specific media must be developed for the optimum growth of an individual serovars or a general medium suitable for the range of leptospires likely to be encountered must be used. During the 1960s, there were considerable advances in the knowledge of the nutrient requirements of leptospires (Turner, 1970) and this resulted in the

formulation by Ellinghausen and McCullough(1965) of a serum-free medium containing two important growth promoting substances; bovine serum albumin fraction V and Tween or Polysorbate 80. This medium was improved by Johnson and Harris (1967) with the formulation of Ellinghausen, McCullough, Johnson and Harris (EMJH) medium. A commercial preparation of EMJH* was used by Hathaway (1978), Hellstrom (1978) and Ryan (1978) in this laboratory and initially it was considered to be the most suitable medium for the growth of the six serovars known to be endemic in N.Z. However, serovar *hardjo*, which is a fastidious organism, grew slowly in this medium. Towards the end of 1977 this laboratory tested a modification of EMJH which has the addition of sodium pyruvate and was described by Johnson and Seiter(1977). This new Johnson/Seiter (JS)medium was evaluated and was shown to be more suitable for the isolation of *hardjo* than the Difco EMJH, and was used for all subsequent work (Hellstrom, 1978). For these reasons the JS medium was considered most suitable for use during the present study.

A semi-solid medium, made by the addition of 0.2% agar to liquid medium, was used for primary isolation of leptospires from blood, urine and kidney homogenates by Hathaway (1978), Hellstrom (1978) and Ryan (1978). The agar appears to promote more rapid, dense and sustained growth than liquid forms and it has been suggested (Turner, 1970) that it may act by absorbing leptospiral metabolites or inhibitory substances in the inoculum or by contributing some nutrient factor.

Factors affecting the isolation and culture of leptospires were discussed by Turner (1970) who emphasised the inhibitory effects of imperfectly cleaned glassware, improperly prepared media, the presence of inhibitory substances in the inoculum and contamination by other microorganisms. Avoidance of the first two inhibitory effects requires constant care and attention to detail in the laboratory. The effect of

*EMJH, Difco Laboratories, Detroit, Michigan, USA.

inhibitory substances in the inoculum can be reduced by using small volumes of inocula or by diluting the inoculum with sterile saline. Contamination with other microorganisms is probably the most difficult problem to overcome when culturing leptospires, especially from urine samples collected in the field. Johnson and Rogers (1964) described the use of a pyrimidine analogue, 5-fluorouracil (5FU), in leptospiral media as a selective inhibitor of contaminants and recommended concentrations of 200-400µg/ml, In a number of trials in this laboratory, Hathaway (1978), Hellstrom (1978) and Ryan (1978) compared the culture of contaminated samples in non-selective media with selective media containing either 5FU or neomycin. The best overall results were obtained with a medium containing 200µg 5FU/ml. The least contamination was obtained in media containing 400µg 5FU/ml, although some inhibition of leptospiral growth was experienced. During their investigations these workers routinely used both non-selective medium and selective media with two levels of 5FU (200 and 400µg/ml) and these three types of media have been used throughout the present study.

Direct microscopic detection of leptospires : In addition to culturing leptospires from tissues and body fluids, it is also possible to detect them by direct microscopy. The two most common techniques are dark field microscopy of blood, urine, cerebrospinal fluid and tissue homogenates, and transmission microscopy of stained leptospires either in fixed smears or paraffin embedded tissues (Turner, 1970). Other techniques include fluorescent antibody tests, tissue imprints and direct staining, but none of these three techniques was used in this study.

Dark field microscopy (DFM): This is the simplest and quickest method for the detection of leptospires in body fluids. A simple trial, using serial dilutions of leptospires from 5×10^7 to 5×10^1 organisms/ml showed that at a concentration of 5×10^3 approximately one leptospire

per field could be seen. This level of sensitivity is similar to that reported by Turner (1970). Previous workers (Hathaway, 1978; Hellstrom, 1978; Ris and Hamel, 1978) have confirmed that DFM is not as sensitive for detecting leptospires as the urine culture method reported here which can detect as few as ten leptospires/ml. However, in very heavily contaminated samples DFM may detect leptospiruria when cultural methods may not. For these reasons DFM is a useful adjunct to culture.

Transmission microscopy : This is used predominantly for the examination of histological sections. Spirochaetes can be demonstrated in fixed tissue sections by silver impregnation techniques such as the modified Warthin and Starry method (Young,1969) and the Levaditi method (Culling, 1974).

INVESTIGATIONS AND DEVELOPMENT OF TECHNIQUES

PART A : Precision of the MAT : Ryan (1978) tested the precision of the MAT by testing 72 pig sera twice and found there was no significant difference between the results of the two tests. He found that 93% of results were within ± one doubling dilution. Other authors have also shown that 90 - 100% of MAT titres were repeatable within ± one doubling dilution (Cole *et al*,1973; Brown, 1978; Hellstrom, 1978). Alterations in antigen concentration and errors in reading, serum identification and serum dilution accounted for some of the observed variations (Hellstrom, 1978). The first three of these factors relied on careful laboratory methods. However, the accurate dilution of serum relied on the precision and accuracy of the saline dispensing equipment, which were not known.

Investigation 1. : Tests were carried out to determine the precision and accuracy of the automatic saline dispenser*. Each of the eight droppers should

* Minipipetter - Cooke Engineering Co., Alexandria, Virginia, USA.

dispense 25µl. A total of 50 individual drops were weighed from each dropper and the weights ranged from 23.2 to 26µg with an average of 24.7 ± 0.2µg, i.e. an error of approximately 1%.

Investigation 2. : An autopipette * , which was used to make the original 1:6 dilution of serum in the SRP, was tested by weighing 20 individual drops when it was set on 25µl. The drops ranged from 23.8 to 24.5µg and averaged 24.5 ± 0.14 µg in weight, i.e. an error of approximately 2%. A combined error of ± 3% in the level of the final dilution is insignificant when the accepted accuracy of the MAT is ± one doubling dilution, i.e. an error of ± 100% (Cole *et al*,1973; Brown, 1978; Ryan, 1978). The conclusion drawn from this result is that the saline dispensing equipment does not appreciably affect the precision of the MAT.

Part B : An experiment to investigate the effect of two anticoagulants, heparin and EDTA, on the survival of *pomona* in blood samples collected for blood culture.

Introduction : Turner (1970) advocated the culturing of human blood directly into media "at the bedside", before it clotted. Under field conditions it is usually necessary to collect blood samples and transport them to the laboratory for culture. It is therefore convenient to use unclotted blood to simplify blood culturing procedures. Thus it is important to use an anticoagulant that is not deleterious to leptospires. Wolff (1954) preferred sodium oxalate to sodium citrate and cited van der Hoeden and van Thiel who found that leptospires could survive at least six days in the former, whereas the latter sometimes exerted "a detrimental effect". In a six day trial, Nervig and Ellinghausen (1978) found that heparin, potassium oxalate and sodium citrate "did not alter the viability of leptospires whereas EDTA had a detrimental effect on them after six hours.

* Pipetman - Gilson, 95400 Villiers-le-bel, France.

However, these workers used laboratory adapted cultures of *grippotyphosa* which were added to normal seronegative pig blood, and thus the trial was somewhat artificial. EDTA is commonly used as an anticoagulant when blood is collected for haemograms and if it was also suitable for blood cultures it would simplify blood sampling procedures. The opportunity arose to test, under natural conditions, the effect of EDTA and heparin on the survival of *pomona* in blood samples taken from experimentally infected calves during the leptospiraemic phase.

Materials and Methods : Using the method described in Part C of this chapter, leptospiraemia was detected in two calves experimentally infected with *pomona* (Thompson, pers comm). Two calves had approximately 10^4 and 5×10^3 leptospires per ml of blood respectively. Evacuated collecting tubes containing EDTA and heparin were used to collect blood from the jugular vein of each calf. Blood was cultured, as described in Chapter Three, at the following times after collection : ½ hour, 3 hours, 6 hours, 13 hours, 24 hours, 48 hours, 72 hours and 96 hours.

Results : The results of blood cultures are shown in Table 2.1. Heparin appeared to have no deleterious affect on the survival of *pomona* up to 96 hours after collection. EDTA had no measurable adverse affect on the viability of leptospires up to six hours after collection, but thereafter it reduced the survival time of leptospires and by 24 hours after collection no leptospires could be cultured from EDTA samples from either calf.

TABLE 2.1 : THE AFFECT OF THE ANTICOAGULANT, EDTA AND HEPARIN, ON THE VIABILITY OF *POMONA* IN BLOOD

Anti Coagulant	Calf No.	Hours after collection ½	3	6	13	24	36	48	72	96
EDTA	1	+	+	+	+	-	-	-	-	-
EDTA	2	+	+	+	-	-	-	-	-	-
heparin	1	+	+	+	+	+	+	+	+	+
heparin	2	+	+	+	+	+	+	+	+	c

+ leptospires isolated. c contaminated.

Discussion : These results, which were obtained under relatively natural conditions, appear to be almost identical to those obtained by Nervig and Ellinghausen, (1978) under artificial conditions. Heparin was the better of the two anti- coagulants tested for collecting blood samples for culture. However, EDTA is probably satisfactory as long as the blood is cultured within six hours of collection.

PART C : Development of a microhaematocrit method for the detection of leptospiraemia :

The detection of leptospires in unclotted blood requires the removal of blood cells. Wolff (1954) described a double centrifugation technique whereby the blood cells are spun down at a slow speed and the serum is removed and examined by DFM. If no leptospires are seen then the serum is spun at high speed and the deposit is examined directly by DFM or by transmission microscopy after staining. This method is time consuming and Hathaway (1978) found it difficult to differentiate leptospires from protoplasmic extrusions of erythrocytes, "pseudospirochaetes"and other debris. Consequently a rapid method for the detection of leptospiraemia was developed (Mackintosh and Thompson, 1979) based on the first stage of Wolff's technique. A microhaematocrit tube was filled with unclotted blood and centrifuged for five minutes at 12,000 r.p.m. in a microcapillary centrifuge*. The tube was then broken 1 cm above the buffy coat and a wet smear of plasma from the broken end of the tube was examined by DFM. Gloves were worn to avoid puncturing the skin with broken glass. Leptospires, if present, were easily seen as the field was clear of cells and debris except for platelets and, if intravascular haemolysis had occurred, erythrocyte "ghosts". With this method very few "pseudospirochaetes" or blood derived protoplasmic filaments were found to be present.

* International Electric Company, Needham Hts., Mass. USA.

The microhaematocrit method was also used for clarifying and examining tissue homogenates and it was usefulfor estimating the number of leptospires present in kidney homogenates used for inocula.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

Collection of blood and urine samples : Blood samples were usually obtained from the caudal tail vein in cattle, the jugular vein in sheep, the ear veins in pigs and the jugular and cephalic veins in dogs using plain 10 ml vacuum tubes*. Health Department personnel assisted in a serological survey (Chapter Five) by taking blood samples from the brachial vein of dairy farmers. Blood samples for leptospiral culture or dark field examination were collected in heparinised tubes.

Female animals were used in experiments and trials because urination could be induced simply and midstream samples collected with the minimum of contamination. Urine samples were obtained from cattle by perineal stimulation, from sheep by smothering until urination commenced (Webster and Reynolds,1955) and from dogs by exercising them on a lead after a period of confinement or by administering a diuretic** which resulted in urination within 30 minutes. Sterile 20 ml universal bottles were used for such collections. Uncontaminated urine samples were obtained from some dogs by introducing a 19 gauge 1½ inch needle through the ventral abdominal wall directly into the bladder and aspirating urine into a 10 ml syringe (bladder paracentesis).

Serological method : The blood samples were allowed to clot at room temperature for 12 to 18 hours and the serum was decanted, centrifuged at 3000rpm for five minutes and stored at -20°C until tested. The serological test used for this study was a modification of the MAT originally described by Galton *et al* (1965), modified by Cole *et al* (1973) and adapted for this laboratory at Massey University by Ryan (1978). Serum samples were diluted

* Vacutainer : Becton, Dickinson and Co. Rutherford, New Jersey, 07070,USA. and Venoject: Terumo Corporation, Tokyo, Japan.
** "Lasix" - Frusemide, Hoechst (NZ) Ltd., Auckland, New Zealand.

1:6 with normal saline and stored in flat-bottomed microtitre serum reference plates (SRPs) which have 8 rows of 12 wells. These plates were sealed with cellophane and stored at -20°C until tested. Round-bottomed microtitre test plates, also with 8 x 12 wells, were used in the MAT. Twenty five µl of normal saline were dispensed into all the wells of the test plates using a Minipipetter*. A Minidiluter* with 12 x 25 µl diluters was then used to transfer a row of 1:6 diluted serum from the SRP into the first row of the test plate. After stirring, 25 µl were transferred from each well to the next row of wells, and so on, resulting in doubling dilutions down the plate from 1:12 in the first row to 1:1536 in the eighth row. Either a modified Cornwall syringe** with an attached Minipipetter dispensing head was used to dispense 25 µl of antigen into each well, a row at a time, or a disposable 25 µl pipette*** was used for individual wells, resulting in final doubling dilutions of serum from 1:24 to 1:3072. A plate cover was used to prevent evaporation. The test plate was gently shaken, incubated at 37°C for 90 minutes then read. An 8-prong dipper (Ryan, 1978) was used to transfer a drop from each of the 8 wells simultaneously, representing all the dilutions of a sample, onto a polished microscope slide. Four rows of 8 drops could easily be accommodated on each slide. A microscope with a dry dark field condenser, 10X objective and 15X eye pieces was used to read the test. The titre was considered to be the highest dilution in which there was 50% or more agglutination of leptospires.

The antigens used routinely were live 4 to 7 day old cultures of the six serovars known to be present in N.Z.; *hardjo*, *pomona*, *tarassovi*, *copenhageni*, *ballum* and *balcanica*. These were grown in liquid JS medium and contained approximately 1 x 10^8 to 5 x 10^8/ml. Serovar *australis* was included in the battery of antigens when its isolation in Northland was reported

* Cooke Engineering Co., Alexandria, Virginia, USA.
** Becton and Dickinson Co., Rutherford, New Jersey, USA.
*** "Dispo" pipette, Cooke Laboratory Products Ltd.,Singapore.

(Thompson, 1980), and serovar *canicola* was used during the survey of dog sera because of its widespread distribution overseas.

Standard antisera, which were supplied by the Center for Disease Control (CDC) *, were used to test the antigens for alterations in sensitivity or mislabelling.

New isolates were also tested against a range of CDC standard antisera to determine their serogroup. For serovar typing antiserum was made by injecting 4 ml of culture of the unknown isolate intravenously into a rabbit on three occasions at weekly intervals and sacrificing the rabbit one week after the last injection. The antiserum and cultures of the isolates were sent to CDC or to the WHO/FAO Leptospirosis Reference Laboratory, Queensland. Towards the end of this study the restriction endonuclease technique, developed for identifying leptospires by Marshall *et al* (1981), became available and limited use was made of this new technique to differentiate serovars within the Hebdomadis serogroup (see Chapters Eight and Ten).

Analysis of serological results : Doubling dilutions of serum form a logarithmic scale and for statistical analysis titres must be treated as geometric measurements. The geometric mean titre (GMT) is the average of a number of titres (Paul and White, 1973). For convenience, the GMT was calculated by using coded titres where the code is the rank of the well in which the endpoint occurred; that is to say 1:24 = 1, 1:48 = 2, and so on to 1:3072 = 8. The coded titres form an arithmetic scale and a simple average was taken and then converted back to a true titre or GMT. These manipulations can be expressed in the following mathematical formulae :

* Center for Disease Control, WHO Leptospirosis Reference Laboratory, Atlanta, Georgia, USA.

Mean coded titre = $\bar{c} = \frac{\Sigma c}{n}$ where c = coded titre

n = number of titres

$$GMT = \frac{1}{12 \times \text{antilog}(\bar{c} \times \log 2)}$$

where the coded titre is $\geqslant 1$

The standard error (SE) and the 95% confidence limits of the mean coded titre are derived by the following formulae:

$$SE = \pm \sqrt{\frac{\Sigma (c - \bar{c})^2}{n^2 (n - 1)}} \quad \text{or} \quad \pm \frac{\text{standard deviation of c}}{\sqrt{n}}$$

Mean coded titre ± 95% confidence limits

$$\bar{c} \pm 1.96 \times SE$$

The 95% confidence limits of the GMT are expressed as true titres after calculation using coded values.

Media : JS medium was prepared strictly to its formula (Appendix I) using analar grade chemicals and glass distilled, deionised water. Each batch of medium was tested for contamination by incubation at 37°C for three days, then at 27°C for three days and then examined for evidence of bacterial growth. Each batch was also checked for its ability to support growth by inoculating a trial sample with tenfold dilutions of a recent *hardjo* isolate. JS medium was used in two forms, liquid and semisolid. The liquid form contained no agar and was used for growing laboratory adapted organisms for use as antigens in the MAT. Recent isolates were also subcultured into liquid medium for typing. Semisolid (s/s) medium

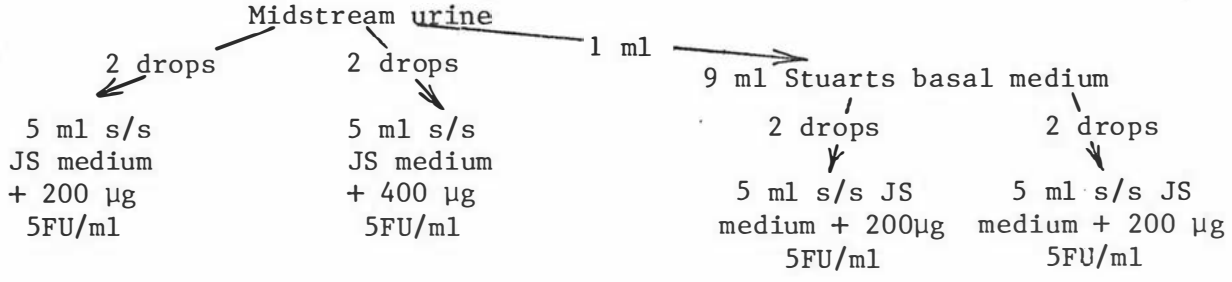
contained 0.2% agar, and was used for primary isolation of leptospires from blood, urine and kidney homogenates. This mediumwas also used for storing stock cultures at room temperature for two to six months between subcultures.

Culture methods : All media inoculation was carried out in a laminar-flow cabinet to reduce contamination.

Blood culture : Blood samples for culture were usually taken from the jugular vein and the vacutainer was removed from the holder before the needle was withdrawn from the skin to avoid contamination of blood. Two drops of blood were pipetted from the vacutainer into s/s JS mediumand the samemedium containing 200 µg 5FU/ml.

Urine culture : Urine samples from dogs were cultured within 30 minutes of collection and samples from cattle were cultured within 90 minutes. The time between collection and culture was kept as short as possible because leptospires have been reported to survive for as little as 90 minutes in undiluted urine (Kirschner and Maguire,1957; Nervig and Ellinghausen,1978). Transport medium as recommended by Flint and Liardet (1980a)was not considered necessary because of this short time interval between collection and culture. Two drops (0.1ml) of urine and two drops of a tenfold dilution of urine with Stuarts basal medium* were cultured in four bottles of s/s JS media containing 200 or 400 µ 5FU/ml, as shown in Figure 3.1.

FIGURE 3.1 : URINE CULTURE METHOD



* Becton,Dickinson and Co., Cockeysville, Maryland, USA.

Urine samples collected by bladder paracentesis or by aspiration from the bladder at necropsy were cultured in both plain s/s medium and s/s medium containing 200 µg 5FU/ml.

Kidney culture : Necropsies of hamsters used in this study were carried out in a laminar flow cabinet after they had been anaesthetised with ether, killed by cervical dislocation, exsanguinated and swabbed with alcohol. All instruments were flamed, the abdomen was opened and the kidneys were removed and transferred to a 5 ml syringe. The kidney was then forced from the syringe into a 10 ml bottle of Stuarts basal medium. This was stirred on a blender for five seconds to provide the homogenate for media inoculation.

Kidneys from cattle, sheep and dogs were removed at necropsy with their capsule intact and transported to the laboratory where they were sampled within two hours in a laminar flow cabinet. A 20-30gm portion of kidney was removed aseptically, flamed and placed in a gamma-sterilised plastic bag together with 100 ml of Stuarts basal medium. It was then homogenised on a Colworth 400 stomacher* and the homogenate was used for media inoculation.

Kidney homogenates were cultured by inoculating two drops of the homogenate into both s/s JS medium and s/s JS medium containing 200 µg 5FU/ml, and repeating the procedure using two drops of the homogenate diluted tenfold with Stuarts basal medium.

Cultures : All cultures were incubated at $27^{o}C$ and, at three week intervals, a loop of culture was transferred onto a polished glass slide and examined by DFM. If no growth was detected after 12 weeks, cultures were discarded. Isolates were subcultured into liquid medium for typing.

* A.J.Seward & Co.Ltd., 6 Stamford St., London SE1 9UE, England.

Dark field microscopy : DFM was used as an adjunct to culture in the detection and monitoring of leptospiruria. Wet smears of unconcentrated urine were examined at a magnification of 150X using a microscope with a dry dark field condenser. Blood samples, tissue homogenates and urine samples containing excessive amounts of debris were cleared using the microhaematocrit method described in Chapter Two.

Histological examinations : For histological examination, tissue samples were fixed in 10% formalin. Paraffin embedded sections 5-6µm thick, were prepared and stained by haematoxylin and eosin. For the demonstration of spirochaetes a modified Warthin and Starry method (Young,1969) was used.

CHAPTER FOUR

THIRTY YEARS OF HUMAN LEPTOSPIROSIS : A RETROSPECTIVE ANALYSIS OF PUBLISHED STATISTICS.

INTRODUCTION

In Chapter One an overall review of the epidemiology of human leptospirosis was presented, together with a summary of factors that have been found to be associated with human infections in this country. Dairy farm workers were the occupational group found to be at greatest risk and the factors shown to be associated with the incidence of human leptospirosis in this group were related to dairy farming practices, the seasonal production of milk on factory supply dairy farms and environmental conditions. However, most of the previous studies have been of only small numbers of clinical cases over short periods of time. The incidence of human leptospirosis in N.Z. has risen dramatically over the last 30 years and during this time there have been major changes in dairying practices and livestock management. However, there have been no published analyses of these changes in farming practices and the possible associations they have had with this increased incidence of disease.

During the last three decades a considerable amount of information has been published on the reported incidence of human leptospirosis, the distribution of livestock, dairy farming practices and meteorological data. The sources of this information are listed under materials and methods. The present study is an attempt to investigate further the associations which have already been identified and to examine the possible effects that changes in farming practices have had on the incidence of occupationally acquired leptospirosis during this 30 year period.

MATERIALS AND METHODS

Information and statistics were obtained from the following sources : annual reports of the Director General of Health (NZDH annual reports), N.Z. Department of Statistics Bulletin on Agriculture, 1974-5 (NZDS Ag Bull, 1974-5), N.Z.Department of Statistics, 1976 census of population (NZDS 1976 census), N.Z.Official Yearbook 1979, N.Z.Dairy Board annual reports (NZDB annual reports), N.Z.Dairy Board farm production reports (NZDB farm production reports), and personal communications from the N.Z.Dairy Board, N.Z.Milk Marketing Authority, N.Z.Department of Statistics (NZDS), N.Z.Meteorological Service (NZMS) and the District Office of the Hamilton Health District.

Linear regression analysis was carried out on the data to investigate the relationships between variables using a Texas Instrument 59 calculator. The regression line of y on x is described by the formula :

$y = mx + b$* where m is the slope of the line and b is the y intercept.

$$m = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}} \quad *$$

$$b = \frac{\Sigma y - m\Sigma x}{n} \quad *$$

A measure of the relationship between two sets of variables is given by the correlation coefficient (r) where

$$r = \frac{m \ Sx}{Sy} *$$

Sx = standard deviation of x

Sy = standard deviation of y

The significance of the correlation coefficient was tested by calculating a t-statistic using the formula

$$t = \sqrt{\frac{Vr^2}{(1-r^2)}} \quad * \text{ where } V = n - 2$$

* Reference : Owner's manual for the TI59 calculator, Texas Instruments Inc., U.S.A.

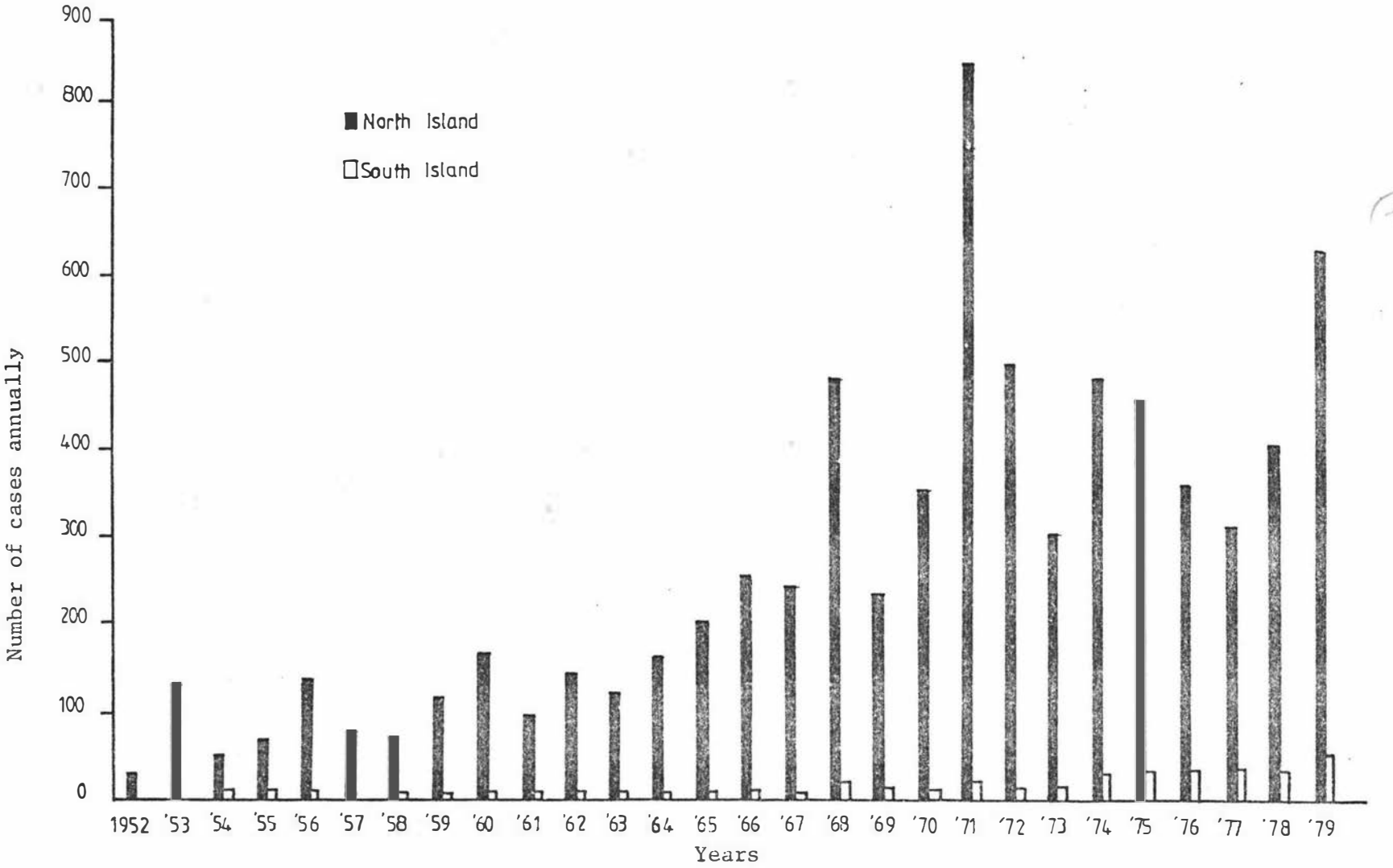
RESULTS

Since human leptospirosis was made a notifiable disease in 1952 the reported incidence has risen from an average of 89 cases annually in the 1950s to an average of 488 cases annually in the 1970s, with the greatest rate of increase occurring in the late 1960s (NZDH annual reports) (Fig.4.1). There were however, wide variations in the annual number of notifications and there appeared to be a cyclical pattern with a periodicity of three to five years. The greatest number of cases reported in one year was 860 in 1971, while the second highest, 677, was in 1979. Apart from these two exceptional years there have been approximately 300 to 500 cases reported annually for the last 12 years. The three health districts with the highest number of annual notifications were Hamilton, Whangarei and New Plymouth (Table 4.1). Together, these three districts accounted for approximately 58% of notifications in the 1950s, 70% in the 1960s and 77% in the 1970s. This increasing proportion of the annual total indicates that in these areas the incidence of leptospirosis has become more common or there has been a greater awareness of the problem (Fig. 4.2).

By analysing published data on human incidence, dairy cattle numbers and human population statistics (summarised in Table 4.2) together with meteorological data and statistics from the dairy industry several associations were found.

1. Cattle associations: Human leptospirosis is associated with dairy cattle. For the 10 year period, 1970-79, the number of human cases of leptospirosis in a health district is strongly correlated with the number of milking cows in the district (NZDS Ag.Bull. 1974-5) (correlation coefficient $r = 0.9667$, $P<0.001$) (Fig. 4.3). The rate of human notifications in each health district is shown in Table 4.2, and these rates are represented

FIG.4.1 : ANNUAL INCIDENCE OF NOTIFIED CASES OF HUMAN LEPTOSPIROSIS IN THE NORTH AND SOUTH ISLANDS (1952 - 1979)



(Copy table)

TABLE 4.1 : NOTIFICATIONS BY QUINQUENNIUM OF HUMAN LEPTOSPIROSIS (1952-1979)

Period	Health Districts recording the highest incidences					North Island (% NZ total)		South Island (% NZ total)		N.Z.total	Decade averages
	Hamilton	New Plymouth	Whangarei	Total for the three districts	(% of N.Z. total)						
1952* - 54	53	31	43	127	(56.4)	214	(95)	11	(5)	225	89
1955 - 59	121	66	95	282	(58.0)	466	(96)	20	(4)	486	
1960 - 64	260	78	120	458	(63.0)	687	(94)	40	(6)	727	224
1965 - 69	624	317	159	1100	(72.9)	1458	(97)	50	(3)	1508	
1970 - 74	1390	414	230	2034	(79.6)	2479	(97)	75	(3)	2554	488
1975 - 79	1020	501	211	1732	(74.6)	2157	(93)	166	(7)	2323	

* Leptospirosis was not a notifiable disease before 1952.

FIG.4.2 : CHANGES IN NOTIFICATIONS OF LEPTOSPIROSIS PER QUINQUENNIUM IN DIFFERENT HEALTH DISTRICTS.

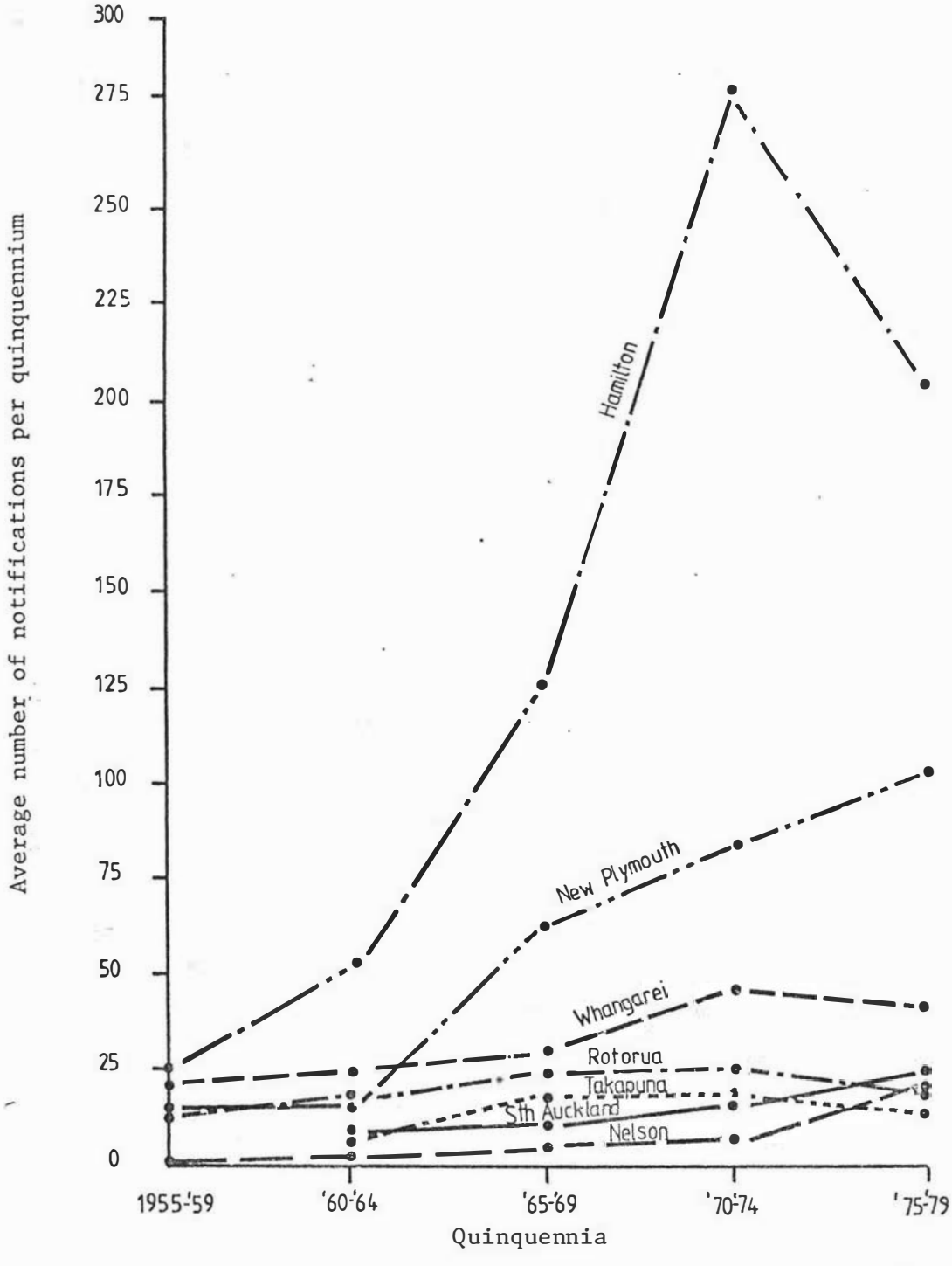
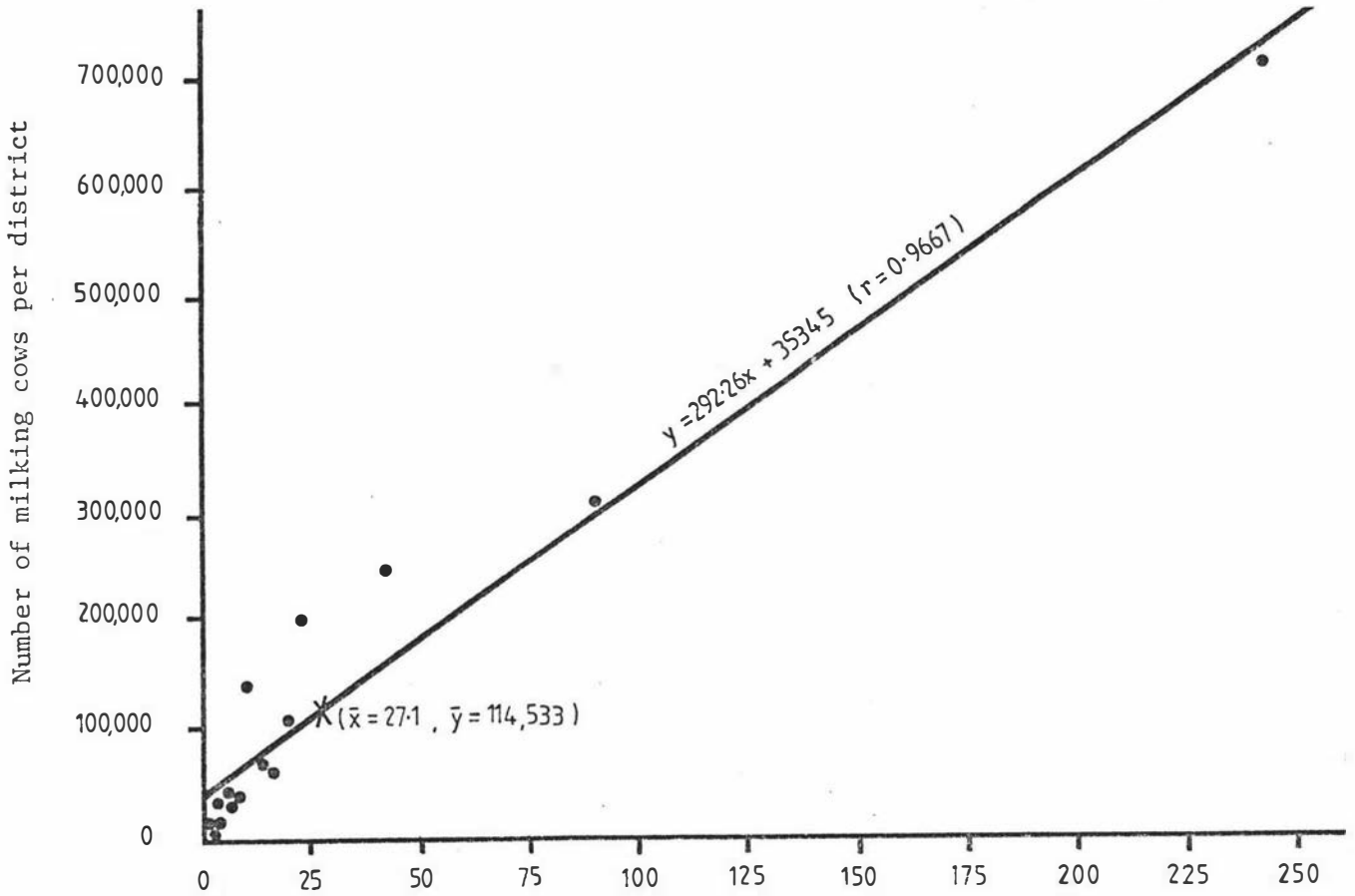


TABLE 4.2 : ASSOCIATION BETWEEN HUMAN NOTIFICATIONS AND NUMBERS OF DAIRY CATTLE

Health Districts	Human ** Population 1974	Average annual * notifications of human leptospirosis 1970-79	(%)	No. of milking ** cows ≥ 2 years old 1974/5	(%)	No. of ** dairy herds	Mean human annual incidence /100,000 people	Mean human annual incidence /100,000 cows	Average herdsize
Whangarei	100,470	44.1	(9.0)	249,280	(13.0)	2,615	43.9	17.7	95.3
Takapuna	261,340	15.5		63,102		797	5.9	24.6	79.2
Auckland	296,730	3.5		24		7	1.2	-	-
South Auckland	235,550	19.1		102,214		1,205	8.1	18.7	84.8
Hamilton	265,770	241.0	(49.4)	712,228	(34.5)	6,585	90.7	33.8	108.2
Rotorua	184,540	21.0	(4.3)	202,353	(10.6)	1,909	11.4	10.4	106.0
Gisborne	63,560	4.3		31,982		633	6.8	13.4	50.5
Napier	123,770	1.2		8,841		411	1.0	13.5	21.5
New Plymouth	97,430	91.5	(18.8)	310,837	(16.3)	3,161	93.9	29.4	98.3
Wanganui	87,460	5.2		39,478		810	6.0	13.2	48.7
Palmerston North	142,680	9.2		147,926	(7.8)	1,924	6.5	6.2	76.9
Hutt	189,840	7.6		40,205		521	4.0	18.9	77.2
Wellington	199,280	0.4		-		-	0.2	-	-
North Island	2,248,420	463.6	(95.1)	1,908,470	(92.6)	20,578	20.6	24.3	92.7
Nelson-Greymouth	125,930	13.5		65,421		949	10.7	20.6	68.9
Christchurch	344,160	6.4		35,757		893	1.9	17.8	40.6
Timaru	108,140	2.2		13,165		552	2.0	16.7	23.8
Dunedin	158,060	1.1		18,278		536	0.7	6.0	34.1
Invercargill	117,690	0.9		20,500		798	0.8	4.4	25.7
South Island	853,980	24.1	(4.9)	153,121	(7.4)	3,728	2.8	15.8	41.1
New Zealand	3,102,400	487.7	(100.0)	2,061,567	(100.0)	24,306	15.7	23.7	84.8

* N.Z.Department of Health Annual Reports
** N.Z.Department of Statistics

FIG.4.3 : REGRESSION OF THE AVERAGE NUMBER OF HUMAN CASES OF LEPTOSPIROSIS NOTIFIED ANNUALLY BETWEEN 1970 AND 1979 ON THE NUMBER OF MILKING COWS IN EACH HEALTH DISTRICT.



cartographically in Fig.4.4(a&b)The highest rate of human leptospirosis per 1000 dairy cows in the 1970s occurred in the Hamilton Health District, followed in descending order by the New Plymouth, Takapuna, Nelson, Hutt, South Auckland, Christchurch, Whangarei, Timaru, Napier, Gisborne, Wanganui, Rotorua, Palmerston North, Dunedin and Invercargill Health Districts. The Auckland and Wellington Health Districts have been excluded because there are few cattle (less than 100) in these districts. Thus, although the numbers of cases in the South Island were small, rates of human notifications/1000 dairy cattle in some of these health districts were just as high as those in the North Island. The rate for the Palmerston North Health District was much lower than the other North Island districts and three of the South Island districts.

There is also a correlation between the number of human cases in a health district and the number of dairy herds (NZDB Ag.Bull. 1974-5) ($r = 0.9543$, $P<0.001$) (Fig. 4.5) and with the average herd size in the district ($r = 0.559$, $P<0.05$) (Fig. 4.6). However the regression line for this latter association appears to be fitted to a curve and by taking the logarithm of the human notifications a much better fit is obtained ($r = 0.897$, $P<0.001$) (Fig. 4.7). This suggests that there is a non-linear relationship between herd size and the number of notifications of human cases. For example, with a doubling in herd size there may be more than twice the number of notifications.

2. Seasonal association: There is an association between human cases and the month of the year. There is a strong correlation ($r = 0.8956$, $P < 0.001$) between the average number of cases occurring in a particular month for the last ten years (NZDH annual report) and the average tonnage of milk processed for that month (NZDB annual reports)(Fig.4.8). Thus the

FIG. 4.4(a) : MEAN ANNUAL HUMAN INCIDENCE RATE OF LEPTOSPIROSIS (1970-79) PER 100,000 DAIRY COWS IN NORTH ISLAND HEALTH DISTRICTS.

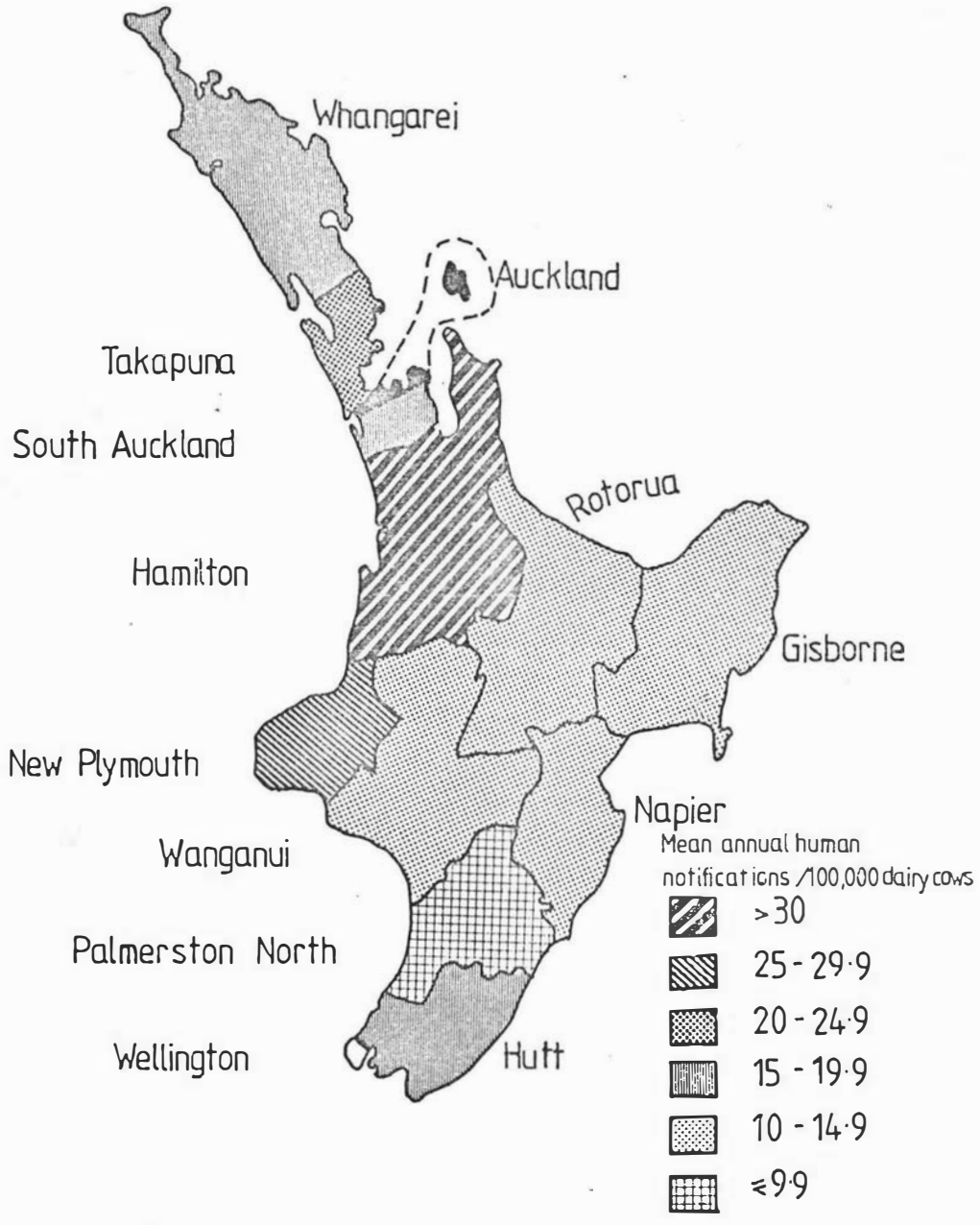


FIG.4.4(b) : MEAN ANNUAL HUMAN INCIDENCE RATE OF LEPTOSPIROSIS (1970-79) PER 100,000 DAIRY COWS IN SOUTH ISLAND HEALTH DISTRICTS.

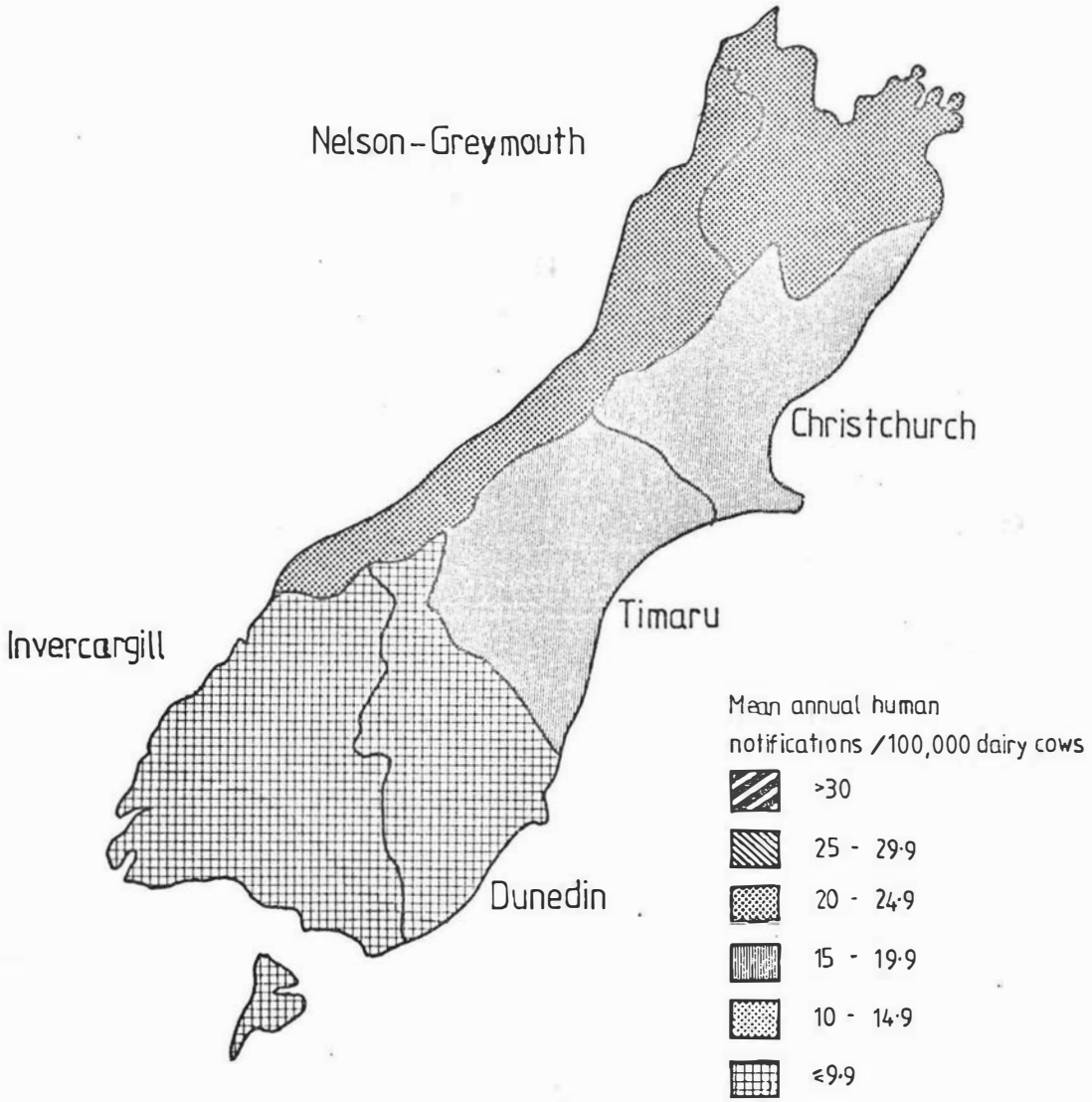


FIG.4.5 : REGRESSION OF THE AVERAGE NUMBER OF HUMAN CASES OF LEPTOSPIROSIS NOTIFIED ANNUALLY BETWEEN 1970 AND 1979 ON THE NUMBER OF DAIRY HERDS IN EACH HEALTH DISTRICT.

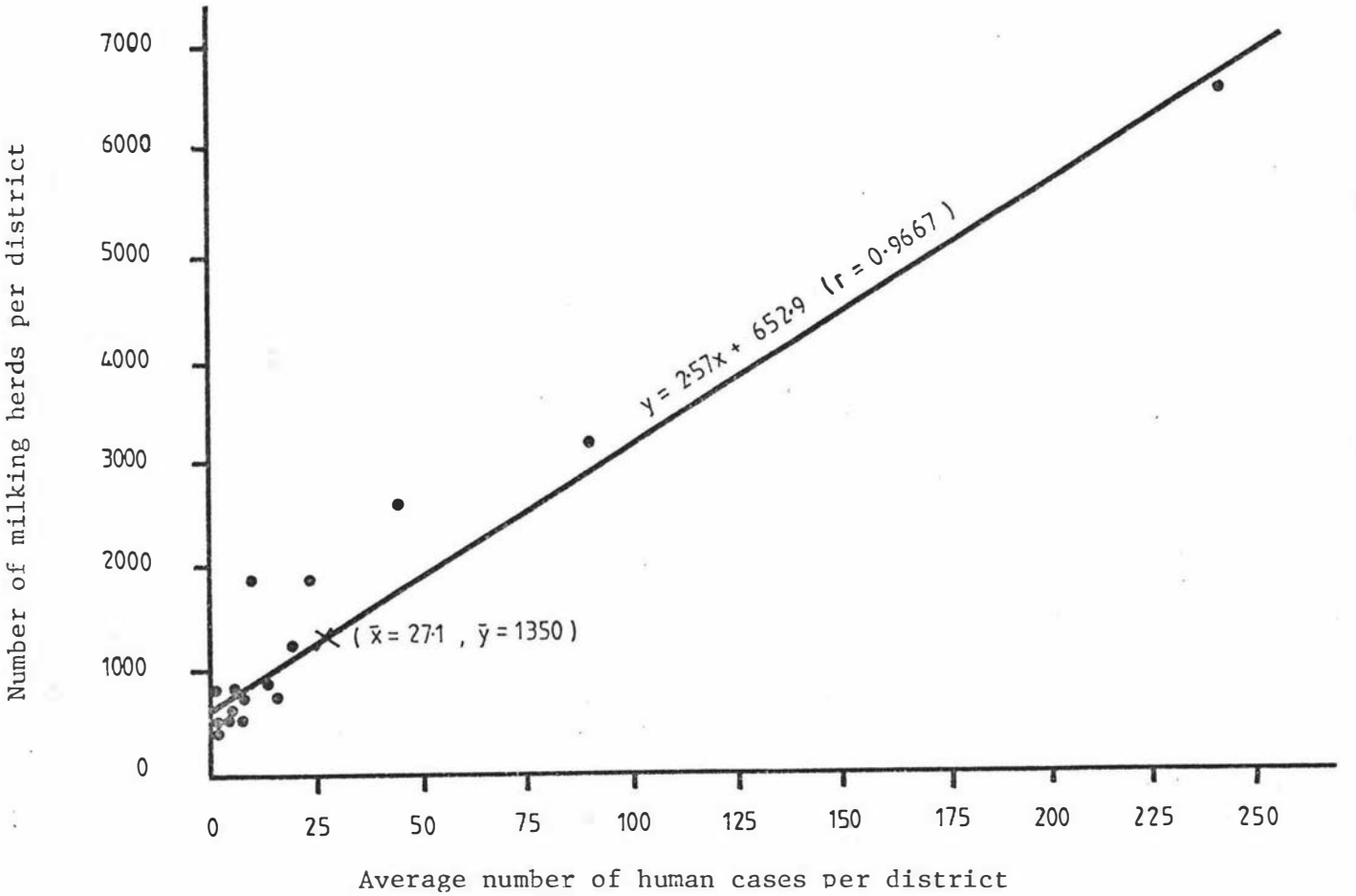


FIG.4.6 : REGRESSION OF THE AVERAGE NUMBER OF HUMAN CASES OF LEPTOSPIROSIS NOTIFIED ANNUALLY BETWEEN 1970 AND 1979 ON THE AVERAGE HERD SIZE IN EACH HEALTH DISTRICT.

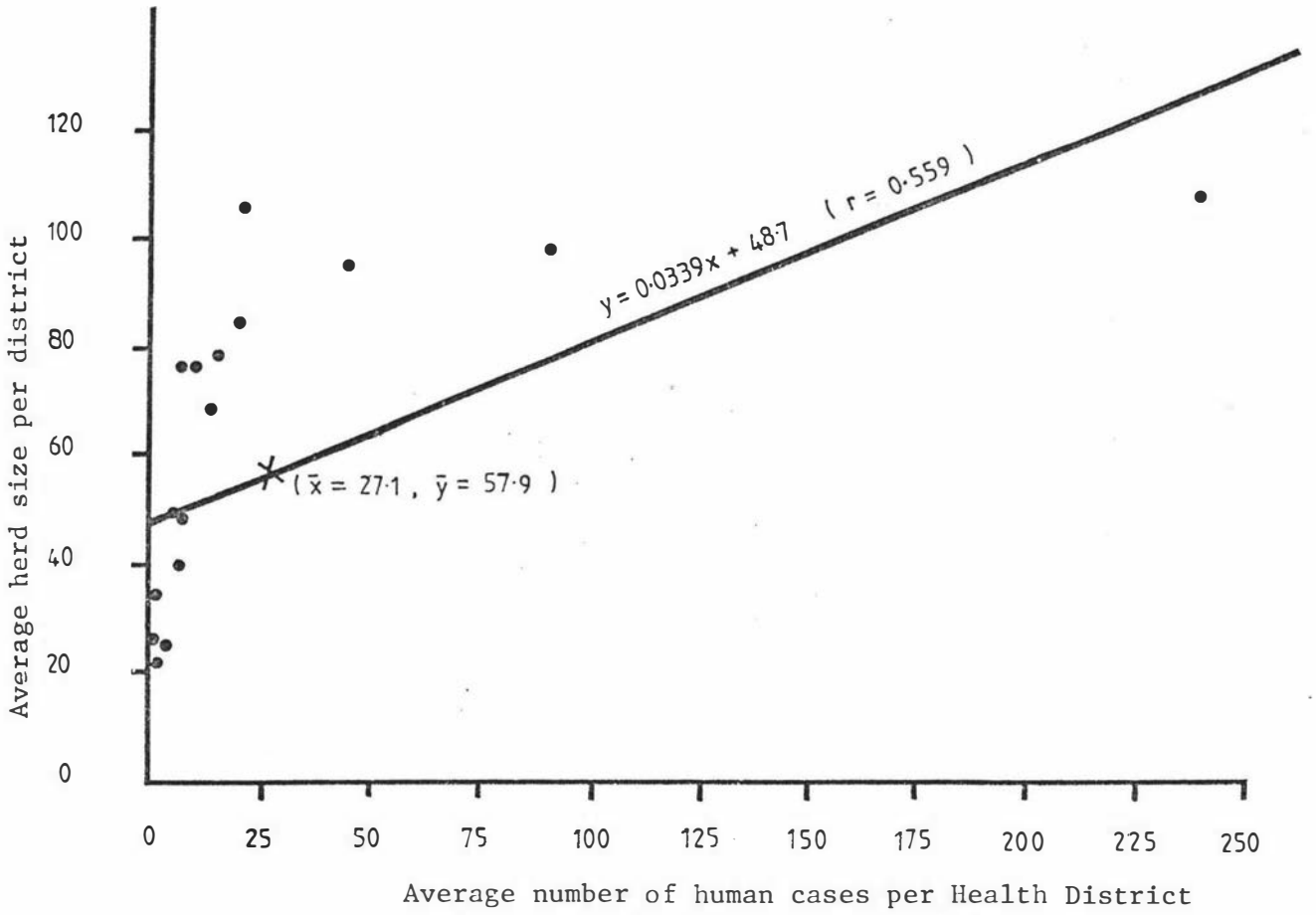


FIG. 4. 7 : REGRESSION OF THE LOGARITHM OF THE AVERAGE NUMBER OF HUMAN CASES OF LEPTOSPIROSIS NOTIFIED ANNUALLY BETWEEN 1970 AND 1979 ON THE AVERAGE HERD SIZE IN EACH HEALTH DISTRICT.

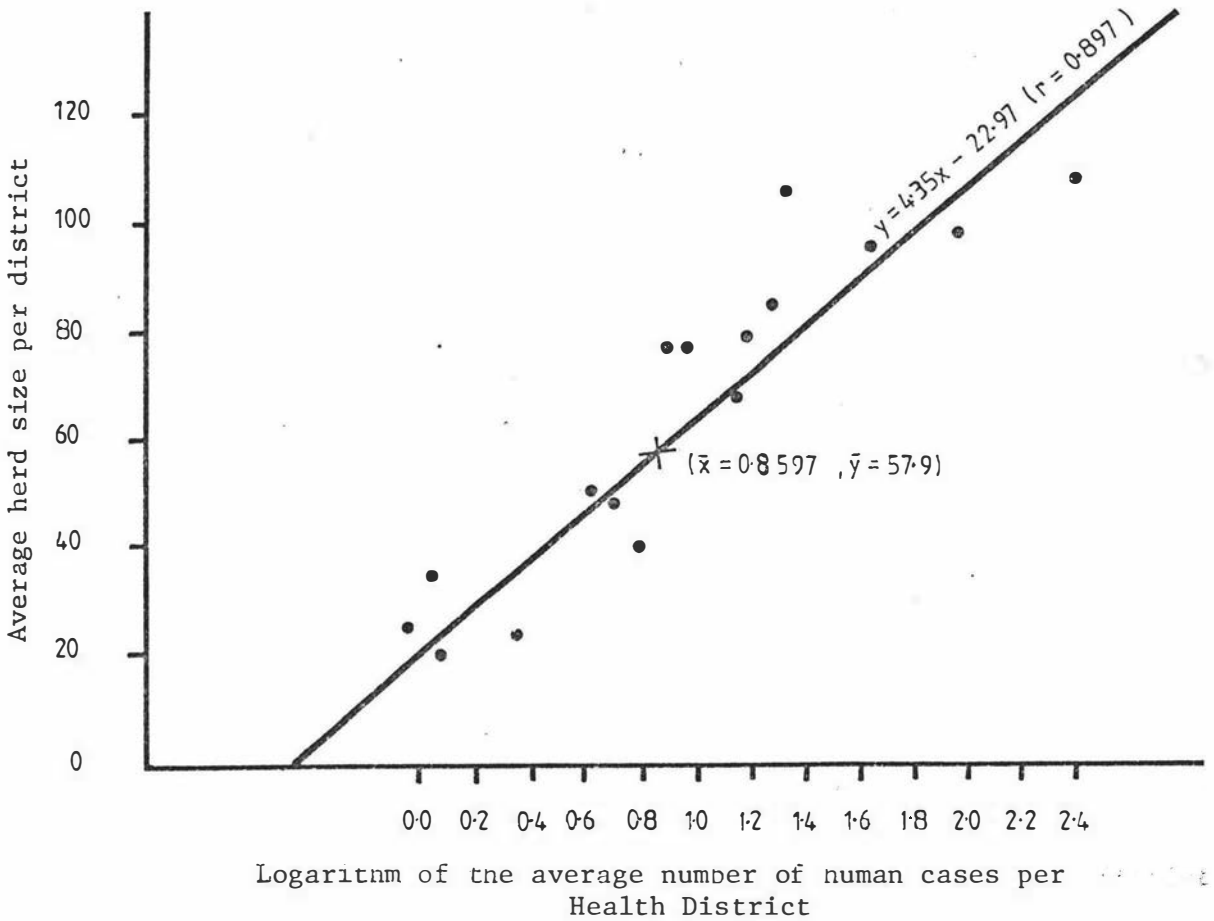
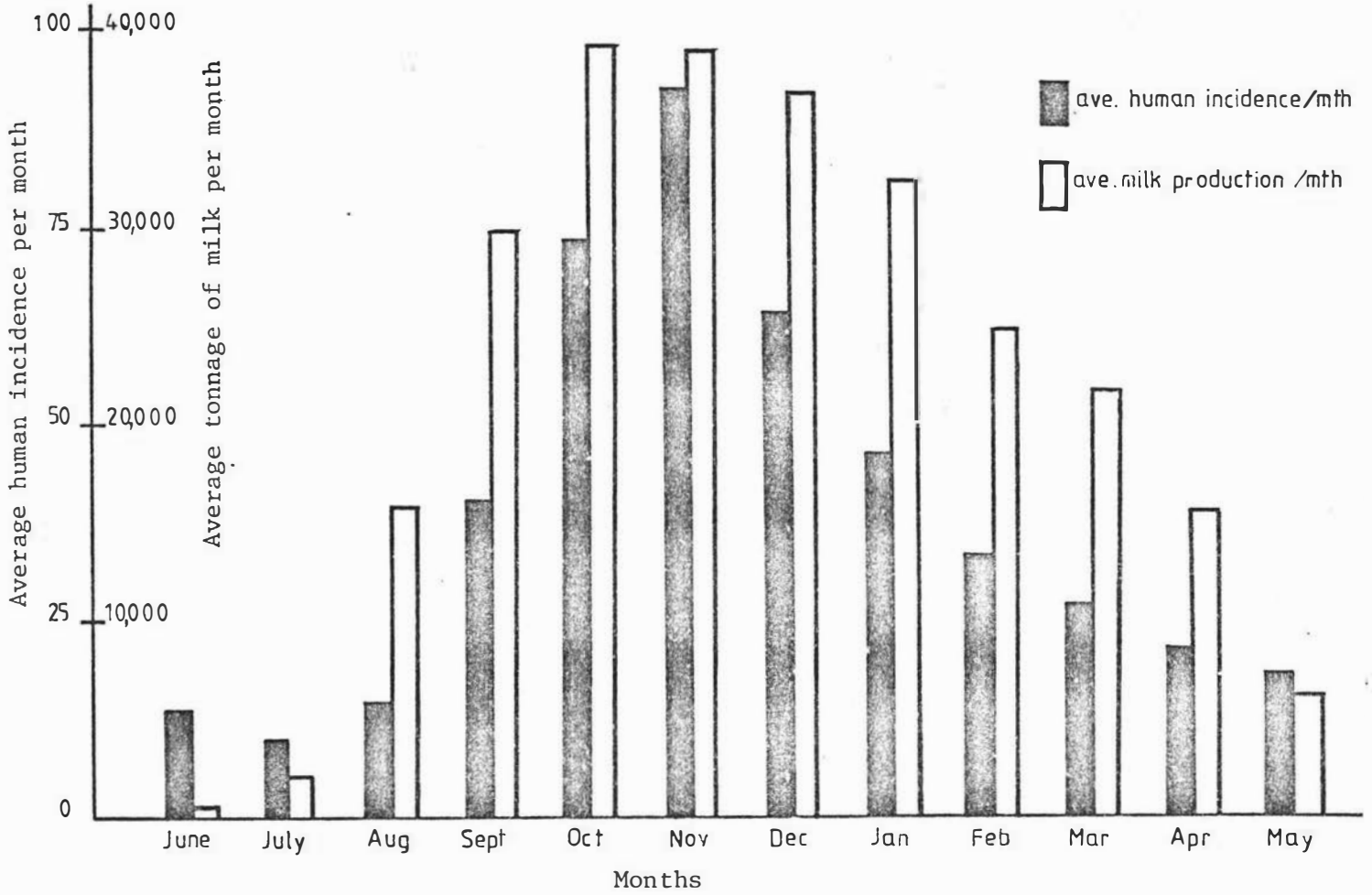


Fig. 4. 8 : COMPARISON BETWEEN THE AVERAGE HUMAN INCIDENCE PER MONTH FROM JUNE 1969 TO MAY 1979 AND THE AVERAGE MILK PRODUCTION PER MONTH ON FACTORY SUPPLY FARMS FROM JUNE 1974 TO MAY 1979.



October/November peak of milk production on factory supply farms, on which over 90% of N.Z. dairy cows reside, coincides with the seasonal peak in notifications of human cases of leptospirosis in November. It is at this time of the year that the cows take longest to milk. Consequently the milker spends the most time in the milking shed and is therefore in close contact with the cows for a longer period. This seasonal trend appears to have become more marked in the 1970s (Fig. 4.9). However, further analysis shows that the proportion of cases occurring in the September to December period in each five year period over the last 25 years was relatively constant (approximately 60%). The only apparent changes since the 1950s have been a slight decline, from 18% to 12%, in the proportion of cases reported in the winter months, May to August, and a corresponding increase for the period, January to April.

3. Rainfall in spring: Variations in the annual number of human cases is related to the amount of rainfall in the spring. Because rainfall tends to be a geographically localised event the analysis was performed using only the cases notified to the Hamilton Health District(Penniket,pers,comm.) and rainfall data from the Ruakura meteorological station(Dyke ,pers.comm.). This showed that in the last 12 years the spring rainfall was strongly correlated with the number of human cases which occurred in that milking season, June to May (Table 4.3, Fig. 4.10). Seasons with greater than average spring rainfall had above average numbers of human cases notified and *vice-versa*. The analyses (Table 4.3) show that the strongest correlations were with the total rainfall for September to November ($r = 0.7346$, $P<0.01$) and with the rainfall in September and October ($r = 0.6931$, $P<0.05$). The inclusion of August rainfall figures did not alter these correlations.

4. Sex and age of patients : There are correlations between patients with leptospirosis and their sex and age. The great majority of all cases of

FIG 4.9 : SEASONAL VARIATION IN HUMAN LEPTOSPIROSIS FOR EACH QUINQUENNIUM DURING THE LAST TWENTY-FIVE YEARS.

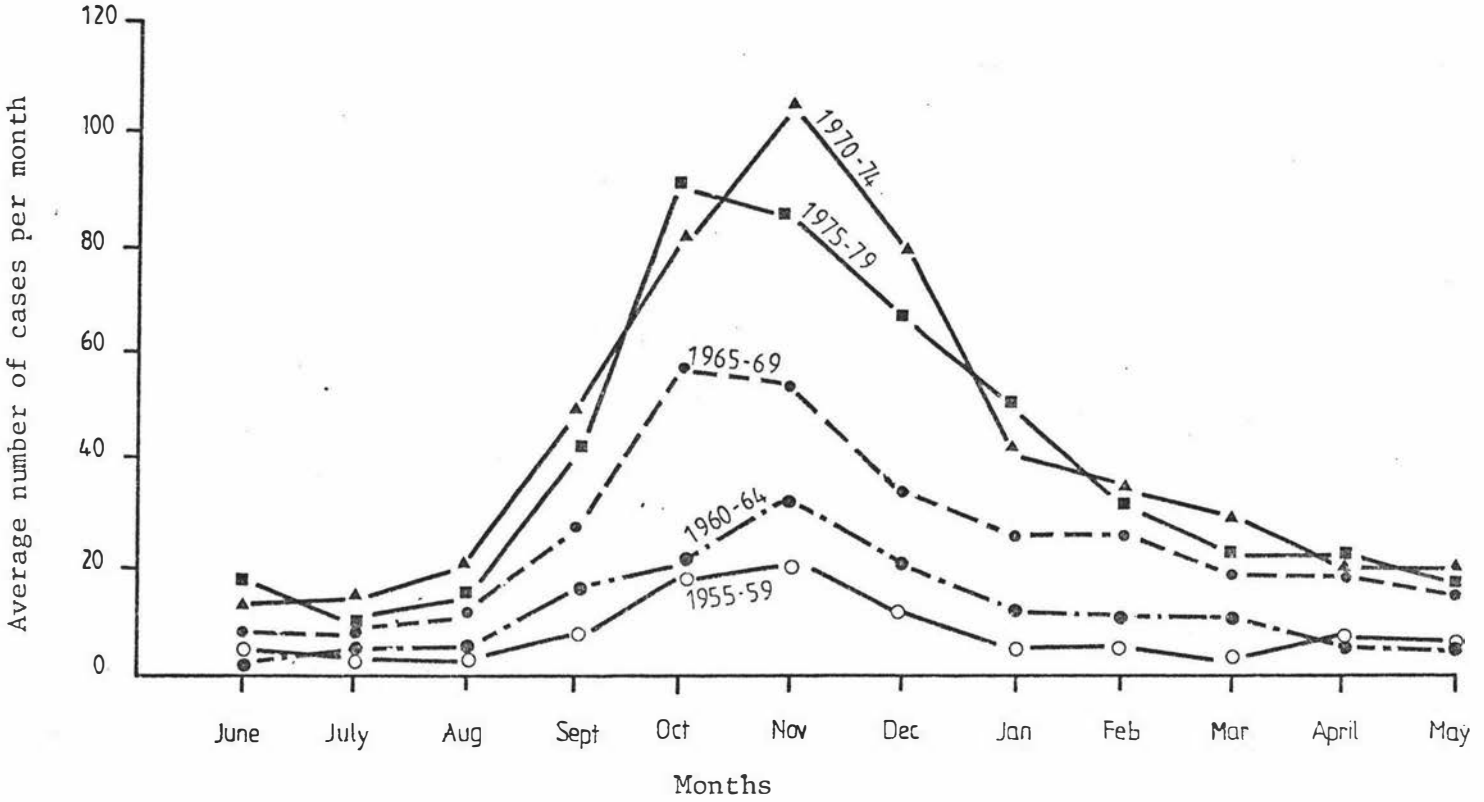
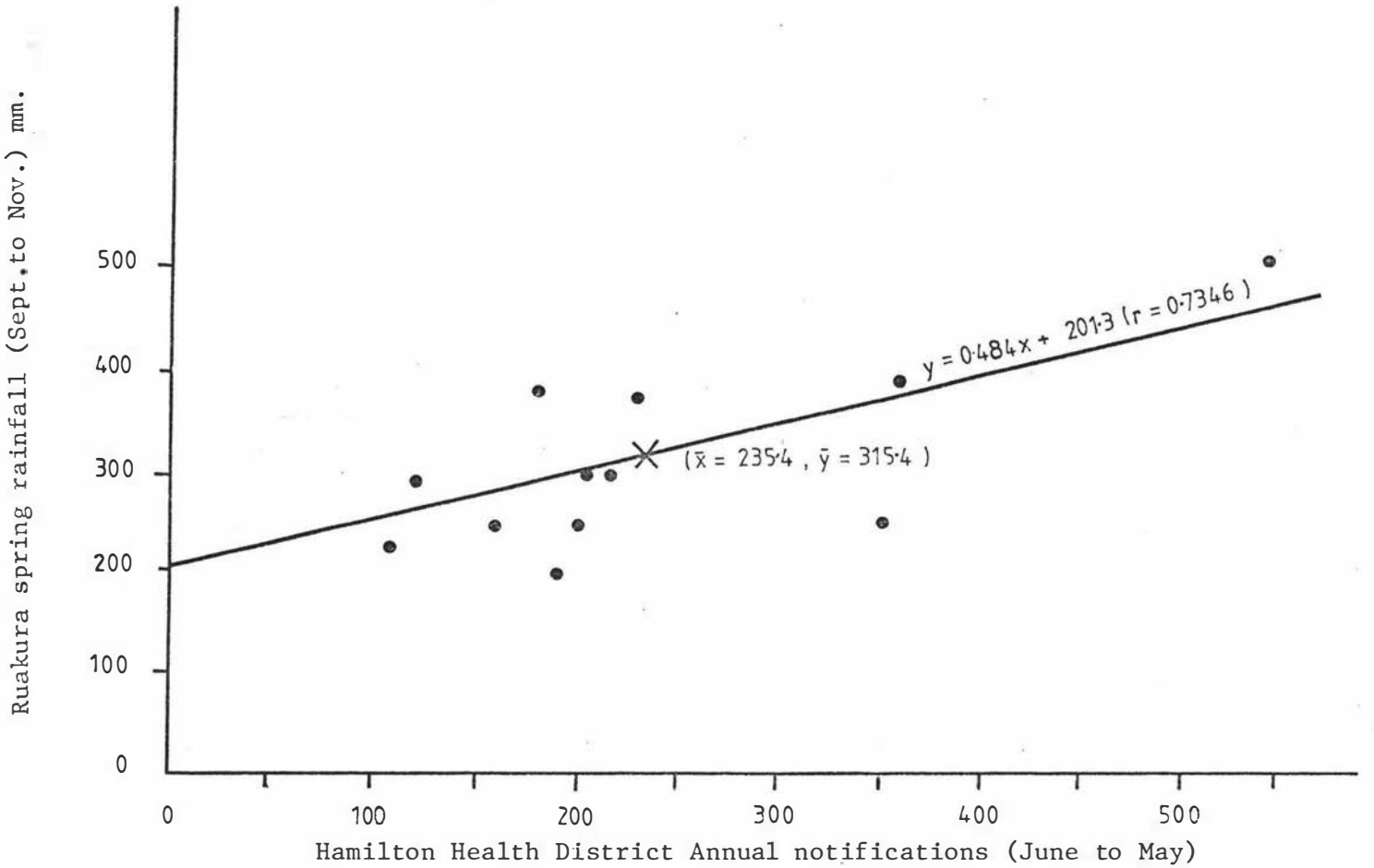


TABLE 4.3 : CORRELATIONS BETWEEN THE ANNUAL INCIDENCE OF HUMAN LEPTOSPIROSIS BASED ON THE DAIRY SEASON (JUNE TO MAY) AND THE SPRING RAINFALL, IN THE HAMILTON HEALTH DISTRICT.

Dairy season (June to May)	Notifications in the Hamilton Health District	Rainfall at Ruakura (mm) Aug/Oct	Sept/Oct	Sept/Nov.
1968/69	205	363	221	298
1969/70	110	275	168	225
1970/71	231	494	316	371
1971/72	542	488	371	512
1972/73	163	276	160	246
1973/74	214	278	172	299
1974/75	355	384	233	250
1975/76	199	283	164	247
1976/77	179	462	283	376
1977/78	121	283	213	294
1978/79	144	251	161	278
1979/80	362	315	230	389
Correlation coeff (r)		0.567	0.6931	0.7346
t statistic (11 d.f.)		1.9	3.04	3.424
Significance		N.S.	$P<0.05$	$P<0.01$ *

* see Fig. 4.10 for regression line.

FIG 4.10 : REGRESSION OF THE ANNUAL INCIDENCE OF HUMAN LEPTOSPIROSIS BASED ON THE DAIRY SEASON (JUNE TO MAY) ON THE SPRING RAINFALL, IN THE HAMILTON HEALTH DISTRICT.



leptospirosis notified to the Health Department (NZDH annual reports) were males, although over the last 20 years the proportion of females increased; 5.2% of notified cases for the period 1960-64 were women, 6.17% for 1965-69, 6.3% for 1970-74 and 8.9% for 1975-79 (NZDH annual reports).

In 1976, there were approximately 120,000 workers employed in the farming industry of which approximately 18% were women (N.Z.Official Yearbook, 1979). Approximately 40,000 workers, or 3% of the total workforce, were employed on dairy farms, but the percentage of these that were women is not reported and therefore it is not possible to compare attack rates for male and female workers. Neither is it possible to determine if the proportion of women workers has increased since the 1960s.

Over the last 20 years, 80% of notified human cases of leptospirosis have been between 15 and 44 years of age (NZDH annual reports). The percentage of male patients increased from 77.9% in the 1960-64 period to 84.6% in the period 1975-79. The increase in women patients has been even greater; from 50% in the 1960-64 period, to 84.1% in the 1975-79 period. Although only 42.9% of the population were between 15 and 44 years of age (NZDS, 1976 census), approximately 73% of the total workforce are in this age group (N.Z.Official Yearbook, 1979).The strong association between the incidence of leptospirosis and males of working age is typical of an occupational disease (Blackmore and Schollum,1980).

5. Farming practices : Trends in human leptospirosis appear to be related to changes in farming practices over the last 30 years. While the annual reported incidence of human leptospirosis has increased over the last 30 years (Fig. 4.1), especially in the Hamilton, New Plymouth and Whangarei Health Districts (Fig. 4.2) there appears to have been a change in the

proportion of cases caused by serovars *hardjo* and *pomona*. In the early 1950s the majority of cases of leptospirosis were attributed to *pomona* (Kirschner *et al*, 1952; Faine and Kirschner, 1953) and the disease was termed "swineherd's disease". Kirschner and Maguire (1957) coined the name "dairy farm fever" when they recognised that dairy farmers were the occupation group most at risk. At this time *pomona* was still the most commonly reported serovar. However, it was not until April 1956 that a Hebdomadis serogroup antigen was included in the battery of antigens used in the MAT (Josland *et al*, 1957). Before this time there were many cases diagnosed clinically as being typical cases of leptospirosis but which failed to be confirmed by serological or cultural techniques (Kirschner, 1954; Josland *et al*, 1957). It has subsequently been shown (Christmas *et al*, 1974b) that *hardjo*, which was probably responsible for many of these early cases, was a difficult organism to isolate using the media available at that time. Also, the Hebdomadis serogroup antigen *medanensis*, used from 1956 until the early 1970s in the MAT, is much less sensitive for detecting *hardjo* antibodies than the homologous antigen. The problem may have been compounded by the use of a minimum titre of $\geqslant$ 1:300 as a diagnostic criterion. Consequently, many *hardjo* infections may have been undetected or may not have been confirmed serologically. Nevertheless, human infections due to Hebdomadis serogroup organisms were detected in the mid 1950s (Josland *et al*, 1957) and Philip and Tennent (1966) recorded the incidence of human infections due to this organism in the Hauraki Plains in the early 1960s. By 1965 Hebdomadis serogroup titres were more commonly detected than titres to any other serogroup in dairy farmers with leptospirosis in the Hauraki Plains. In 1971, Christmas *et al* (1974_a) isolated *hardjo* for the first time in N.Z. and in a survey of clinically affected people in the Hauraki Plains area they found that two-thirds of patients were infected with this serovar, while one-third were infected with *pomona*. Brockie (1976) analysed National Health Institute records for 1971-74 and similarly found that two-thirds

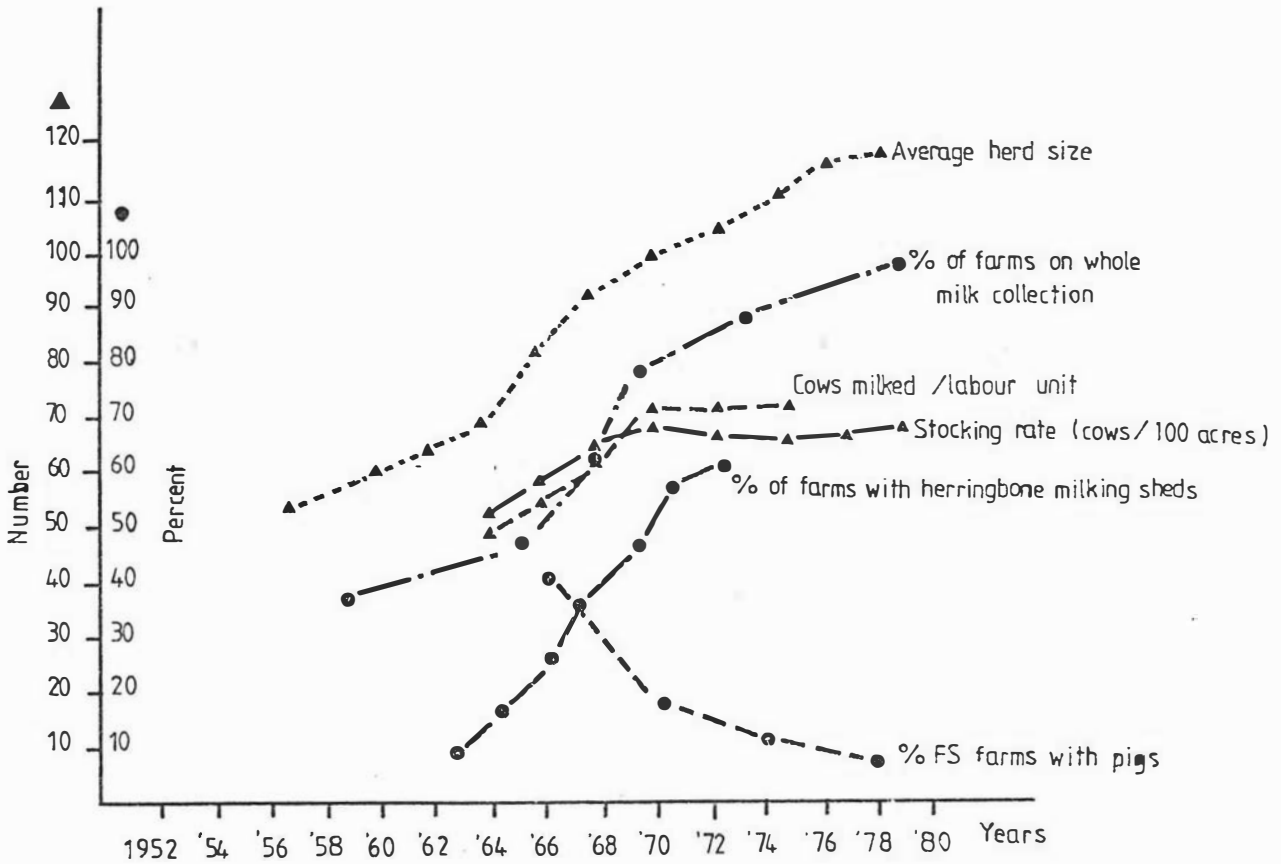
of cases were attributed to *hardjo* and one-third to *pomona*, with less than 1% due to other serovars. Hellstrom (1978) provided convincing evidence to show that there has been a decline in the incidence of *pomona* infections in dairy cattle over the last 30 years and an increasing incidence of *hardjo* infections. It seems likely that the incidence of human *pomona* infections has also declined and the rise in the incidence of cattle *hardjo* infections has resulted in the increased incidence of human infections due to this serovar since the 1950s.

Although the annual number of notified cases of human leptospirosis has increased it was not possible to determine what proportion of this increase was due to improved diagnosis or increased awareness and reporting. However, some authors (Philip and Tennent, 1966) were convinced that there had been a real increase in the annual incidence in their district. It has been suggested (Philip and Tennent, 1966; Jamieson *et al*, 1970) that changes in farming practices have contributed to this increase.

Over the last 30 years there has been an increase in the total volume of milk produced and improved efficiency of milk production in the dairy industry, largely as a result of the following factors :

(a) There has been a change from cream collection to whole milk collection, with a consequent decline in the number of pigs that were kept on dairy farms to consume unsaleable skim milk. This transition started in the early 1950s and by 1959/60, 38% of the North Island and 14% of South Island factory supply dairy farms were on whole milk collection and by 1978/79, 97% of North Island and 88% of South Island farms were on whole milk collection (NZDB annual reports) (Fig.4.11). In the early 1950s most factory supply farms kept pigs but by 1965/66 only 42%

FIG. 4.11 : CHANGES IN DAIRY FARM PRACTICES AND MANAGEMENT DURING THE LAST TWENTY-FIVE YEARS.



of factory supply farms had pigs, and by 1978/79 this figure had declined to 8.8% (NZDS annual report)(Fig.4.11).

(b) In 1950, there were only 1.85 million milking cows in the country. This number of milking cows increased until it reached a peak of 2.3 million in 1969/70 before declining to 2.1 million in 1978/79 (NZDB annual report) (Fig.4.11).

(c) There has been a steady decline in the total number of dairy farms over the last 30 years, with a reduction from 37,000 in 1950 to 15,774 in 1978 (NZDS annual report) (Fig. 4.11). At the same time there has been a dramatic increase in the average herd size, from 50 cows in 1950, to 61 cows in 1960, to 100 in 1970 and to 118 in 1978 (NZDB annual report)(Fig. 4.11). The greatest rate of increase occurred in the 1960s.

(d) Perhaps the greatest incentive to increasing herd size has been the introduction of herringhbone sheds which allowed the efficient milking of a large number of cows by a small number of milkers in a relatively short time. They first appeared in the early 1960s, and in 1963, 10% of herds which used artificial insemination had herringbone sheds, and by 1972, 63% had changed to this type of shed (NZDB farm production reports) (Fig. 4.11). In the 1967/68 season a survey of the main North Island dairy areas showed that 44% of dairy farms had herringbone sheds (Anon,1969). In the Hauraki Plains area in 1971, Christmas *et al* (1974) reported that approximately 50% of farms had herringbone sheds. A random survey of Manawatu farms in 1979, as reported in Chapter Five, showed that 77% had herringbcne, 6% had rotary and 17% had conventional walkthrough sheds.

(e) The number of cows milked per labour unit has increased (NZDB farm production reports) and this is probably associated with the transition to herringbone and rotary sheds (Fig.4.11).

(f) The changes to whole milk collection, improved pasture management and better animal husbandry have resulted in increased stocking densities (Fig.4.11). In the 1963/64 season there was an average of 53 cows/100 acres (133 cows/100 hectares) and by 1977/78 this had risen to 71 cows/100 acres (175 cows/100 hectares) (NZDB farm production reports).

DISCUSSION

Leptospirosis is the most commonly notified infectious occupational disease in this country and this appears to be associated with a high prevalence of leptospirosis in dairy cattle and the frequent close contact that occurs between workers and cows during milking (see Chapters One and Five). In a workforce of 40,000 the annual attack rate for dairy farm workers from 1970-79 was approximately 1100 per 100,000 compared with a crude attack rate of 18 per 100,000 for the whole of N.Z.'s population.

The associations with the age and sex of infected persons are related to the occupational nature of the disease, and the geographical distribution of cases is related almost entirely to the distribution of dairy cattle. The geographical features and climatic conditions necessary for efficient pasture production and dairy farming probably favour the environmental survival and transmission of leptospires. The best dairy farming areas,in the Waikato and Taranaki districts, also have the highest rates of human leptospirosis/1000 dairy cows. These districts have the highest stocking rates and the largest herds, and this latter factor is correlated strongly with human incidence rates. However, these rates are based on notifications

by doctors to the local Department of Health office, and in these areas of intensive dairy farming there has probably been an increased awareness of human leptospirosis and greater expertise in its diagnosis.

The seasonal incidence of human leptospirosis is related to seasonal milk production on factory supply farms on which over 90% of N.Z. dairy cattle are milked. Kirschner (1954) attributed the high incidence in spring to the "rainy season", although in most dairy areas the wettest time of the year is the winter (NZMS). Philip and Tennent (1966) were the first to record the relationship between the peak of human cases in November and the calving season which occurs from July to November on factory supply farms. Brockie (1976) noted that this peak of human notifications was related to the period of maximum milk production and suggested that it was because the farmer spent more time in the milking shed at that time of year, and therefore received the greatest exposure to potentially infected cow urine. From an analysis of National Health Institute records for the years 1970-74, Brockie (1976) also found that the seasonal patterns of *hardjo* and *pomona* infections were different. The highest incidence rates for *hardjo* infections were reported in November and were lowest in June and July, while *pomona* infections occurred at a relatively constant level throughout the year, with only a small increase in spring. The peak of human *hardjo* infections which occurs in spring is probably related to the pattern of *hardjo* infection in cattle, as well as to the increased time spent in the milking shed. It has been shown by Hellstrom (1978) that where *hardjo* is endemic in a herd, most new infections occur in spring when susceptible replacements are introduced to the herd and environmental conditions are most conducive for cow-to-cow transmission. *Pomona* infections in cattle, on the other hand, occur at any time of the year depending on when infection is introduced into the herd. These infections may only be apparent when they cause either bovine abortions in the autumn

and winter or ''redwater'' in calves in spring.

The cyclical pattern of human cases and the association between years of high human incidence and years of higher than average rainfall suggest that wet conditions enhance the risk of infection for man, possibly by favouring the survival of leptospires in the environment. However, the risk to man of direct infection from urine may also be increased if wet conditions allow infection in a herd to spread quickly to all susceptible cattle early in the milking season. Wet seasons may also favour the spread of *pomona* from pigs to cattle via contaminated water. In seasons of less than average rainfall, the environmental survival of leptospires may not be as great and infection within a herd may not spread as rapidly or extensively.

The apparent increase in the incidence of human leptospirosis, especially in the Waikato, Taranaki and North Auckland areas, and the change from *pomona* to *hardjo* as the most common infecting serovar, appears to have been due primarily to the intensification of dairy farming over the last 30 years. Until the early 1960s most herds were small, the cows were milked in walkthrough sheds and dairy farmers also kept pigs. However, with the transition to whole milk collection, and the disappearance of skim milk as a farm byproduct, the majority of farmers ceased to keep pigs. As a result the incidence of *pomona* infection has declined in both cattle and man. The change to herringbone sheds allowed dairy farm operations to be intensified and has resulted in an increase in the size of herds. Jamieson *et al* (1970), Christmas *et al* (1974a)and Brockie (1976) all suggested that large dairy herds contributed to the high incidence of human leptospirosis in this country. Hellstrom (1978) found a significant association between the prevalence of titres to *hardjo* in cattle on Manawatu dairy farms and herd size, and suggested that larger herd size

increased the opportunity for transmission of infection. Gsell (1952) noted that the incidence of "swineherd's disease" in Switzerland had increased in the first half of this century, and attributed this to changes in animal husbandry, especially to the increase in the size of sow herds. It appears that increased herd size, together with increased stocking rates may have contributed to the increased prevalence of *hardjo* infection in N.Z. dairy cattle. Milkers working in herringbone sheds appear to be at greater risk than milkers in conventional milking sheds due to their greater exposure to urine splash while working below the level of the cows. This was first suggested by Philip and Tennent (1966) and supported by Christmas *et al* (1974a) and Brockie (1976). Herringbone sheds also allow a greater number of cows to be milked per man thereby increasing even further the milker's exposure to bovine urine. A survey conducted by the Department of Health during the 1967-68 season in the Waikato and Taranaki districts showed that there was an association between farmers with leptospirosis and milking in herringbone sheds. The rate of infection for workers in herringbone sheds was twice that for workers in walkthrough sheds. They also found an association between cases occurring in workers in herringbone sheds and herd size. Larger herds were associated with an increased risk of leptospirosis for the milkers.

Apart from all the associations and observations so far discussed, there are probably a number of other factors which have contributed to the increased prevalence of leptospiral infections in livestock in this country. For example the movement of cattle may have assisted the dissemination of *hardjo* throughout N.Z.. The Tuberculosis Eradication Scheme which started in the 1950s and the Brucellosis Eradication Scheme introduced in 1969, have resulted in large numbers of cattle being culled and forced farmers to "open their herds" to buy in replacements. The reduction in the number of dairy herds and the increasing herd sizes of the remaining dairy farms

have resulted in smaller herds being sold and dispersed to larger units. Sharemilkers often move from farm to farm with their herds at the completion of the dairy season in late autumn and this results in the movement of large numbers of cattle through N.Z. at this time of the year. The buying and selling of dairy beef calves and yearlings increases the movement of stock between farms, as does the buying and selling of pedigree stock.

There have also been changes in dairy farm practices in recent years. These include putting on the milking machine cups without first washing or stimulating the udder, and the wearing of aprons, especially while milking in herringbone and rotary milking sheds. It is possible that some of these factors may reduce the exposure of the dairy farm worker to infected urine. This lack of knowledge about the associations between dairy farm practices and the risk of the dairy farm worker contracting leptospirosis led to the planning and implementation of the survey of Manawatu dairy farmers described in Chapter Five.

SUMMARY

A retrospective analysis of published statistics indicated that :

1. Leptospirosis is an occupational disease of dairy farmers and in the last ten years an average of 488 cases have been notified annually and approximately 90% of these have been reported in dairy farm workers. In a workforce of 40,000 the attack rate is approximately 1100per 100,000 compared with a crude attack rate of 18 per 100,000 for the whole of N.Z.'s population.

2. Leptospirosis is most commonly reported in male dairy workers between 15 and 44 years of age.

3. The distribution of human cases is associated with the distribution of

dairy cattle in N.Z.(r = 0.9667). Hamilton and Taranaki Health Districts had the greatest number of cattle and the greatest herd sizes and also had the greatest number of human cases reported per 1000 dairy cattle.

4. The majority of human cases occur between October and November each year and this coincides with the peak of milk production on factory supply dairy farms.

5. In the Hamilton Health District in the last 12 years, seasons with greater than average spring rainfall have been associated with seasons of higher than average numbers of notifications of human leptospirosis.

6. Notifications of human leptospirosis have risen from an average of 58 per year in the 1950s to 488 per year in the 1970s. This dramatic increase has probably been due in part to changes in farming practices over the last 30 years. These changes include a transition from cream to whole milk collection, increased herd sizes, a transition from walkthrough to herringbone milking sheds, increased numbers of cows milked per man and increased stocking rates. These factors have led to an increased prevalence of *hardjo* infection in dairy cattle and have increased the exposure of the dairy worker to leptospirosis. The number of pigs kept on dairy farms has decreased and this has led to a reduced prevalence of *pomona* infection in dairy cattle and a reduced incidence of infection with this serovar in dairy workers.

7. The movement of livestock over the last 30 years has probably contributed to the increased incidence of leptospirosis in dairy workers by assisting the spread of leptospirosis between herds throughout N.Z.

CHAPTER FIVE

MANAWATU DAIRY FARM SURVEYS

INTRODUCTION

In a study of the epidemiology of leptospirosis of dairy farm workers it is essential to know the incidence of infection in order to study the risk factors associated with infection. Most surveys of human leptospirosis in N.Z. (see Tables 1.4 and 1.5) have been of clinical cases and, although some attempts have been made to study certain causal relationships, they lacked adequate controls. Two exceptions have been the study of the risk factors associated with working in herringbone sheds conducted by the Department of Health (Anon, 1969) and a small case-control survey carried out in the Waikato by Penniket (1977). The only data available on the occurrence of human leptospiral infections in N.Z. as a whole are the numbers of cases reported to the Department of Health. The true number of human infections is likely to be higher than the number reported since some cases may have been misdiagnosed, not reported or not seen by doctors.

To obtain a more accurate estimate of the true incidence of leptospirosis in different occupational groups in N.Z. it was considered necessary to conduct serological surveys which would give a measure of the prevalence of infection. Hellstrom (1978) and Blackmore *et al* (1979) respectively produced evidence to show that bovine and human leptospiral titres of 1:24 or greater lasted for approximately ten years, and therefore a serological point prevalence approximates the incidence of infection for the last ten years. Surveys of this kind also assist in identifying risk factors associated with an increased prevalence of titres.

The only major cross-sectional serological studies of leptospirosis in occupational groups at risk in N.Z. have been confined to those employed in the meat industry (see Table 1.5) (Blackmore *et al*,1979; Blackmore and Schollum, 1980). They found a 10.2% prevalence in meat inspectors and a 6.3% prevalence in meat workers, of leptospiral titres of 1:24 or greater. These surveys also examined possible causal associations and the most important findings were firstly, the correlation between the prevalence of human leptospiral titres and working on the killing chain and secondly, the correlations between *pomona* and *tarassovi* titres and killing and inspecting pigs.

This chapter describes a cross-sectional serological survey of Manawatu dairy farmers carried out with the assistance of Department of Health personnel and a subsequent case-control study of a sample of "high risk" and "low risk" farms. Although the Manawatu is not a major dairying area and has a relatively low reported incidence of leptospirosis, it has over 700 dairy farms and previous surveys have shown that the prevalence of titres to *hardjo* in Manawatu cattle is similar to that found in the major dairying areas of the Waikato, Taranaki and Northland districts (Hellstrom, 1978). Therefore, the first survey was intended to measure the serological prevalence of leptospiral titres in farm workers and to investigate the correlations between these titres and personal attributes and dairying practices. The subsequent survey investigated the prevalence of leptospiral titres in animals from farms selected from the initial survey and gathered information on livestock management practices. This latter survey was conducted as a case-control study as this was the most efficient method of examining these factors with the limited resources available.

MATERIALS AND METHODS

Part I : Cross-sectional serological survey of Manawatu dairy farm workers

Four initial meetings were held with Health Department personnel to implement and design this survey, which was conducted in conjunction with a survey on the use of iodophors on dairy farms (Anon, 1979b).

There are approximately 630 seasonal or factory supply (FS) and 70 town supply (TS) dairy farms in the area served by the Manawatu Cooperative Dairy Company and the Palmerston North Milk Processing Company within the Palmerston North District. A random sample of 100 FS farms and all 70 TS farms were selected for this survey. Each farm was visited by employees of the Health Department and questionnaires were completed pertaining to the farm and to each person over the age of 15 years who resided or worked on the farm and was willing to cooperate.

The questionnaire consisted of two main parts. The first contained questions on farm variables including : farm type (FS or TS), herd size, shed type, average time spent in the shed at each milking, concurrent keeping of pigs, history of leptospirosis in the herd and milking techniques such as teat washing, stimulation of milk "let-down" and the use of teat sprays (see Appendix II). The second part concerned the person being interviewed and related to personal details including age, sex, milking experience, current milking status, use of protective clothing and medical history over the last five years, including specific diseases such as leptospirosis and brucellosis as well as non-specific symptoms and disorders (see Appendix III). Each person had the variables pertaining to their fa added to their personal data file to allow cross-tabulation between "farm" and "person" variables.

Serum samples were tested for antibodies to *Leptospira interrogans* serovars *hardjo, pomona, ballum, copenhageni, tarassovi* and *australis* using the MAT as described in Chapter Three. For the purposes of analysis people with leptospiral titres were considered in three categories : people seropositive to any leptospiral serovar(L pos), people seropositive to *hardjo* (H pos) and people seropositive to *pomona* (P pos).

The majority of the statistical analyses were carried out with the aid of a computer using the methods described by Nie *et al* (1975). Cross-tabulations were analysed by chi-square tests. For 2 x 2 tables, Fishers exact test was applied when there were fewer than 21 cases. Yates corrected chi-square was used for all other 2 x 2 tables. Where possible, tables with "expected" cell sizes of less than five were regrouped by combining two or more similar categories. Interval and scaled data were subjected to analysis of variance or other standard techniques. When a t-test was applied a two-tail probability was used to test the null hypothesis (Ho : $\mu_1 = \mu_2$) when the relationship between two samples was not known. However, when the alternative hypothesis, that one sample mean was greater than another ($H_1 : \mu_1 > \mu_2$), was tested then a one-tailed probability was used (Nie *et al*,1975). Analysis of the results involved three groups of information : "farm" and "person" variables from the questionnaire and serological results. It was important to define any associations in the first two areas which may have confounded subsequent analyses involving serological results. For example, a correlation between herd size and shed type may have confounded the correlation between shed type and the prevalence of farmers with leptospiral titres.

Part 2 : Case-control survey

The 30 milkers who had a leptospiral titre of $\geq$1:96 in the cross-

sectional serological survey (Part 1) were approached by telephone and 25 agreed to take part in a case-control study of the prevalence of leptospiral titres in their livestock and an investigation of management practices that might be associated with an increased risk of leptospirosis to the milkers. Thirty farmers who were seronegative in the cross-sectional survey were approached and 27 agreed to take part as negative controls. Each farm was visited and ten dairy cows from the milking herd were bled, together with any dogs, and a sample of the pigs or sheep on the farm which were available, and the sera from these animals were tested as decribed above. In the majority of cases the cattle were randomly selected from the two to five year old cows in the herd. In the others a sample of ten cows was taken at random from those which were available. As the survey took place in autumn, the cows in a small number of factory supply herds had ceased lactating and some of the animals were temporarily absent from the farm. A questionnaire relating to variablesnot covered by the initial survey was completed for each farmer. These questions concerned farm variables such as, topography, drainage, livestock numbers and movements, breeding practices and whether the cattle had been vaccinated for leptospirosis. Personal details such as smoking habits, drinking raw milk and the home-killing of animals were also collected (see Appendix IV). The questionnaire data and the results of serological tests of the blood samples from animals were added to each person's data-file collected in the prior survey.

For the purposes of analysis, the 52 farms were divided into three comparative groups according to the serological status of the milker determined in the initial survey and consisted of the following numbers of farms :

Group 1 : (a) 20 Hpos farms on which the milker had a titre to *hardjo*.

(b) 32 Hneg farms, i.e. all the control farms together with those farms where the farmer had a titre to *pomona* or *ballum* only.

Group 2 : (a) 10 Ppos farms on which the milkers had a titre to *pomona*.

(b) 42 Pneg farms, i.e. all the control farms together with those farms where the farmer had a titre to *hardjo* or *ballum* only.

Group 3 : (a) 25 Lpos farms on which the milker had a titre to any leptospiral serovar.

(b) 27 Lneg control farms.

Analyses were carried out as described above in Part 1.

RESULTS

Part 1 : Cross-sectional survey

A total of 226 people on 79 FS and 57 TS farms completed questionnaires and, of these, 213 people on 74 FS and 57 TS farms provided blood samples. Of the people originally approached, 79% of people on FS and 81% of people on TS farms responded to the questionnaire and blood samples were collected from 94% of the people questioned.

Farm variables :

Farm type : The results from a comparison of farm variables between FS and TS farms are shown in Table 5.1.

FS farms differed significantly from TS farms in that more FS farmers kept pigs for sale ($P<0.05$) and leptospirosis had been diagnosed in a greater percentage of FS herds than in TS herds. All other FS/TS differences were non significant.

Herd size : Table 5.2 shows the results from an analysis of some

TABLE 5.1: A COMPARISON OF FARM VARIABLES BETWEEN FACTORY-SUPPLY AND TOWN-SUPPLY FARMS

FARM VARIABLE	TOTAL	FACTORY SUPPLY No.	(%)	TOWN SUPPLY No.	(%)	SIGNIFICANT DIFFERENCES
SHED TYPE - herringbone	103	61	(77.2)	42	(73.7)	
- rotary	11	5	(6.3)	6	(10.5)	NS.
- walkthrough	22	13	(16.5)	9	(15.8)	
HERD SIZE - average number cows	114.8	119.6		108.6		NS.
COWS TAILS DOCKED - none	36	23	(29.1)	13	(22.8)	
- some	5	2	(2.5)	3	(5.3)	NS.
- all	46	28	(35.4)	18	(31.6)	
- not known	49	26	(33.0)	23	(40.4)	
PIG KEEPING - none	72	40	(50.6)	32	(56.1)	
- for home consumption	51	28	(35.4)	23	(40.4)	
- for sale	13	11	(13.9)	2	(3.5)	P<0.05
TEAT WASHING - -ve.	21	10	(12.7)	11	(19.3)	NS.
- +ve.	115	69	(87.3)	46	(80.7)	
CLINICAL HISTORY OF LEPTOSPIROSIS IN THE HERD - none	105	55	(69.6)	50	(87.7)	
- suspected	23	17	(21.5)	6	(10.5)	
- clinical diagnosis	4	3	(3.8)	1	(1.8)	
- laboratory confirmed	4	4	(5.1)	0		
- suspected, clinical diagnosis and lab. conf. combined	31	24	(30.4)	7	(12.3)	P<0.01

NS = not significant

TABLE 5.2: AN ANALYSIS OF FARM VARIABLES WITH RESPECT TO HERDSIZE.

FARM VARIABLE		HERDSIZE (No. of milking cows)	SIGNIFICANCE OF ANALYSIS OF VARIANCE
CLINICAL HISTORY OF LEPTOSPIROSIS IN THE HERD	- none	110.2	
	- suspected	118.0	
	- clinically diagnosed	168.8	P<0.01
	- laboratory confirmation	166.3	
SHED TYPE	- walkthrough	75	
	- herringbone	119	P<0.01
	- rotary	155	
APRON WEARING	- -ve.	105	P<0.05
	- +ve	132	
PIG KEEPING	- none	109.6	
	- for home consumption	122.1	NS
	- for sale	116.3	

NS = not significant

farm variables and herd size.

An analysis of variance showed a strong correlation between herd size and shed type. The average number of milking cows on farms with a walkthrough shed was 75, a herringbone shed 119 and a rotary shed 155. Herds with a history of leptospirosis also tended to be larger ($P<0.01$). There were positive correlations between the wearing of aprons and both herd size and herringbone sheds ($P<0.05$).

In addition, farms with larger herds tended to use plant sanitisers to a greater extent and to use pasture spray or ponds for effluent disposal. The practice of stimulation of milk let-down was not correlated with herd size.

Personal variables :

Of the 213 people bled, 174 (81.7%) were males and 39 (18.3%) were females. The milking status of these participants was divided into three categories : full time (more than nine milkings attended per week), part time (1 to 9 per week), and never or rarely. Of the 177 full time milkers, 161 were males and 16 were females. In addition there were 16 part time milkers (12 males, 4 females) and 20 non milkers (1 male, 19 females).

There were significant correlations between several personal and farm variables. People with a history of clinical leptospirosis more frequently had a medical history of headache, anorexia, weight loss and insomnia and were more likely to work with larger dairy herds.

The length of milking experience, which was very closely related to age, was correlated with a number of factors. Older, more experienced farmers milked in walkthrough sheds more frequently than younger farmers, their herds were smaller than average, the average time spent in the shed at each milking was less and they were more frequently factory suppliers than town suppliers.

Serological Results :

The results of the serological examinations carried out on the 213 blood samples are summarised in Table 5.3. A total of 84 positive titres (≥1:24) were detected; 48 to *hardjo* (57.1%), 29 to *pomona* (34.5%), four to *copenhageni* (4.8%), three to *ballum* (3.6%) and none to *tarassovi*. These titres were detected in a total of 66 people (31%), 52 of whom had a titre to one serovar only and 14 had titres to two or more serovars (see Table 5.4). Twelve (5.6%) people had concurrent *hardjo/pomona* titres and this was a significantly higher percentage than would be expected to occur if they were independently contracted ($P<0.01$). All four *copenhageni* titres were present concurrently with *pomona* titres which were at least one dilution higher.

Analysis of questionnaire data and serological results :

A summary of results and analyses is presented in Table 5.5. There were significant correlations between people with leptospiral titres (Lpos, Hpos and Ppos) and a number of variables. These correlations were; between Lpos and milking status, sex, a clinical history of leptospirosis, the wearing of shorts and milking experience; between Hpos and milking status, sex, a clinical history of leptospirosis, the average time spent in the shed at each milking, the wearing of aprons and milking experience; between Ppos and milking status, the concurrent keeping of pigs for sale, and milking experience.

Milking status : In this survey, only those people actively engaged in milking cows had leptospiral titres. The attack rates for *hardjo* positive full time, part time and non milkers were 26.6%, 6.3% and zero % ($P<0.05$). As all non milkers were seronegative to all serovars they were excluded from subsequent analysis.

TABLE 5.3: SEROLOGICAL RESULTS FROM DAIRY FARM RESIDENTS

	Serovar *hardjo*	*pomona*	*tarassovi*	*copenhageni*	*ballum*	Total
Titres 1:24	17	11	0	2	0	30
48	9	9	0	1	1	20
96	15	5	0	0	1	21
192	3	2	0	1	1	7
384	2	2	0	0	0	4
768	1	0	0	0	0	1
1536	0	0	0	0	0	0
3072	1	0	0	0	0	1
No. of titres ≥1:24	48	29	0	4	3	84
GMT* of titres ≥1:24	1:63	1:53	0	1:48	1:96	
Percentage of sero-positives	57.1	34.5	0	4.8	3.6	100%
Serological prevalence of survey pop.** (%)	22.5	13.6	0	1.9	1.4	
Serological prevalence of milkers *** (%)	24.8	15.0	0	2.1	1.6	
No. of TS farmers with titres ≥1:24	23	10	0	3	2	38
No. of FS farmers with titres ≥1:24	25	19	0	1	1	46

Number of people with a titre ≥ 1:24 to one or more serovars = 66 (31%)

Of those 66, 48 (72.7%) had a *hardjo* titre

and 29 (43.9%) had a *pomona* titre

while 14 (21.2%) had titres to two or more serovars.

* GMT = Geometric Mean Titre

**n = 213

***n = 193 (full time and part time)

TS = Town supply

FS = Factory supply

TABLE 5.4: MULTIPLE SEROLOGICAL RESPONSES OF DAIRY FARM RESIDENTS.

Case No.	Serovar	Titre	Serovar	Titre	Serovar	Titre
1	*pomona*	1:24	*hardjo*	1:24		
2	"	1:24	"	1:24		
3	"	1:24	"	1:48		
4	"	1:24	"	1:48		
5	"	1:48	"	1:24		
6	"	1:48	"	1:48		
7	"	1:48	"	1:96		
8	"	1:48	"	1:96	*ballum*	1:192
9	"	1:48	"	1:96	*copenhageni*	1:24
10	"	1:96	*copenhageni*	1:24		
11	"	1:192	*ballum*	1:48		
12	"	1:192	*hardjo*	1:48		
13	"	1:384	*hardjo*	1:384	*copenhageni*	1:48
14	"	1:384	"	1:768	"	1:192

TABLE 5.5 CORRELATIONS BETWEEN SEROLOGICALLY POSITIVE FARMERS AND 'FARM' AND 'PERSON' VARIABLES

Variable	Categories	No. in Group	Hpos farmers			Ppos farmers			Lpos farmers		
			No.sero-positive	Attack Rate%	Significance	No.sero-positive	Attack rate%	Significance	No.sero-positive	Attack rate%	Significance
MILKING STATUS	part time	16	1	6.25		3	19		4	25	
	full time	177	47	26.6		26	14.7		62	35	
	all milkers	193	48	24.8		29	15	*	66	34.2	
	non milkers	20	0	0	P<0.05	0	0	P<0.05	0	0	P<0.01
SEX (milkers only)	male	173	48	27.7	P<0.05	29	16.8	*	66	38.2	
	female	20	0	0		0	0	P<0.05	0	0	P<0.01
CLINICAL HISTORY OF LEPTOSPIROSIS IN MILKERS	-ve.	168	32	19.0		20	11.9		46	27.4	
	+ve.	25	16	64	P<0.01	9	36	P<0.05	20	80.0	P<0.01
TIME SPENT IN SHED/MILKING	Up to 2 hours	133	27	20.3		20	15		40	30	
	2 or more hours	60	21	35	P<0.05	9	15	NS	26	43.3	NS
WEARING OF SHORTS IN SHED	-ve.	66	12	18.2		6	9		15	10	
	+ve.	127	36	28.3	NS	23	18	NS	51	40	P<0.05
WEARING OF APRON IN SHED	-ve.	46	5	10.9		8	17.4		10	21.7	
	+ve.	147	43	29.3	P<0.05	21	14.3	NS	56	39	NS
SHED TYPE	Walkthrough	29	4	13.8	NS	6	20.7		7	24	
	Herringbone plus rotary	164	44	26.8	χ^2=1.6	23	14.0	NS	59	36	NS
FARM TYPE	Town supply	88	24	27.3		9	10		27	30.7	
	Factory supply	105	24	22.8	NS	20	9	NS χ^2=2.27	39	37.1	NS
CLINICAL HISTORY OF LEPTOSPIROSIS IN THE HERD	-ve.	146	35	24		18	13		44	30	
	+ve.	47	13	27.7	NS	11	23.4	NS χ^2=2.6	22	46.8	NS
PIG KEEPING	Home consumption	80	16	20		10	12.5	P<0.05	22	27.5	
	For sale	20	3	15 NS	NS	7	35.0		8	40 NS	NS
	None	93	29	31		12	13.0	P<0.05	36	38.7	

* Fisher's exact test

NS not significant

Sex : Males were significantly more at risk to leptospirosis than females ($P<0.01$). None of the 20 female milkers (four part time and 16 full time) had leptospiral titres compared with attack rates for the 173 male milkers (12 part time and 161 full time) of 38.2% positive to any serovar, 27.7% positive to *hardjo* and 16.8% positive to *pomona*.

Clinical history of leptospirosis : There was a significant correlation ($P<0.01$) between a clinical history of leptospirosis and a serological titre at the time of the survey. Clinical histories were divided into three categories - no history of clinical leptospirosis, diagnosed clinically but not confirmed serologically, and serologically confirmed leptospirosis. The percentages in the second and third categories were almost identical and therefore these categories were combined to give a larger cell size. Although 80% of the 25 milkers who had a history of leptospirosis were seropositive, only 30% of those who were seropositive had a history of clinical leptospirosis. The only two farmers who reported having had leptospirosis twice had titres to both *hardjo* and *pomona*.

Time spent in the shed at each milking : There was a significant correlation between Hpos and the average time spent in the milking shed. On farms where the milking took two or more hours, 35% of the milkers had titres to *hardjo*, compared with 20.3% of the milkers on farms where milkings took less than two hours ($P<0.05$). The prevalence of titres to *pomona* was identical (15%) in both categories, and was therefore not associated with the time spent milking.

Shorts : The wearing of shorts was significantly correlated with the prevalence of leptospiral titres. The attack rate for people who did not wear short trousers was 10% compared with an attack rate of 40% for people who wore short trousers ($P<0.05$).

Aprons : There was a significant correlation between Hpos and wearing aprons ($P<0.05$). However, other variables such as farm type, herd size and shed type were shown to confound this correlation and there is probably no direct causal relationship between these two factors. There were no correlations between wearing aprons and Lpos or Ppos.

Udder washing and teat stimulation : There were no statistical correlations between these factors and the prevalence of leptospiral titres in milkers.

Shed type : Rotary and herringbone shed categories were combined as the milkers working in them had a similar serological prevalence and appeared to be exposed to similar risk factors associated with milking cows from a pit. The prevalence of titres to *hardjo* of milkers working in these sheds was 27% compared to 14% of milkers working in walkthrough sheds. Although this is not statistically significant there were relatively few walkthrough sheds. Herd size also affects this comparison as walkthrough sheds were only present on farms having less than 150 milking cows.

Farm type : There were no significant differences between FS and TS farms with regard to the overall serological prevalence of leptospiral titres although there was a trend towards a higher prevalence of *pomona* titres on FS farms where the attack rate was 19% compared to 10% on TS farms ($\chi^2 = 2.27$).

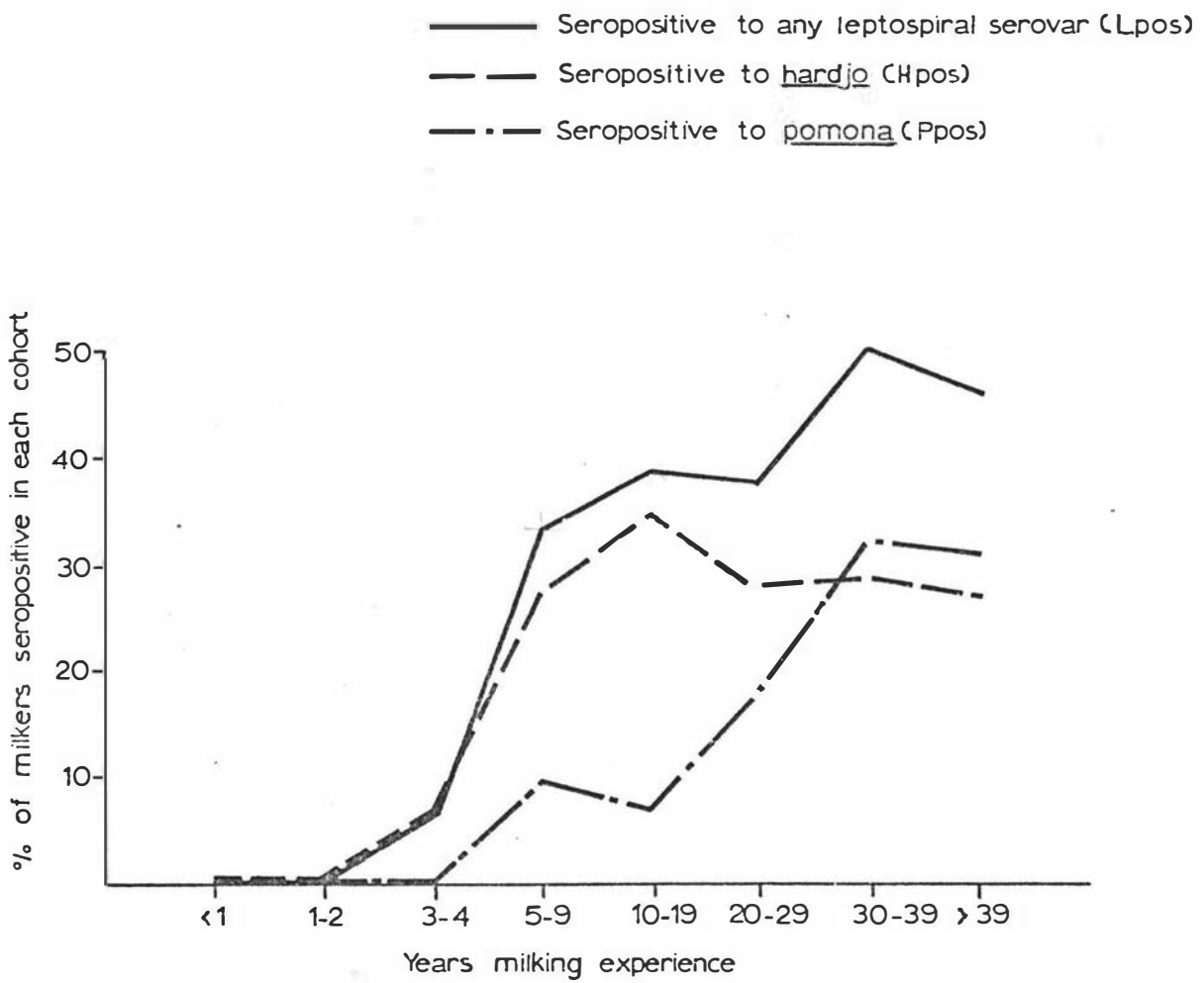
Herd size : A t-test indicated a trend towards a positive correlation between increasing herd size and increasing risk ($P<0.09$), although a χ^2 test, using four herd size categories, was less significant.

History of leptospirosis in the herd : There was a trend towards a positive correlation between a history of bovine leptospirosis in the herd and Ppos farmers.

Pigs : There was a statistically significant correlation between people with *pomona* titres and the keeping of pigs for sale. Of the farmers who kept pigs for sale, 35% had titres to *pomona*, compared to 12.5% of farmers who kept pigs for home consumption and 13% of farmers with no pigs ($P<0.05$).

Length of occupational exposure (milking experience) : As shown in Figure 5.1 the serological prevalence of leptospirosis increased with experience until it reached a maximum of 50% in the 30 to 39 year group. Lpos is almost entirely a summation of Hpos and Ppos, both of which have a different distribution with respect to experience. The prevalence of *hardjo* titres in milkers quickly rose to 25% in the 5 to 9 years experience group, reached a maximum of 33% in the 10 to 19 years group and then declined slowly to 23% in the >39 years group. The prevalence of *pomona* however remained negative until the 5 to 9 years group and then gradually increased to a maximum of 31% in the 30 to 39 year group. An analysis of the distribution of farmers keeping pigs for sale with respect to milking experience showed that this also reached a maximum of 15.6% in the 39 to 39 years experience, compared to an overall average of 10%.

FIG.5.1 : THE RELATIONSHIP BETWEEN THE SEROLOGICAL PREVALENCE OF LEPTOSPIRAL TITRES IN MILKERS AND THE NUMBER OF YEARS FOR WHICH THEY HAVE BEEN MILKING.



Part 2 : Case-control survey

A total of 52 farms, 25 Lpos and 27 Lneg farms, were investigated (see Table 5.6). The overall ratios of FS to TS farms in the case-study group (16:9) and the control farms (17:10) were similar, and were not significantly different from the ratio of farms (13:9) in the initial survey.

Serological results :

The results of serological examinations of the 520 cattle sera are summarised in Table 5.7. At least one of the ten cows from each of the 52 herds sampled had a titre to *hardjo* and the average sample prevalence was 66.3% and this was assumed to be similar to the herd prevalence. Eleven farms (21.2%) had one or more cattle with titres to *pomona* and of the 520 cattle tested, 27 (5.2%) had titres to *pomona*. Similarly 4.8% of cattle had titres to *tarassovi*, 4.4% to *copenhageni*, and 2.9% to *ballum*. Of the 360 Hebdomadis serogroup titres, 345 were to *hardjo* and 340 to *balcanica*. The GMT of 1:57 to *hardjo* was significantly higher on a paired T-test ($P<0.05$) than the GMT of 1:51 to *balcanica* although the actual difference was small. The majority of titres to the other four serovars occurred concurrently with titres to other serovars (see Tables 5.8 - 5.11). Most, if not all, the titres to *copenhageni* were considered to be cross-reactions or non-specific reactions because they were all 1:48 or less, their GMT was 1:27 and all except one were concurrent with titres to other serovars. Analysis showed that there was a significant association between the concurrence of *pomona* and *copenhageni* titres ($P<0.05$), with the *pomona* titres usually one to four dilutions higher than the concurrent *copenhageni* titres. There was also a strong trend ($P<0.10$) towards a similar association between concurrent *hardjo* and *copenhageni* titres. Nearly half the *tarassovi* titres occurred as single reactions and there were no associations between

TABLE 5.6 : DISTRIBUTION OF FARMS ACCORDING TO FARM TYPE (FS or TS) AND THE SEROVAR TO WHICH THE CASE-STUDY FARMERS HAD TITRES.

Farm Type	Control Farms	Case Study Farms	Serological Responses of Farmers*		
			hardjo	*pomona*	*ballum*
FS	17	16	12	6	1
TS	10	9	8	4	1
	27	25	20	10	2

* includes 5 dual titres : 4 *hardjo/pomona* and 1 *hardjo/ballum*

1 multiple titre : *hardjo/pomona/ballum*

TABLE 5.7 : SEROLOGICAL RESULTS FROM BOVINE SERA

	hardjo	*balcanica*	*pomona*	*copenhageni*	*tarassovi*	*ballum*
Titre 1:24	104	125	8	19	12	11
48	115	115	4	4	4	2
96	81	61	9		6	1
192	29	27	3		1	1
384	13	10	2		2	
768	3	2	1			
1536						
3072						
n cattle 520						
No. titres ≥ 1:24	345	340	27	23	25	15
GMT of titres ≥ 1:24	1:57	1:51	1:74	1:27	1:51	1:33
Percentage of seropositives (excluding *balcanica*)	79.3		6.2	5.3	5.7	3.4
Serological prevalence	66.3	65.4	5.2	4.4	4.8	2.9
No. herds 52						
No. herds with one or more seropositive	52		11	14	13	9
Percentage of herds with one or more seropositives	100		21.2	26.9	25	17.3

TABLE 5.8: TITRES TO OTHER SEROVARS IN BOVINE SERA WITH TITRES TO *POMONA*

pomona	*copenhageni*	*tarassovi*	*ballum*	*hardjo*	*balcanica*
768	48			96	96
384	24			24	48
384	24			24	24
192	24			24	24
192	24			24	24
*192					
96	48			192	192
96	24			48	96
96	24			48	24
96				48	24
96	24				
96				24	24
96				24	24
* 96					
* 96					
48	24	192	24	24	24
48	24			24	24
48				24	24
48				24	24
24	24		24	96	24
24	24				
24				48	48
24				24	48
24				24	24
24		24			24
* 24					
* 24					

* 5 serum sample reacting to *pomona* only.

TABLES 5.9 : TITRES TO OTHER SEROVARS IN BOVINE SERA WITH TITRES TO *TARASSOVI*.

tarassovi	*pomona*	*copenhageni*	*ballum*	*hardjo*	*balcanica*
384		24		48	48
*384					
192	48	24	24	24	24
96				24	24
96				24	
96					24
* 96					
* 96					
* 96					
48				48	48
* 48					
* 48					
* 48					
24				384	384
24		48		192	192
24				96	96
24				96	96
24				96	48
24				48	48
24				48	24
24	24				24
* 24					
* 24					
* 24					
* 24					

* 11 serum samples reacting to *tarassovi* only.

TABLE 5.10 : TITRES TO OTHER SEROVARS IN BOVINE SERA WITH TITRES TO *COPENHAGENI*

copenhageni	*pomona*	*tarassovi*	*ballum*	*hardjo*	*balcanica*
48	768				
48	96			48	96
48	96			192	192
48		24		192	192
24	384			24	48
24	384			24	24
24	192			24	24
24	192			24	24
24	96			48	24
24	96				
24	48	192	24	24	48
24	48			24	24
24	24			96	48
24	24				
24		384		48	48
24				768	384
24				96	192
24				96	24
24				48	48
24				24	24
24				24	
24				24	
*24					

* 1 serum sample reacting to *copenhageni* only.

TABLE 5.11 : TITRES TO OTHER SEROVARS IN BOVINE SERA WITH TITRES TO *BALLUM*

ballum	*pomona*	*copenhageni*	*tarassovi*	*hardjo*	*balcanica*
* 192					
96				768	384
48	24	24		96	24
48				24	24
24	48	24	192	24	24
24				768	768
24				192	192
24				96	96
24				96	48
24				96	48
24				96	48
24				48	24
24				48	24
24				24	24
* 24					

* 2 serum samples reacting to *ballum* only.

tarassovi titres and *pomona* or *hardjo* titres. The GMT for *ballum* titres was 1:33 while the GMTs for *tarassovi, hardjo, balcanica* and *pomona* were all greater than 1:50 and the distributions of these titres all reflected high proportions of recent infections.

Blood samples were obtained from 65 dogs on 37 farms. Of these dogs, 29 had titres to one or more leptospiral serovars : 18 (28%) to *hardjo*, 16 (25%) to *balcanica*, six (9%) to *ballum*, four (6%) to *pomona*, two (3%) to *copenhageni*, one (1.5%) to *tarassovi* and one (1.5%) to *canicola*. These results are discussed more fully in Chapter Six.

Analysis of serological results and farm data :

Factors associated with titres in the farmer.

(a) Hpos farms : There was a significantly ($P<0.05$) higher serological prevalence of titres to *hardjo* in cattle from the "high risk" (Hpos) farms than from the control farms (see Table 5.12). This was even more marked on the FS farms ($P<0.01$) but was non-significant on TS farms. However, if a minimum titre of 1:192 is used as an indication of more recent bovine infection, there were significantly higher ($P<0.05$) prevalences of these higher titres on both FS and TS "high risk" farms than on control farms. The distribution of herd *hardjo* prevalences (see Figure 5.2) shows that over half the "high risk" farms had a prevalence of titres to *hardjo* of 90 - 100% and a mean prevalence of 60% in the control herds. The GMT for each herd was also significantly higher on Hpos farms (see Table 5.12). This association was significant for both FS and TS Hpos farms when a one-tailed probability was applied to the t-test statistic. Greater herd size was associated with an increased risk on FS Hpos farms when the one-tailed t-test statistic was used ($P<0.05$) but not on TS Hpos farms. The distribution of

TABLE 5.12 : DIFFERENCES IN A NUMBER OF FARM VARIABLES BETWEEN HPOS AND HNEG CASE-CONTROL FARMS

Variable	Farm type	Hpos farms			Hneg farms			Significance
		(n)	range %	Mean %	(n)	range%	mean %	P
Prevalence of titres to *hardjo* 1:24	factory supply	(12)	60-100	83.3	(21)	10-100	53.8	< 0.01
	town supply	(8)	10- 90	66.3	(11)	40-100	71.8	NS(0.64)
	combined	(20)	10-100	76.5	(32)	10-100	60.0	< 0.05
Prevalence of titres to *hardjo* 1:192	factory supply	(12)	0- 70	15.0	(21)	0- 40	5.2	< 0.01
	town supply	(8)	0- 30	13.75	(11)	0- 10	4.5	< 0.05
	combined	(20)	0- 70	14.5	(32)	0- 40	5.0	< 0.01
Geometric mean titre (GMT) of titres to *hardjo*	factory supply	(12)		1:62	(21)		1:27	0.051 +
	town supply	(8)		1:69	(11)		1:51	0.074 +
	combined	(20)		1:65	(32)		1:46	< 0.01
Herd size (No.of cows)	factory supply	(12)		161.9	(12)		115.1	< 0.066 +
	town supply	(8)		94.6	(11)		115.6	NS (0.32)
	combined	(20)		135.0	(32)		115.3	NS (0.27)

+ 2 tail t-test statistic but if 1-tail statistic used then P<0.05.
NS = not significant even if 1-tail statistic used.

hardjo titres of cattle in case-study and control farms is shown in Figure 5.3.

The age specific prevalence rates of titres to *hardjo* of cattle are shown in Table 5.13. In the two to three year old cattle the highest prevalence of titres to *hardjo* of 1:24 or greater was 87.8% found on "high risk" (Hpos) FS farms. This was followed by TS Hpos farms (78.1%), TS Hneg farms (75.9%) and FS Hneg farms (40.8%). Similarly, the prevalence of titres to *hardjo* of 1:192 or greater also followed this order as did the GMTs of the samples. These differences in seroprevalence between Hpos and Hneg were significant ($P<0.05$) on factory supply (FS) but not town supply (TS) farms. The GMTs of animals with titres to *hardjo* of $\geq$ 1:24 and the percentages of animals with titres to *hardjo* of $\geq$ 1:192 were higher in the two to three year olds than in the cows three years old and over on the FS and TS Hpos and the TS Hneg farms, while the reverse was the case on FS Hneg farms.

Cross-tabulations with variables collected in both the case-control and the cross-sectional surveys showed that only a history of leptospirosis in the farmer ($P<0.01$) and a history of leptospirosis in the herd ($P<0.05$) were significantly associated with Hpos farms. Variables examined, but found to be non-significantly associated with Hpos study farms included; the topography and drainage of the property, the keeping of pigs, the buying in of calves, heifers, cows, bulls, weaner pigs, sows and sheep, the use of a bull or artificial insemination, the homekilling of livestock, smoking in the milking shed, drinking raw milk, the keeping of beef cattle, sows and sheep on the property, whether the cows' tails were docked or not, and the washing and stimulating of cows' udders prior to milking.

(b) Ppos farms : There were no significant associations between Ppos farms and any farm variables. However, a number of variables showed

TABLE 5.13: AGE SPECIFIC PREVALENCE RATES AND GEOMETRIC MEAN TITRES (GMTs) OF TITRES TO *HARDJO* IN CATTLE

Farm type	Age (years)	No.of cattle	No. and (%) of cattle with titres ≥ 1:24		GMT of cattle with titres ≥ 1:24	No and (%) of cattle with titres ≥ 1:192	
Hpos	2-3	41	36	(87.8)[a]	3.167[b]	15	(36.6)[c]
FS* farms	3-4	34	28	(82.4)[d]	2.143	2	(5.9)
	4-5	16	13	(81.2)	2.077	0	(0)
	5 +	28	22	(78.5)	2.227	1	(3.6)
Hneg	2-3	76	31	(40.8)[a'f]	1.806[b']	2	(2.6)[c']
FS Farms	3-4	61	36	(59) [d']	2.139	4	(6.6)
	4-5	21	15	(71.4)	2.153	2	(9.5)
	5 +	40	28	(70.0)	2.036	3	(7.5)
Hpos	2-3	41	32	(78.1)[x]	2.969[e]	10	(24.4)[y]
TS* farms	3-4	12	10	(83.3)	2.5	1	(8.3)
	4-5	6	6	(67)	1.83	0	(0)
	5 +	3					
Hneg	2-3	29	22	(75.9)[f'x']	2.5[e']	4	(13.8)[y']
TS farms	3-4	20	17	(85)	2.47	0	(0)
	4-5	12	10	(83.3)	1.8	0	(0)
	5 +	28	21	(75)	2.238	1	(3.6)

* FS = factory supply
TS = town supply

a a', b b', c c', d d', e e', f f' pairs significantly different at 5% level
x x', y y' pairs not significantly different at the 5% level.

FIG.5.2 : FREQUENCY OF THE PREVALENCE OF TITRES TO *HARDJO* IN CATTLE ON HPOS AND HNEG FARMS.

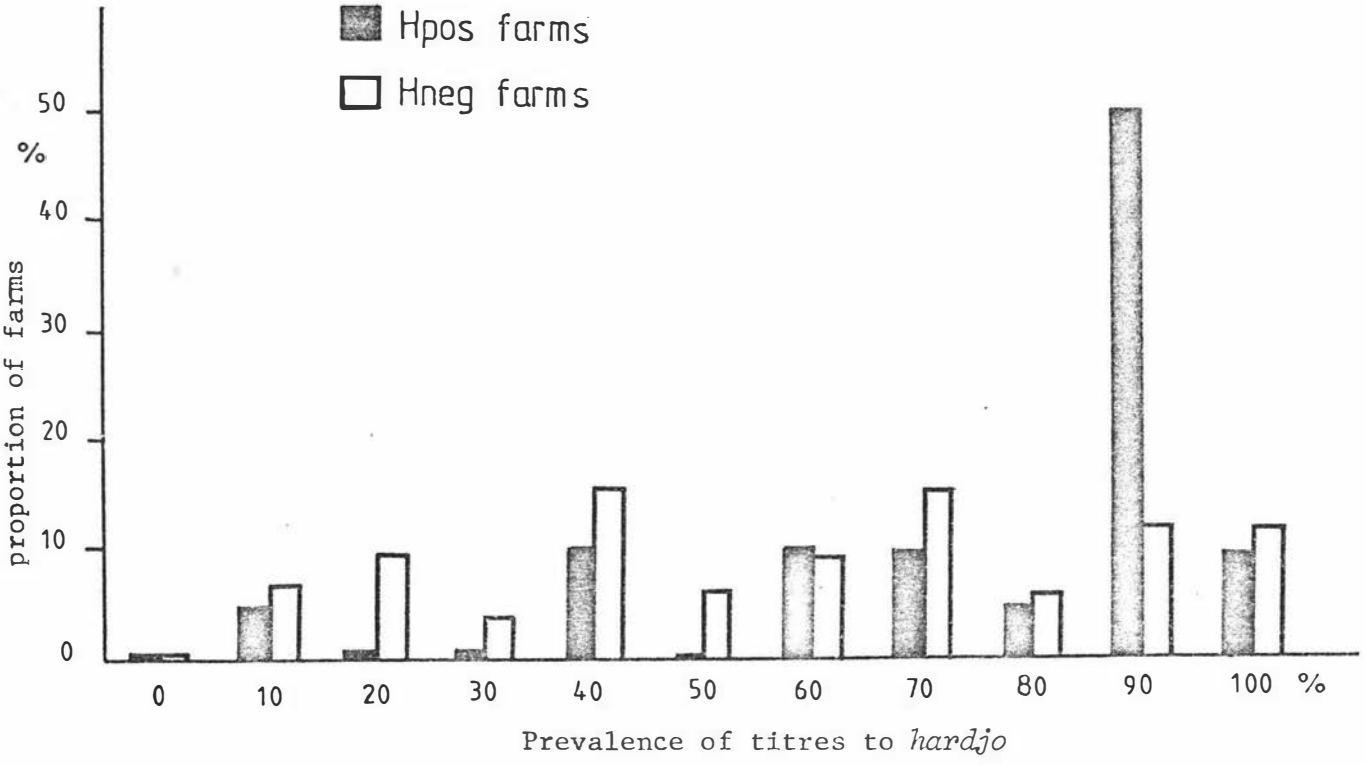
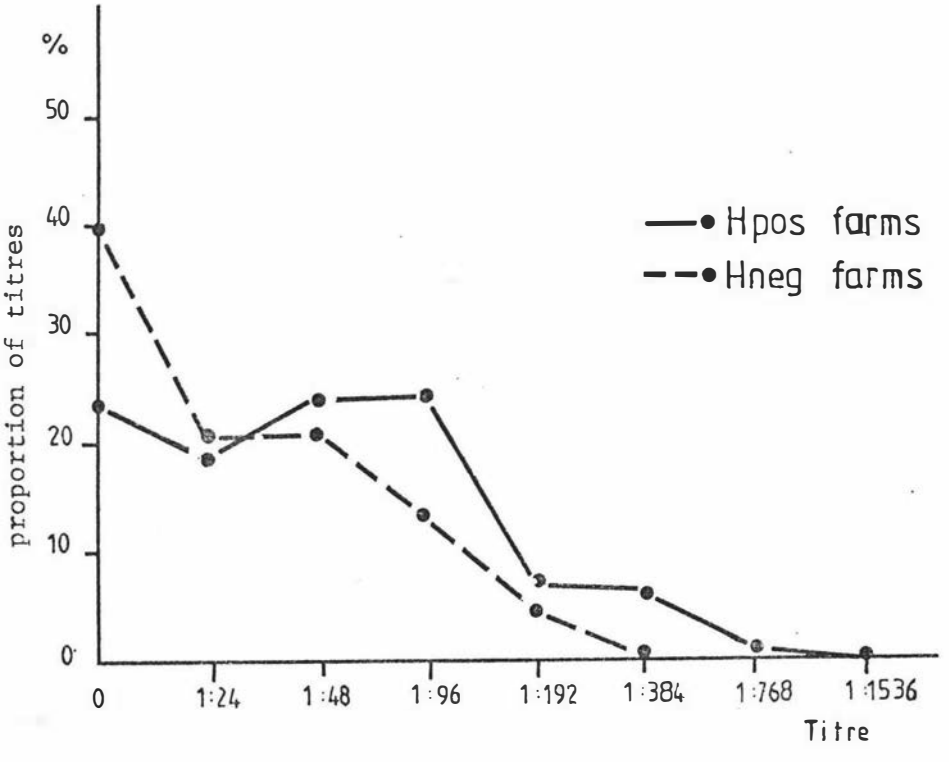


FIG.5.3 : DISTRIBUTION OF TITRES TO *HARDJO* IN CATTLE ON HPOS AND HNEG FARMS.



strong trends towards such associations and included the prevalence of *pomona* titres in cattle (P = 0.16), the herd size (P = 0.07), the number of beef cattle including dairy beef (P = 0.07) and the number of breeding sows on the farm (P = 0.13) (see Table 5.14). Cross-tabulation showed strong trends towards associations between Ppos farms and the buying in of calves (P = 0.09) and the home killing of pigs (P = 0.13). It should be noted that the number of Ppos farms was small and two of the ten farms had recently experienced an outbreak of *pomona* infection in their dairy beef calves. These two cases were largely responsible for the associations between Ppos and the buying in of calves and the number of beef cattle kept.

Factors associated with titres in cattle

(a) Hardjo titres : There was a significant association between the prevalence and the GMT of the titres to *hardjo* ($P<0.01$) and a strong trend towards an association between the prevalence of titres to *hardjo* and the buying in of bulls (P = 0.06).

(b) Pomona titres : In herds with a 10% or greater prevalence of titres to *pomona* more calves ($P<0.05$) and cows ($P<0.05$) were bought in and there was a trend towards an association with the keeping of beef cattle (P = 0.14). These trends were probably associated with the buying in of calves for dairy beef production.

Factors associated with farm type

There were no significant differences between town supply and factory supply farms regarding the prevalence or GMT of titres to *hardjo*. The average herd size of town supply farms (106.8) was smaller than that of factory supply farms (132.1) although this difference was not significant at the 5% level (one-tailed t-test P = 0.0825).

TABLE 5.14 : DIFFERENCE IN A NUMBER OF FARM VARIABLES BETWEEN PPOS AND PNEG FARMS

Variable	Ppos farms (n=10) (%)	Pneg farms (n=42) (%)	Significance (P=) 2-tail	1-tail
Average prevalence bovine titres $\geqslant$1:24 to *pomona*	12	3.6	0.32	0.16
Average herd size (No. of cows)	161	113.8	0.14	0.07
Average No. of beef cattle	62.7	17.0	0.14	0.07
Average No. of breeding pigs	5.8	0.88	0.26	0.13

Factors associated with seropositive dogs

(a) Dogs with titres to hardjo : Dogs with titres to *hardjo* were associated with Hpos farms ($P<0.01$), with farms which had a serological prevalence of titres to *hardjo* of 40% or greater in their cattle ($P<0.05$) and with farms where the herd size was above average ($P<0.05$).

(b) Dogs with titres to pomona : Dogs with titres to *pomona* were not significantly associated with any farm variables but only a small number of animals was involved.

DISCUSSION

The results of these surveys demonstrate a high prevalence of leptospiral titres in dairy farm residents and highlight some of the risk factors that might be causally associated with *hardjo* and *pomona* infections in this occupational group.

The ratio of *hardjo* to *pomona* titres in milkers was nearly 2:1 which is similar to that found by Christmas *et al* (1974a) and Brockie (1976) in surveys of clinical cases of human leptospirosis. There was no significant difference in the clinical histories of disease caused by these two serovars , a finding previously reported by Christmas *et al* (1974a). Therefore, it was assumed that the overall prevalence of milkers with a history of clinical leptospirosis was not biased towards infection with either serovar. In addition, the serological prevalences of serovars other than *hardjo* and *pomona* were relatively low and were unlikely to contribute significantly to the incidence of clinical leptospirosis in dairy farm workers.

An extrapolation of the serological prevalence from this survey to the total population of dairy farm workers in the Manawatu, according to the

relative proportions of town supply and factory supply farms, suggests that approximately 344 (expected range 266 to 431) of an estimated 950 milkers would have a leptospiral titre of 1:24 or greater to one or more serovars. Of these, it could be expected that 104 (81 to 131) milkers would have had a history of clinically diagnosed leptospirosis, of whom 52 (40 to 65) would have been affected in the last five years. It is further estimated that approximately 26 (20 to 37) of this latter group would have been serologically confirmed. However, in the five years prior to the survey only 20 cases of clinical leptospirosis in the survey area were reported to the Department of Health. There is a large difference between the estimated 52 clinical cases and 20 reported cases although this latter figure is similar to the estimated number of serologically confirmed cases. These discrepancies are most likely to be due to under-reporting and incorrect diagnoses by medical practitioners.

These results indicate that approximately two-thirds of those who were serologically positive were either subclinically or mildly affected, or their leptospirosis was not diagnosed. A similarly high proportion of subclinical and/or misdiagnosed cases was reported by Blackmore *et al* (1979) in a serological survey of leptospirosis in New Zealand meat inspectors.

When analysing the data from both surveys it was necessary to assume that the information on the farm and person variables existing when the survey was conducted had remained constant for some time. It is possible that some seropositive farmers may have contracted leptospirosis on other properties and that some control farmers may have been infected in the past with a particular serovar and that their titre had decayed to less than 1:24 by the time of the survey. However, it was not possible to control these

possible errors which, if random, would not have biased the results but would have reduced the strength of possible associations.

All the analyses were based on serological data and not on clinical information and therefore it was necessary to assume that the presence of a leptospiral titre of 1:24 or greater indicated past infection with that serovar and that the risk of contracting infection was the same as contracting clinical leptospirosis.

As leptospirosis is a classical anthropozoonosis, human infection must be contracted by direct or indirect contact with infected animals. However, the epidemiology of *hardjo* is quite different to that of *pomona* on dairy farms (see Chapter One). *Hardjo* is maintained endemically in cattle populations while *pomona* causes outbreaks of infection in cattle but is maintained in pig populations. Therefore it is important to discuss the results and analyses of related risk factors for these two serovars separately.

Hardjo : The results of the case-control study confirmed the hypothesis that the risk to the milker of contracting *hardjo* infection was greater on farms where there was a high prevalence of titres to *hardjo* in the cattle and especially where there was evidence of an endemic cycle of infection in the milking herd. This appeared to be particularly so on factory supply farms where the prevalence of titres to *hardjo* in the case-study cattle was significantly greater than in the factory supply control cattle, and the higher GMT and prevalence of titres of 1:192 or greater indicated that there was an endemic cycle of infection in the herd, predominantly in the two to three year olds. The differences in the prevalence of titres to *hardjo* and the GMT between town supply case-study and control farms were

not as marked. Hellstrom (1978) suggested that it is easier for *hardjo* to remain endemic on town supply than factory supply farms because on town supply farms there is a constant mixing of susceptible stock with infected cattle. On factory supply farms the endemic cycle relies on a small proportion of the herd carrying infection from one season to the next, and a greater herd size may increase the opportunity for this to occur by increasing the number of leptospiruric and susceptible animals. This latter conjecture is supported by the finding that the prevalence was significantly correlated with herd size on factory supply but not town supply farms.

Although the overall prevalence of titres to *hardjo* was lower on the town supply Hpos farms than the factory supply Hpos farms, the milkers on TS farms were exposed to cattle throughout the year compared with only nine to ten months of the year in the case of factory supply milkers. If *hardjo* was cycling endemically in the milking herds the frequent introductions of animals into the town supply herds throughout the year may have resulted in a more constant exposure to the milkers, whereas on factory supply farms, the majority of susceptible animals probably became infected soon after introduction into the herd in the spring and the farmers were maximally exposed at that time.

The age-specific bovine serological prevalences on the factory supply control farms, showed two patterns of titres in the two year old cows. Either there was a high prevalence of titres with a low GMT, indicating that there was an endemic cycle of infection in the young replacement cattle rather than in the herd, or there was an overall low serological prevalence indicating that *hardjo* was not endemic on those farms or was endemic at a low level and was not infecting all the susceptible two year olds in their first season in the herd. Hellstrom (1980) reported similar patterns of

infection in a number of herds in the Taranaki district. Some of the herds with a low serological prevalence of Hebdomadis titres may have experienced sporadic infections with *balcanica* as described in Chapter Eight.

Leptospirosis has been recognised as an occupational disease of dairy farmers in New Zealand for many years, and on a dairy farm milking is the activity most associated with frequent close contact with dairy cattle and exposure to urine. Thus it was not surprising to find that only those people actively engaged in milking cows had leptospiral titres. The prevalence of titres to *hardjo* in full time, part time and non milkers were 26.6%, 6.3% and zero % respectively ($P<0.05$). Because non milkers were all serologically negative they were not included in analyses with other farm variables.

The amount of time spent in the shed at each milking was positively correlated with titres to *hardjo* in the milkers. Brockie (1976) also demonstrated a similar relationship between the time spent in the shed each day and the incidence of human clinical leptospirosis. It is reasonable to conclude that the longer the milker is exposed to infected cows the greater the risk of contracting leptospirosis.

It is surprising to find no serological evidence of leptospirosis in women milkers compared to 38.2% in male milkers, despite the fact that 16 of the 20 women surveyed were full time milkers. In a study of dairy farm associated leptospirosis in the Hamilton Health District, Penniket (1977) also found a low incidence of clinical leptospirosis in women. Of the 90 notified cases only 10 (11%) were women, although women comprised 30% of the case control negative group. Penniket suggested that women take more care with personal hygiene and are less likely to have open cuts and abrasions

on arms and hands than men. He also suggested that women are more likely to wear gloves while milking. However, in the present survey only eight milkers wore gloves and only one of these was a women. None of the glove-wearers were *hardjo* positive, but one was *pomona* positive and he also kept pigs for sale (see below).

The analysis of the prevalence of titres to *hardjo* in milkers, according to the length of time that they have been milking shows that *hardjo* has been responsible for the majority of clinical cases in the last ten years. There is a rise in the prevalence of titres to *hardjo* as the length of milking experience increases up to ten years, and the prevalence then remains at this level. Blackmore *et al* (1979) also found a similar plateau in the serological prevalence of leptospiral titres in meat inspectors who had been working for ten or more years. These authors suggested that leptospiral titres in humans last for approximately ten years and if the rate of exposure is relatively constant, then the serological prevalence should plateau after ten years exposure, as the number of new cases should approximately balance the number of previously infected cases which have lost their titre. Eventually, the prevalence in the more experienced group would tend to decline as the proportion of susceptible people in that cohort declined. There is evidence to suggest that, in cattle, reinfection with the same serovar rarely if ever occurs (Hellstrom, 1978). However, cattle rarely survive longer than ten years.

The initial survey also investigated some of the risk factors associated with milking. The most obvious is the likelihood of an increased risk associated with working from a pit. This association was first suggested by Philip and Tennent (1966) and was supported by a Health Department report (Anon, 1969). Christmas *et al* (1974 a) also found a correlation between

herringbone sheds and reported human cases, although the effects of herd size were not taken into consideration. However, Penniket (1977) found no correlation between shed type and clinical cases of leptospirosis. In the present survey although the serological prevalence was much lower in walkthrough sheds (13.8% compared with 26.8% for herringbone sheds), the difference was not statistically significant because of the small number involved (only 15% of the milkers in the survey worked in walkthrough sheds). This indicates the increased popularity of herringbone sheds since the 1971 survey by Christmas *et al* (1974 a). Apart from the obvious difference between working from a pit and working on the same level as the cow,the trend towards the use of herringbone sheds has usually been associated with increased herd sizes. As detailed in Chapter Four this change has been associated with an increase in the number of cows milked by each man, and possibly a longer time spent in the shed at each milking. Both of these factors may have contributed to the increased exposure of milkers to leptospirosis.

The association between milkers with titres to *hardjo* and the wearing of "protective" clothing is somewhat enigmatic. The wearing of aprons, which was positively correlated with titres to *hardjo*, appears to offer no protection from infection. However,this association was confounded by herd size and shed type, because milkers in herringbone sheds milking larger herds more frequently wore aprons.

The wearing of shorts also appeared to constitute a risk factor and over two-thirds of milkers wore shorts at some time during the year. The increased risk is probably associated with the increased exposure of cuts and abrasions on the legs to urine splash. Also, the milker who wears shorts in the shed is likely to wear shorts at other times and is, therefore, more likely to have scratches and abrasions on the legs.

Pomona : In the initial survey nearly a third of the seropositive farmers had titres to *pomona* and in the case-control study the ratio of Ppos to Hpos farmers was 1:2. However, the seroprevalence of titres to *pomona* in the 520 cattle tested was only 5.2% compared with 66.3% which had titres to *hardjo*. The initial survey showed that, as was the case with *hardjo*, there was a significant association between dairy farm residents with titres to *pomona* and the milking of cows. However, the time spent in the shed at each milking was not correlated with Ppos farmers suggesting that some other activity, such as the keeping of pigs, also exposed them to *pomona*.

The pig is recognised as the reservoir or maintenance host for *pomona* in New Zealand and can excrete the organisms in the urine for over a year (Ryan, 1978). The importance of pigs in transmitting *pomona* to man and cattle was recognised by Kirschner *et al* (1952). Calves that became infected with *pomona* may die from "redwater" or haemolytic anaemia, while a proportion of pregnant cattle infected in the third trimester of pregnancy will abort. However, many adult cattle do not show any clinical signs of infection and may be leptospiruric for up to three months (Hellstrom, 1978). In the initial survey there was a significant correlation between farmers with *pomona* titres and the keeping of pigs for sale on their farms. The keeping of pigs for home consumption did not constitute a risk factor, probably because pigs for this purpose are usually bought in small numbers at about 8 to 12 weeks of age while still protected by maternal antibody and are thus free from infection. The keeping of pigs for sale usually involves keeping a much larger number of pigs, including a herd of breeding sows and it has been shown that *pomona* is present in over 50% of commercial pig breeding herds in New Zealand (Ryan, 1978). Traditionally, pigs were

kept on factory supply farms and fed skim milk while the cream was sent to the factory. There has been a transition through the 1960s to whole milk collection from factory supply farms and a consequent decline in this type of pig keeping (see Chapter Four). However, this survey showed that factory supply farmers had a higher prevalence of *pomona* titres than town supply farmers and that pigs were still more commonly kept for sale on factory supply farms than on town supply farms.

The positive correlation between the amount of dairy farming experience and farmers with *pomona* titres and the similarity to the correlation between dairy farming experience and pig keeping is further evidence of the pig/*pomona* association. The more experienced farmer is more likely to have kept pigs in the 1950s and 1960s and is probably more likely to keep pigs now or to have kept them until recently. Human leptospiral titres of 1:24 or greater may persist for up to ten years or more (Blackmore *et al*, 1979) and therefore *pomona* titres in these more experienced farmers may reflect both past and present pig association. The very low prevalence of *pomona* in the group of farmers with less than 10 years experience supports the contention that the relative importance of *pomona* on the modern dairy farm is decreasing (see Chapter Four). The association between pigs and human *pomona* infection is supported by the results of a survey of New Zealand meat workers and inspectors in whom *pomona* titres were significantly correlated with pig killing and inspection (Blackmore and Schollum,1980).

In the case-control study there were no significant associations between farmers with titres to *pomona* and farm variables, although a number showed strong trends towards associations and included the prevalence of *pomona* titres in the herd, the size of the herd, the number of beef cattle

and breeding sows on the farm, the buying in of calves and the home-killing of pigs. These factors support the argument that *pomona* infection is not endemic in the cattle but that sporadic epidemics occur when *pomona* is introduced to the herd, either by buying infected cattle, or by their exposure to water or pasture contaminated by infected pig urine. Two of the ten case-study farmers with titres to *pomona* had recently bought in dairy beef calves from a wide variety of sources and experienced outbreaks of *pomona* infection in their calves. Infection had then apparently spread to the milking herd. These two farmers may have contracted *pomona* infection either whilst handling infected calves or milking infected cows.

These surveys highlight some of the differences between the epidemiology of *hardjo* and *pomona* infections in dairy farm residents. The titres to other serovars found in these people are probably of little significance. The *copenhageni* titres found in four farmers all occurred concurrently with *pomona* titres which were at least one dilution higher and were considered to be cross-reactions. The *ballum* titre of 1:192 occurred concurrently with a *hardjo* titre of 1:96 and a *pomona* titre of 1:48 and these titres probably reflect past infections with all three serovars. *Ballum* infection in man is most likely to have resulted from direct or indirect contact with rats, mice or hedgehogs which have been shown to carry this serovar in N.Z. (Brockie, 1977; Brockie and Till, 1977; Hathaway, 1978), although sporadic infections of cattle have been recorded (Ris *et al*, 1973).

Dogs on dairy farms appear to be susceptible to leptospiral infections but are probably accidental "dead end" hosts, and as discussed in Chapter Six, are probably not significantly involved in the epidemiology of *hardjo* or *pomona* on dairy farms.

The results of these two surveys suggest that the person on a dairy farm at greatest risk is a male full time milker, who has been farming for about five years, works in a herringbone shed, wears shorts and an apron, milks a large herd, keeps a breeding herd of pigs and buys in calves. The greatest risks to the farmer of contracting *hardjo* infection are associated with milking cows and, although the covering of cuts and abrasions on the hands, arms and face and the avoidance of urine splash to the face may reduce these risks, the only complete solution is to eliminate *hardjo* infection from the dairy herd. Similarly, the complete control of *pomona* infection in farmers depends on the elimination of *pomona* infection from pigs and cattle.

SUMMARY

1. A serological survey of 213 randomly chosen dairy farm residents in the Manawatu showed that 66 (34%) of the people who milked cows had leptospiral titres $\geq$1:24.

2. Of these 66 people, 48 had titres to *hardjo* and 29 had titres to *pomona*. Dual *hardjo/pomona* titres occurred in 12 people. *Ballum* and *copenhageni* titres accounted for 8.4% of titres and the majority of these were probably due to cross-reactions. No *tarassovi* titres were detected.

3. Twenty farm residents who did not milk cows and all 20 women milkers, both full time and part time, were serologically negative.

4. A third of the seropositive milkers had a history of clinical leptospirosis.

5. Factors which were significantly correlated with leptospiral titres in milkers included the time spent in the dairy shed during milking, the wearing of shorts, the keeping of pigs for sale and the number of years the individual had been working on a dairy farm.

6. The type of shed and the size of the herd were interrelated and both showed strong trends toward a correlation with the serological prevalences of titres in milkers.

7. There was no significant difference between the prevalence of leptospiral titres in town supply and factory supply dairy farm workers.

8. The results of the cross-sectional survey indicate that human *hardjo* infection was acquired predominantly from dairy cows in the milking shed, while *pomona* infections were contracted both inside and outside the milking shed and were associated with keeping of pigs for sale.

9. A case-control followup survey of the herd prevalence of leptospiral titres was conducted on 52 of the 136 farms in the cross-sectional survey. These were selected on the basis of the farmer's leptospiral titre. Twenty-five "high risk" farms where the farmer's titre was $\geq$ 1:96 indicating recent infection, were compared to 27 "low risk" farms where the farmer was serologically negative.

10. The cattle in all 52 herds had at least a 10% prevalence of titres $\geq$ 1:24 to *hardjo*, and the mean prevalence was 66.35%. Twenty-two percent of herds had at least a 10% prevalence of titres to *pomona* and, 5.2% of all the cows had a titre to *pomona*.

11. There was a significant association between "high risk" *hardjo* case-study farms and the prevalence of titres $\geq$ 1:192 to *hardjo* in the cattle indicating the presence of an endemic cycle of infection in the herd. Age specific prevalence rates showed that *hardjo* was cycling in the two to three year old cows in the "high risk" herds. On "low risk" farms there was either a low serological prevalence of titres $\geq$ 1:24 in the two to three year old cows indicating an absence or a low level of active infection in the herd, or the two to three year olds had a high prevalence of low titres indicating that the young stock had become infected with *hardjo* before entering the herd.

12. Herd size was significantly associated with an increased risk to milkers on factory supply but not town supply farms.

13. The serological results from the cattle samples support the hypothesis that endemic *hardjo* infection can be maintained more easily on town supply farms, where there is more frequent mixing of livestock, and that larger herds on factory supply farms increase the likelihood that *hardjo* will be maintained endemically in the herd.

14. "High risk" *pomona* case-study farms showed trends towards associations with a high herd prevalence of titres to *pomona*, increased herd size, the number of breeding sows and the buying in of dairy beef calves.

15. Farm dogs had a 45% serological prevalence of leptospiral titres $\geq$ 1:24, with *hardjo*, *ballum* and *pomona* titres occurring most frequently. Their possible role in the epidemiology of leptospiral infections on dairy farms is discussed in Chapter Six.

CHAPTER SIX

LEPTOSPIROSIS IN DOGS IN NEW ZEALAND

INTRODUCTION

Isolations of leptospires from dogs have been recorded in most countries of the world (Anon, 1966; Anon, 1975e) and include serovars from all the leptospiral serogroups. The serovars most commonly isolated are *canicola, icterohaemorrhagiae, copenhageni, pomona* and *bataviae,* and of these *canicola* is probably of greatest public health importance (Stoenner, 1976). It is generally accepted (Broom and Joshua, 1949; van der Hoeden, 1955; Stoenner, 1976; Willcox, 1976) that the dog is the most important maintenance host for *canicola* in the U.K., Europe and the U.S.A. The ubiquitous *Rattus norvegicus* is the maintenance host for *copenhageni* and *icterohaemorrhagiae* and dogs often become infected with these serovars by contact with infected urine from rats (Alston and Broom, 1958). Infected dogs have been shown to shed *icterohaemorrhagiae* in their urine for one to two months (Monlux, 1948) and dog-to-dog transmission occurs especially where the population density of dogs is high, such as in kennels, and epidemics may result (Corbould, 1968; Michna, 1970; Watson *et al,* 1976a).

New Zealand appears to be one of the few countries in which *canicola* is not endemic in the dog population. The only report of *canicola* occurring in dogs was by Kirschner and Gray (1951) who found three dogs in the Dunedin area with titres to *canicola*. A number of small surveys of dogs (Salisbury, 1954; Hartley, 1956; Anon, 1968b)and surveys of wildlife (Hathaway, 1978), cattle (Hellstrom, 1978) and pigs (Ryan, 1978) have failed to show any serological evidence of *canicola* infection. One human case of "canicola fever" was diagnosed on serological grounds in the early 1950s (West and Whitehead, 1953). However it is possible that the *canicola* titres

in this human case and in the dogs reported by Kirschner and Gray (1951) were paradoxical cross reactions following infection with *copenhageni*. The latter serovar has been isolated from rats in N.Z. and appears to be restricted to the Auckland and Waikato districts in the North Island and the Dunedin area in the South Island (Kirschner and Gray, 1951; Durfee,1976; Hathaway,1978; Carter and Cordes, 1980; Hathaway and Blackmore,pers.comm.). There have been only two reports of infections of dogs with *copenhageni* in the Auckland area (Anon,1978a;Mackintosh *et al*, 1980a)and the investigation of one of these outbreaks will be discussed in this Chapter.

Pomona is the only serovar for which there is direct or indirect evidence of infection occurring commonly in dogs in this country. It was first isolated by Te Punga and Bishop (1953) from farm dogs on a property on which there had been an outbreak of *pomona* abortions in cattle. Salisbury (1954) reported that of 63 dog sera received from the lower North Island, seven had titres to *pomona*. In serological surveys of dogs in Australia (Forbes and Lawrence, 1952; Spradbrow, 1962; Watson *et al*, 1976b) *pomona* titres have been detected commonly, especially in rural dogs. In the U.S.A. *pomona* has also been isolated from rural dogs in association with outbreaks of *pomona* infection in cattle and pigs (Alexander *et al* , 1957; Murphy *et al*, 1958; Morter *et al*,1959). All these infections of dogs have been subclinical or inapparent and experimental inoculations of dogs with *pomona* (Murphy *et al*,1958; Cholvin *et al*,1959; Menges *et al*,1960) also produced subclinical infections but with a leptospiruria of 15 to 315 days duration.

Prior to this present study the only other serovar for which titres have been reported in the dog in N.Z. was *hardjo* (Anon,1972). These

titres to *hardjo*, of 1:200 and 1:400, occurred in two farm dogs owned by a farmer who had leptospirosis. The epidemiological significance of leptospiral infections of farm dogs in N.Z. has not been previously studied. This chapter describes an investigation of an outbreak of clinical leptospirosis in a pack of hounds and the experimental infection of dogs with *tarassovi*. An assessment of the role of the dog in the epidemiology of leptospirosis on dairy farms is also made.

PART A : AN INVESTIGATION OF AN OUTBREAK OF LEPTOSPIROSIS IN A GROUP OF HOUNDS

History

A hunt pack of 38 hounds was kept on a small property bounded by a tidal estuary on one side and a road on the other, in the South Auckland district. The hounds were from one to 13 years old with more than half under five years. They were continuously kept together as a pack except for bitches on heat and three breeding bitches. These breeding bitches whelped between October and November and they and their pups were kept separate from the pack until July. The pack was hunted twice a week from April to mid-August. Throughout the year the hounds were let out of their enclosure each morning and fed in an adjoining field. In the summer they were sometimes taken to the estuary for a swim. The hounds were under the supervision of the huntsman whenever they were released from their enclosure.

The hounds' enclosure was in two sections, one of which was a concrete floored area of 400 square feet surrounded by a brick wall and partially roofed over, and the other was a 1600 square feet area of open ground surrounded by a wire fence.

The hounds were fed on cow and horse meat from animals which were either killed at the kennels or had recently died from non-infectious conditions. All the meat including the kidneys was fed raw until the time of the study. The rest of the offal from the animals used for food was incinerated.

The pack never hunted with any other packs, but individual hounds from other kennels, especially bitches, came to the establishment to be mated. In late March 1978, about six weeks prior to the first clinical case of leptospirosis in the pack, the hunt held a hound show on the property and 15 of the hounds were shown, together with hounds from other packs.

Rat infestation had been a problem in previous years at this kennel, especially in the killing shed and food preparation areas. Poison had been laid regularly and rats had not been observed on the property in the four months prior to the first clinical case of leptospirosis.

The first clinical case of leptospirosis occurred on 23 May 1978, designated day 0. Target, a three year old male hound, showed signs of severe depression, inappetance and jaundice and was treated by the attending veterinarian with intravenous fluids and streptomycin injections. This animal recovered fully after two to three weeks. The pack was bled on day 6 and serological examinations were carried out in this laboratory. Target had titres of 1:3072 to *copenhageni*, 1:384 to *canicola*, 1:384 to *ballum* and 1:96 to *pomona*. Three other hounds had titres of 1:48 to *copenhageni*, two had titres of 1:192 and 1:96 to *hardjo*, and two had titres of 1:96 and 1:48 to *pomona*. No hounds had titres to *tarassovi*. It was assumed that Target's infection was due to *copenhageni*, that it had been an

isolated case, and that five days of treatment with streptomycin would have prevented any leptospiruria. All the other low titres in the hounds were thought to represent evidence of previous infections and these animals were unlikely to be leptospiruric. For the next six weeks all the hounds hunted twice a week and no further clinical cases of disease were detected. In July and August, 45 to 70 days after Target was affected, three more hounds, aged one, four and five years, showed similar clinical signs and, despite treatment, two died. A post mortem examination of one of the hounds showed extensive jaundice, hepatitis, nephritis and pneumonia. Histological examinations showed severe acute glomerulonephritis with haemorrhage and interstitial infiltration by mononuclear cells. A few leptospires were seen, in silver-stained sections, in the kidney cortex. Liver sections showed swollen and vacuolated hepatocytes, and vaso-congestion. Lung tissue showed signs of haemorrhagic interstitial pneumonia. All these features were consistent with a diagnosis of acute leptospirosis due to an Icterohaemorrhagiae serogroup organism. The other hound that died had a titre of 1:384 to *copenhageni* shortly before death, but circumstances did not allow a necropsy. The one year old hound which recovered had a maximum titre of 1:768 to *copenhageni*. The hounds ceased hunting at the end of August. On day 105 all the hounds were bled again and vaccinated with a commercial *pomona/icterohaemorrhagiae* vaccine*. Serological tests showed that 26 of the 36 hounds had titres to *copenhageni*, including three of 1:768 and five of 1:384. Target's titre had dropped to 1:48 to *copenhageni* and 1:24 to *canicola*, 106 days after being clinically ill. At this test one hound also had a titre of 1:24 to *tarassovi*. On day 130, four weeks after the second bleeding, the kennels were visited in an attempt to isolate the causal agent of the outbreak and make a definitive diagnosis. Urine samples were collected from 12 hounds, six by bladder paracentesis and six by collecting

* Leptovax - I.C.I. Tasman Ltd.

voided urine. These urine samples were cultured within 60 minutes of collection by the method described in Chapter Three. All cultures of the six voided urine samples were grossly contaminated. However, leptospires were isolated from four of the six urine samples collected by bladder paracentesis and these were initially assumed to be *copenhageni*. During subsequent subculturing one isolate died out and could not be recovered. The remaining three were shown to belong to the Tarassovi serogroup by testing them against known antisera. They were subsequently typed as *Leptospira interrogans* serovar *tarassovi* by C.D.C.*, Atlanta, Georgia. More recently they have been shown, by the use of the restriction endonuclease technique (Marshall *et al*, 1981), to give DNA fragment patterns identical to those of porcine isolates of *tarassovi* from this country (Robinson *et al*, in press).

No further clinical cases of leptospirosis occurred in the hounds. They were all bled again two months later on day 185 and 18 urine samples were collected by bladder paracentesis. No further dogs with titres to *copenhageni* were identified but seven hounds were shown to have titres to *tarassovi*. However, two of the hounds, from which isolates had been obtained two months previously, had only a trace of agglutination to *tarassovi* at a serum dilution of 1:24. One new isolate of *tarassovi* was obtained from an animal with a titre of 1:24 to that serovar. Two months later, on day 246, the pack was bled for the last time and urine samples were collected from 23 hounds. No further titres to *tarassovi* were identified and only four of the seven hounds which had been seropositive previously still had titres to this serovar. A *tarassovi* isolate was obtained from the urine of one of the four hounds which had been leptospiruric four months previously. This animal, which still had a titre of 1:24, was treated with streptomycin for

* Center for Disease Control.

three days. Unfortunately it was not possible to revisit the property to determine whether or not the treatment was successful in eliminating kidney infection.

Throughout the outbreak the two bitches with pups, which were in an isolated compound, remained seronegative to all serovars.

Discussion

There appeared to have been two epidemics of leptospiral infection in this pack during the nine month period of study. The first was due to an Icterohaemorrhagiae serogroup organism assumed to be *copenhageni* and the second was due to *tarassovi*. The original outbreak which clinically affected four hounds, two of which died, eventually infected 26 of the 38 hounds before the pack was vaccinated. This outbreak is similar to one described by Watson *et al* (1976a) in kennelled greyhounds in Australia in which one adult and six pups were clinically affected. The adult and three pups died, and 26 of the 143 dogs in the kennel had titres of 1:100 or greater to *copenhageni*. Those authors attribute the origin of the outbreak to the presence of rats, known to be infected with *copenhageni*, in a neighbouring piggery, together with a high population density of dogs running in close contact in damp environmental conditions. In the present study similar conditions existed and the hounds exhibited a considerable amount of urine sniffing and licking of vulvas. It is assumed that the source of *copenhageni* was infected rats coming into the hunt kennels, although it is possible that infection could have been contracted from other infected hounds at the hunt show. *Copenhageni* has also been isolated from cattle in the South Auckland area (Dodd and Brakenridge,1960) and prior to the outbreak, raw kidneys from cattle were fed to the hounds. The hounds may also have been exposed to other sources of infection outside the kennels during the course of a hunt. It was assumed that once *copenhageni* had been

introduced into the pack subsequent transmission was by direct contact and that the high population density of hounds facilitated a propogating epidemic.

The outbreak of *tarassovi* infection is unlikely to have been detected if the clinical cases of *copenhageni* infection had not occurred. All infections with *tarassovi* were clinically inapparent, as were 80% of the *copenhageni* infections, and produced remarkably low serological responses. No titres to *tarassovi* over 1:48 were detected and two hounds which were leptospiruric had no detectable titres. Leptospiruria was detected in five hounds and one of these hounds was leptospiruric for at least four months. Infection was detected in only ten of the hounds, although it is possible that other hounds may have been infected but did not have detectable titres or leptospiruria at the time of the tests. The apparent failure of *tarassovi* to infect more than a third of the pack suggests that dog-to-dog transmission did not occur as readily with this serovar as occurred with *copenhageni*.

The source of the *tarassovi* infection was most likely to have been infected pigs or cattle. Although no porcine kidneys were fed to the hounds, pigs are considered to be the maintenance hosts for this serovar in New Zealand (Ryan, 1978) and the hounds may have been exposed to infected pig effluent during the course of a hunt. Farina *et al*(1965),in Italy, detected titres to *tarassovi* in a number of dogs living near infected pigs. Alternatively, infected bovine kidneys may have been fed to the hounds. Although there have been no reported isolations of *tarassovi* from cattle in N.Z., Hellstrom (1978) found that 9% of cattle had titres to this serovar.

PART B : EXPERIMENTAL INFECTION OF DOGS WITH *TARASSOVI*

Introduction

In attempting to investigate the epidemic of *tarassovi* infection in the hounds described in Part A of this chapter, it became apparent that there was no basic information on the response of dogs to infection with this serovar. Furthermore, the isolation of *tarassovi* from dogs was particularly interesting as the epidemiology of this serovar in N.Z. is largely unknown.

Materials and Methods

Five apparently healthy stray adult bitches, ranging in weight from 15 to 30 kg were confirmed as serologically negative to *tarassovi*, *hardjo*, *pomona*, *ballum* and *copenhageni*. They were vaccinated with a live attenuated distemper/hepatitis vaccine*, treated with Task** for enteroparasites and had their vocal cords excised. They were fed on a diet of dog sausage, and housed in five adjoining cages separated by wire mesh. After a ten day settling-in period, during which time they were bled again and found to be still seronegative, they were all inoculated by intraperitoneal injection with 1ml of a four day old subculture of *tarassovi* containing 5.6×10^8 leptospires/ml. This organism had been isolated from a hound two months previously and had undergone three subcultures in liquid medium.

The bitches were observed for clinical signs of disease, their temperatures recorded twice daily for two weeks and then daily for a further

* D H vaccine - Websters

** Task - Shell (ICI Tasman)

six weeks. Blood samples were taken for culture on days three and seven pi. Urine samples for culture were taken eight days prior to the commencement of the experiment, at the time of inoculation, twice weekly for five weeks pi, and then periodically until the experiment was terminated. To facilitate the collection of urine from these bitches an intramuscular injection of Lasix*, a diuretic, was given at a rate of 0.5 ml/15kg body weight, and 30 minutes later they were led out onto a patch of grass where they had been trained to urinate. A 10 to 20 ml midstream sample was collected in a sterile universal bottle and the urine was subcultured within 30 minutes as described in Chapter Three. The urine samples were also examined by dfm, and biochemical test strips** were used to test for protein, blood, haemoglobin, glucose, ketones, nitrite, urobilinogen and bilirubin and to determine the pH. Blood samples were collected on the same day as the urine, and serological tests were performed as described in Chapter Three.

On day 154 pi a young adult male dog (No. 6) which was serologically negative, was introduced into the dog compound and was run alternately with bitches Nos. 2 and 5 in their cages. After running in contact with these bitches for 100 days with negative serological and cultural results, dog No. 6 was isolated for seven days and inoculated intraperitoneally with 5ml of urine, containing at least 10^3 leptospires/ml. This had been collected from the bladder of bitch No. 2 at necropsy. Cultures of tenfold dilutions of this urine were also made. The dog (No. 6) was monitored for evidence of infection in the same way as were the bitches.

A small trial was conducted during the course of this experiment to determine the effect of Lasix on the recovery of leptospires from dog urine.

* Lasix - Frusemide, Hoechst

** Combur-8 test. Boehringer, Mannheim.

On five occasions, paired urine samples were collected, with and without the use of Lasix, from the two bitches, No. 3 and No. 4, both of which had been shown to be leptospiruric 21 days earlier (day 246). The bitches were trained to urinate, without the use of Lasix, when they were exercised on a patch of grass and samples were collected. They then received a 1 ml intramuscular injection of Lasix and were again taken to the grass 30 minutes later when a second sample was collected. The urine samples were cultured within 15 minutes of collection. The pH, osmolarity and specific gravity of the urine samples were measured using a pH meter*, an osmometer** and a refractometer*** respectively.

At the end of the experiment all dogs were destroyed with intravenous pentobarbitone and necropsies conducted. Half of one kidney was removed and cultured as described in Chapter Three. The rest of the kidney was fixed in formalin for histological examination. A sample of urine was aspirated from the bladder by syringe and cultured.

Results

All five bitches became infected, developed titres to *tarassovi* and shed leptospires in their urine. None of them showed any clinical signs of disease except for transient temperature elevations up to 40.2°C which occurred in all five bitches in the first few days pi. By seven days pi all temperatures were within a range of 38.3 to 39.5°C.

By the 7th day pi all five bitches had developed titres to *tarassovi* ranging from 1:48 to 1:192 (Table 6.1). All five had reached a peak titre of 1:768 by day 14 pi. In bitch No. 2 the titre declined to < 1:24 by day 147 pi while the other four remained serologically positive until destroyed

* Triac DPH-1 pH meter - Triac Controls Ltd. P O Box 45-149,Auckland 8,N.Z.
**Advanced Osmometer - Advanced Instruments,Inc. Newton Highlands,Mass.02161,USA
***Atago Urine S.G. - Atago Optical Works Co.Ltd., Japan

TABLE 6.1 : TITRES OF FIVE BITCHES EXPERIMENTALLY INFECTED WITH *TARASSOVI*

Sampling times Days pi	Reciprocal titres to *tarassovi** Bitch Number 1	2	3	4	5
- 10	0	0	0	0	0
0	0	0	0	0	0
3	0	0	0	0	0
7	192	192	192	48	192
10	384	384	384	384	768
14	768	768	768	768	768
17	768	768	768	768	384
21	384	384	768	768	768
28	384	384	384	384	384
35	192	192	192	192	192
42	192	192	192	192	192
49	192	192	192	192	192
56	192	96	96	192	96
70	96	48	96	192	48
84	192	48	96	192	96
105	96	48	48	96	96
119	96	48	48	96	96
147	48	0	48	96	48
175	48	0	48	96	48
203	48	0	48	48	48
220	96	0	48	48	48
232	48 **				
246		0	48	48	48
261		0 **			
265					48 **
295			48 **	48 **	

* all dogs seronegative to *pomona*, *copenhageni*, *ballum* and *hardjo* throughout the experiment.

** Titre at death

between 232 and 295 days pi when all their titres were 1:48.

Cultures of blood collected on day 3 pi yielded *tarassovi* isolates from three of the five bitches. Cultures from the other two were contaminated by other organisms which prevented the detection of leptospires. On day 7 pi all blood cultures were negative (see Table 6.2).

Urine samples cultured prior to the commencement of the experiment, on the day of inoculation and day 3 pi, were all negative. On day 10 pi *tarassovi* was isolated from four out of the five urine samples, while the fifth sample was grossly contaminated. All five bitches were almost continuously leptospiruric until destroyed between days 232 and 295 pi at which time leptospires were isolated from the urine of three of them, and the kidneys of all five.

The male dog (No. 6), introduced to the bitches at day 154 pi, remained serologically and culturally negative for 100 days despite close contact with leptospiruric bitches. This contact involved licking of vulvas and urine tasting of bitches Nos. 2 and 5. The dog was also observed to mate and "tie" with bitch No. 5. Dog No. 6 also failed to become infected after an intraperitoneal injection of 5ml of urine. This urine was negative on d f examination. However, *tarassovi* was cultured from two drops of a 100 fold dilution of this urine, indicating that there were at least 10^3 leptospires per ml.

The results of the trial comparing the cultural success rate and the survival of leptospires in urine collected with and without the use of Lasix are shown in Table 6.3. This trial, conducted between 265 and 276 days pi, indicated that the use of Lasix enhanced the survival of leptospires

TABLE 6.2 : CULTURAL RESULTS OF BITCHES EXPERIMENTALLY INFECTED WITH *TARASSOVI*.

Bitch No. / Days pi	1	2	3	4	5
			Blood culture		
3	+	+	-(c)	+	- (c)
7	-	-	-	-	-
			Urine culture		
-8	-	-	-	-	-
0	-	-	-	-	-
3	-	-	-	-	-
10	+	+	+	+	- (c)
14	+(c)	-(c)	+	+	+
17	+(c)	+	+	+	+
21	+	+	+	+	+
24	+	+	+	+	+
28	+	+	+	+	+
31	+	+	+	+	+
35	+(c)	+	+	+	+
42	+	+	+	+	+
49	-(c)	+	+	+	+
56	+	+	+	+	+
69	+(c)	+	+	-	+
77	+	+	+	+	+
84	+	+	+	+	+
97	+	+	+	+	+
105	-(c)	+	+	-	+
119	+	+	+	+	+
133	-(c)	+	+	+	+
147	+	+	+	+	+
161	-(c)	+	+	+	+
175	+	+	-	+	+
196	+	+	+	+	+
220	ND	+	ND	+	+
225	+	ND	+	+	ND
246		+	+	+	-
265			+	+	+
266			+	-	
269			+	+	
275			+	+	
276			+	+	
295			-	+	
			Kidney culture		
232	+				
261		+			
265					+
295			+	+	

+ = *tarassovi* isolate
- = negative culture
(c) = contaminated culture
ND = not done

TABLE 6.3 : THE EFFECT OF A DIURETIC+ ON THE ISOLATION OF LEPTOSPIRES FROM THE URINE OF EXPERIMENTALLY INFECTED DOGS

Days pi	Dog No.	Urine*	pH	Specific Gravity	Osmolarity (milliosmols)	Culture results <½ hour U**	<½ hour $u_{\bar{10}}$	2 hour U	2 hour $u_{\bar{10}}$	Summary of isolations 3 Naturally voided urine	4 Naturally voided urine	3 Lasix induced urine	4 Lasix induced urine
265	3	NU	6.3	>1.05	ND	-	-	-	-	-			
	4		6.65	1.05	ND	+	-	-	-		+		
	3	L	6.55	1.002	ND	+	+	+	+			+	
	4		6.85	1.001	ND	+	+	+	+				+
266	3	NU	6.0	>1.05	1737	-	-	-	-	-			
	4		5.95	>1.05	1620	-	-	-	-		-		
	3	L	7.35	1.000	225	+	+	+	+			+	
	4		7.4	1.000	289	+	-	+	-				+
269	3	NU	5.9	>1.05	1859	-	-	-	-	-			
	4		6.5	1.045	1530	+	-	-	-		+		
	3	L	6.45	1.005	305	+	+	+	+			+	
	4		6.8	1.002	301	+	+	+	+				+
275	3	NU	6.3	1.041	1440	+	-	-	-	+			
	4		7.0	1.030	1413	+	+	+	-		+		
	3	L	7.1	1.000	231	+	+	+	+			+	
	4		7.25	1.000	220	+	+	+	+				+
276	3	NU	6.25	>1.05	1794	-	-	-	-	-			
	4		5.8	>1.05	1791	-	-	-	-		-		
	3	L	6.7	1.004	396	+	-	+	-			+	
	4		6.95	1.000	269	+	+	+	+				+
										1	3	5	5

+ Lasix - furosemide

* NU = naturally voided urine
  L = urine induced with Lasix

** U = 2 drops of urine cultured
  $u_{\bar{10}}$ = 2 drops of a 10-fold dilution of urine cultured

in urine and their subsequent isolation. Urine samples from No. 3 were culturally positive on only one of the five occasions when Lasix was not used, whereas urine samples collected after the administration of Lasix were positive on all five occasions. Similarly urine samples from bitch No. 4 were positive on three out of five occasions without Lasix, compared with all five occasions with Lasix (Fisher's exact test P = 0.005).

The survival time of leptospires in urine appeared to be increased when Lasix was used. Of the four positive samples collected without Lasix only one was positive after two hours at room temperature, whereas all ten of the Lasix induced urine samples were positive after two hours (Fisher's exact test P = 0.011).

The urine samples collected after the administration of Lasix, all of which yielded isolates, had lower osmolarities and specific gravities and were less acidic than those collected without the use of Lasix, only 40% of which yielded isolates (see Table 6.3). The urine samples collected without Lasix from which isolates were obtained were also less acidic and had lower osmolarities and specific gravities that those from which isolates were not made.

The use of Lasix on two consecutive days appeared to reduce the chance of isolating leptospires from urine on the second day (Fisher's exact test, P = 0.07), although only a small number of samples (ten) were included in this analysis.

At necropsy all six dogs were in good condition and none showed any gross abnormalities except for Nos. 2 and 4, each of which had two pale circumscribed lesions 1mm in diameter in the kidney cortex, and Nos. 1, 4 and 6 which had up to ten lesions of similar size and appearance on the peritoneal

surfaces of the spleen and liver. Histological examinations showed that the inoculated bitches Nos. 1, 3, 4 and 5 had varying degrees of mild focal chronic interstitial nephritis characterised by aggregations of plasma cells and a few macrophages in their renal cortices. No abnormalities were detected in the kidney of bitch No. 2. The white lesions observed in the kidney cortices appeared on histological examination to be parasitic granulomata in that they consisted of a central caseous mass surrounded by a inner zone of large macrophages and an outer zone of lymphocytes, small macrophages and a few eosinophils. The control dog, No. 6, had a localised chronic pyelitis and a few foci of chronic interstitial nephritis. It is possible that these foci were associated with the chronic pyelitis, but they could have been due to a previous leptospiral infection. No leptospires were seen in any of the sections stained by the Warthin and Starry method.

Discussion

All five inoculated dogs became infected with *tarassovi* and their failure to show any clinical signs of disease other than a transient rise in temperature appeared typical of other reports of dogs experimentally infected with *tarassovi* (Farina *et al*,1965) and *pomona* (Murphy *et al*, 1958; Cholvin *et al*,1959; Menges *et al*,1960). However, both natural and experimental infections of dogs with *icterohaemorrhagiae* (Low *et al*,1956; Yoder *et al*,1957; Watson *et al*,1976a) have been shown to result in clinical signs which ranged from mild to severe,with young dogs being more severely affected. Experimental infections with *canicola* were usually inapparent in adult dogs but were often severe in young dogs (Monlux, 1948; Taylor *et al*, 1970). Hubbert and Shotts (1966) investigated a natural outbreak of *canicola* in a kennel of greyhounds where 14 out of 19 dogs were infected and only one five-month old pup was clinically affected and died. In the natural

outbreak of *tarassovi* infections described in Part A no clinical signs of infection were seen in hounds ranging from one to eight years of age, whereas four adult hounds were clinically affected with *copenhageni* in the previous outbreak and two of these hounds died. Natural *pomona* infections in dogs have been reported as inapparent (Te Punga and Bishop, 1953; Morter *et al*, 1959).

The serological response which was first detected seven days pi, reached a maximum of 1:768 in two to three weeks and then declined. By five months pi the titres had fallen to less than 1:24 in one animal, 1:48 in three animals and 1:96 in one. These titres were higher than those found in dogs naturally infected with *tarassovi* (Part A) and may have been due to the large number of organisms with which the dogs were challenged. Farina *et al* (1965) also detected a rapid decline in titres after infecting five dogs with *hyos (tarassovi)*. In their experiment the dogs' titres reached a maximum of between 1:500 and 1:5000 at 21 to 35 days pi and had declined to 1:200 by 63 days pi. At the termination of the experiment two dogs had titres of 1:10 and 1:50 at 93 and 103 days pi respectively, and *tarassovi* was isolated from the kidneys of the latter dog. In the present experiment all the dogs, including the one which became seronegative after five months, continued to shed leptospires in their urine until killed eight to ten months pi when their titres ranged from $<$1:24 to 1:48. Menges *et al* (1960) recorded similar findings in that the titres of four dogs infected with *pomona* had declined to less than 1:16 within six to nine months pi and one of these dogs continued to shed for ten months pi. Hubbert and Shotts (1966) investigated an outbreak of *canicola* infection in greyhounds and recorded maximum titres of 1:800 which had declined to between $<$1:16 and 1:50 after six months. Similarly an experimental infection of four dogs with *canicola*

(Menges *et al*, 1960) recorded maximum titres of 1:512 to 1:1024 which declined to < 1:16 in ten months. In Part A of this chapter, the *copenhageni* titre of Target, declined from a maximum of 1:3072 to 1:24 in seven months after infection. Similarly, the 25 hounds that had been infected in the preceding three months had a GMT of 1:135 and five months later this had declined to 1:41. It can be concluded from these results, that dogs generally do not produce a very great or persistent antibody response to leptospiral infection as measured by the MAT, unlike cattle and pigs which produce high persistent titres to *pomona* and *tarassovi* (Hellstrom, 1978; Ryan, 1978). It also appears that some dogs can still be leptospiruric after their titres have declined to below 1:24. It is interesting to note that none of the serum samples from the dogs infected with *tarassovi* had any titres to other serovars including *pomona, hardjo, copenhageni, ballum, canicola* and *australis*. This lack of cross-reactivity between *tarassovi* and other serovars is quite different to that seen in dogs infected with *copenhageni, canicola* and *pomona* (Cholvin *et al*, 1959; Menges *et al*, 1960). This was also demonstrated in Part A of this chapter.

Although *tarassovi* could still be isolated from all five dogs' kidneys from 225 to 296 days pi it appears that Lasix enhanced the recovery of leptospires from the urine and increased the intensity of shedding. Nervig and Garrett (1979) also found that Lasix enhanced the recovery of *hardjo* from the urine of cattle and facilitated the collection of urine samples. In the present study it is unlikely that the leptospiruria would have been detected as frequently without the use of Lasix. Urine samples collected without Lasix had a lower pH, higher osmolarity and higher specific gravity than Lasix induced urine. The naturally voided urine had usually been accumulating in the bladder for up to 12 hours compared with 30 minutes for the Lasix induced urine. Leptospires did not survive for more than two hours in the normally voided urine and it is probable that only the leptospires

which passed out of the kidneys into the bladder in the two hours prior to micturition were viable at the time of examination. On the other hand, all the leptospires in the Lasix induced urine would have been in the bladder less than 30 minutes. Nervig & Garrett (1979) also suggested that Lasix has a "flushing" effect causing more leptospires to be flushed out of the tubules than normal. This would explain why it was more difficult to culture leptospires from the urine of dogs which had been given Lasix the previous day.

The trial using naturally voided urine and Lasix induced urine demonstrated that no isolations were made from urines with a pH less than 6.3 and a specific gravity-greater than 1.05. Hubbert & Shotts (1966) obtained isolates of *canicola* from canine urine samples which had a pH as low as 5.5 and a specific gravity of 1.045. Thus there is a suggestion that *canicola* is better able to survive in normal canine urine which has a normal pH range of 5.0 to 7.0 and a specific gravity up to 1.06. The degree and duration of leptospiruria and the survival of leptospires in the urine are important factors in determining whether dogs can act as maintenance hosts for a particular serovar. Dogs are usually considered to be maintenance hosts for *canicola* and able to maintain infection within a population by dog-to-dog transmission. *Canicola* usually causes a moderate degree of chronic interstitial nephritis (Michna, 1970) which may cause a reduction in the kidney's ability to concentrate the urine, resulting in a lower osmolarity and specific gravity and a more neutral pH. The very mild changes seen in the dogs in the present experiment did not appear to affect the pH or osmolarity of their urine. Although it was shown that *tarassovi* can remain in the kidney for at least ten months, the low level of leptospiruria and the inability of the organisms to survive in the urine for more than a few hours indicate that the dog is unlikely to be a maintenance host for this serovar.

In the outbreak described in Part A it appears that less than a third of the pack became infected despite the high population density and moist environmental conditions. It also seems unlikely that the degree of leptospiruria of dogs infected with *tarassovi* is as high as that which occurs in the pig, which is considered to be a maintenance host for this serovar (Ryan, 1978).

Dog No. 6, which was a stray obtained from a farming area, appeared to be resistant to infection and it is possible that it had previously been exposed to *tarassovi* and its titre had declined to less than 1:24.

PART C : INVESTIGATION OF LEPTOSPIRAL INFECTIONS OF FARM DOGS ASSOCIATED WITH OUTBREAKS OF *POMONA*.

Introduction

During the three year period of this study a number of epidemics of *pomona* infection in dairy herds were investigated. Two such epidemics are described in Chapter Nine. During the course of these investigations blood and urine samples were collected from a number of animal species, including dogs, living on the affected farms. In three of these cases *pomona* infection was detected in dogs and these cases are the subject of discussion in this section.

Epidemic I : This epidemic of *pomona* abortions in a dairy herd is described in Chapter Nine. The three farm dogs from this farm were found to have titres of 1:192, 1:384 and 1:1536 to *pomona* a week after the 45 day

abortion epidemic had terminated, and one month later *pomona* was isolated from the urine of two of the dogs. All three were then treated with penicillin/streptomycin at the owner's request. The probable source of the outbreak in the cattle was contaminated pig effluent.

Epidemic II : This epidemic of *pomona* infection, in which 11 cows aborted, occurred on a dairy farm and is referred to in the discussion of Chapter Nine. Four dogs lived on the property. Two of these, which were owned by the farmer, were used most of the time for working the cattle and were housed near the dairy sheds. The other two were owned by the farm worker, but were rarely used on the farm and lived well away from the dairy herd. The two dogs owned by the farmer had titres of 1:384 and 1:3072 and *pomona* was cultured from urine samples from both. The other two dogs were sero-negative and culture of the urine was not attempted. Both of the infected dogs were treated with penicillin/streptomycin. The probable source of infection for the cattle was effluent from pigs which had been kept on the farm until shortly before the first abortions were recorded.

Epidemic III : This epidemic resulted in an outbreak of haemoglobinuria with some deaths in a group of approximately 100 dairy beef calves ranging in age from one to four months. *Pomona* was isolated from a urine sample from an infected calf. The two dogs on the property, one a working cattle dog and the other a boxer house dog, had titres to *pomona* of 1:192 and 1:384 respectively. The boxer dog was allowed to roam freely around the farm and cow shed. No urine samples were collected from these dogs for culture but both were treated with penicillin/streptomycin at the owner's request. *Pomona* was probably introduced to the property by one or more infected animals among 80 calves purchased in the previous three months, from a wide area.

Discussion

In the three case-studies presented here, the cattle were most probably infected by exposure to contaminated pig effluent or to infected calves brought onto the property. It is believed that the dogs became infected by exposure to infected cattle urine or foetal membranes from cattle which had aborted. It seems unlikely that the dogs were responsible for initiating the outbreaks although this possibility cannot be ignored. Similar cases of canine *pomona* infections associated with *pomona* epidemics in cattle have been described previously in N.Z. (Te Punga and Bishop,1953) and the U.S.A.(Morter *et al*,1959). If it is assumed that dogs contract infection from cattle then the high rate of infections seen in these dogs indicates that they are probably highly susceptible to infection or that they were exposed to a high level of environmental contamination or both. All four dog urine samples from which *pomona* isolates were obtained were negative on dfm indicating that there were fewer than 10^3 leptospires per ml, in contrast to the high rate of shedding of *pomona* in leptospiruric cattle and pigs infected with this serovar (Doherty, 1967c; Ryan, 1978). In view of the long period of leptospiruria that has been demonstrated previously in dogs experimentally infected with *pomona* (Cholvin *et al*,1959; Menges *et al*, 1960) it was possible that the dogs in this study represented public health hazards and potential vectors of infection to other farm animals. Consequently all the dogs were treated with penicillin/streptomycin injections as previous work had shown that these antibiotics were effective in controlling leptospiruria in dogs (Meyer and Brunner, 1949; Yoder *et al*, 1957; Hubbert and Shotts, 1966).

PART D : SEROLOGICAL SURVEY OF FARM DOGS IN THE MANAWATU.

Introduction

While conducting a serological survey of dairy cattle on a

number of Manawatu dairy farms, as described in Part B of Chapter Five, blood samples were also collected from farm dogs wherever possible. It was hoped that the results from such a survey would provide information on the prevalence of leptospiral titres in dogs working on dairy farms and possible associations with the prevalance of leptospiral titres in humans and cattle on those farms.

Results

A total of 64 dogs from 37 farms were bled and of these dogs 29 (45.3%)had titres to one or more leptospiral serovars. Eighteen dogs (28%) had titres to *hardjo*, 16(25%) to *balcanica*, 6(9%) to *ballum*, 4(6%) to *pomona*, 2(3%)to *copenhageni*, 1(2%)to *tarassovi* and 1(2%) to *canicola*. All dual reactions to *hardjo* and *balcanica* were assumed to be crossreactions and the majority were assumed to be due to infection with *hardjo*. Apart from these crossreactions, five other dogs had titres to two or more serovars as shown below :

hardjo 1:192/*balcanica* 1:48 /*ballum* 1:96

hardjo 1:24 /*balcanica* 1:48 /*tarassovi* 1:48

hardjo 1:24 /*balcanica* 1:24 /*copenhageni*1:24 /*canicola*1:24

balcanica 1:24/*pomona* 1:24

pomona 1:48 /*copenhageni*1:24

The 37 farms chosen for the case-control study described in Chapter Five included 15 farms where the milker had a titre to *hardjo*, seven with a titre to *pomona* and one with a titre to *ballum*, and 18 where the milker was seronegative. Thus the dogs on these farms were not a random sample and were more likely to live on farms which had a higher risk of workers contracting leptospirosis.

A variable number of dogs was sampled on the 37 farms and not all dogs on a given farm had leptospiral titres.

As stated in Chapter Five, there was a positive association between titres to *hardjo* in milkers and their dogs ($P<0.01$). On 60% of farms on which the milker had a titre to *hardjo* one or more dogs also had a titre to *hardjo* compared with only 31.8% of farms where the milker was seronegative. There was a similar trend between *pomona* titres in dogs and milkers but the numbers were too small to show a significant association. There were also trends towards associations between the prevalence of titres to *hardjo* and *pomona* in the dairy cattle and titres to these serovars in dogs ($P = 0.13$ and $P = 0.11$ respectively in one-tailed t-tests).

Discussion

The high serological prevalence of leptospiral titres in dogs appears to reflect the high serological prevalence of leptospirosis in domestic farm animals (Hellstrom, 1978; Ryan, 1978) and wildlife (Hathaway, 1978). On dairy farms the cattle dog is constantly exposed to mud and urine and can frequently be seen drinking from puddles. The associations between titres to *hardjo* and *pomona* in milkers and titres to these serovars in their dogs is unlikely to be a direct association, but probably reflects a common exposure to infected cattle and pigs (see Chapter Five).
Few serological surveys of dogs reported in overseas literature have included farm dogs. However, Watson *et al* (1976b) examined sera from stray dogs in the West Sydney district including some rural dogs. Using 1:100 as a minimum dilution in the MAT they found a serological prevalence of 6.8%. The most common serological reactions were to *copenhageni* (5%), *pomona* (1%) and *hardjo* (0.5%). Michna (1967) found that six out of 23 country

dogs in the U.K. had Hebdomadis serogroup titres of 1:100 or greater. If a titre of 1:100 or greater is used, it is likely to detect only the more recent infections and the true prevalence of previous infection will be underestimated. In natural and experimental infections in dogs with *copenhageni*, *canicola*, *pomona* and *tarassovi* discussed earlier in this Chapter the level of circulating antibody declined rapidly and did not remain at 1:100 or greater for longer than one year, although some authors (Borg-Peterson and Fennestad, 1962) contended that dogs recovering from *canicola* and *icterohaemorrhagiae* infections had titres of up to 1:300 for more than a year and titres of up to 1:100 "may persist for years". *Tarassovi* infections especially have been shown to promote a poor antibody response, and some dogs were leptospiruric while their titres were 1:24 or less (Parts A and B). Therefore, for these reasons a minimum serum dilution of 1:24 was used in this survey and its use is supported by the demonstration of associations between titres to *hardjo* of 1:24 or greater in the dogs, in the milkers and in the cattle. If a titre of 1:100 or greater had been used only three of the 18 dogs with titres of 1:24 or greater would have been considered, thereby underestimating the true prevalence of seropositive dogs.

All the dogs included in this survey were apparently healthy at the time of sampling and none had a history of clinical leptospirosis. This appears to be typical of natural canine infections with *tarassovi* and *pomona* (Parts A and C) and *hardjo* infections are probably also inapparent (Anon,1972). Durfee (1978) infected two dogs with *hardjo* and neither showed any clinical signs of disease.

In this survey the prevalence of titres to *ballum* was higher than that of titres to *pomona* and this presumably reflects greater exposure to

infected rodents, although sporadic *ballum* infections in cattle have been recorded (Ris *et al*, 1973). The single titre to *tarassovi* of 1:48 is probably a true indication of past infection with this serovar as it was as high as the Hebdomadis serogroup titre with which it was concurrent. The two titres to *copenhageni* were both 1:24 and one was concurrent with a *pomona* titre of 1:48 and was probably a crossreaction. Cholvin *et al*(1959) showed that experimental *pomona* infections produced low *copenhageni* crossreactions. The other *copenhageni* titre occurred concurrently with a *canicola* titre of 1:24, a *balcanica* titre of 1:24 and a *hardjo* titre of 1:24 suggesting nonspecific reactivity of the serum. There were no titres to *australis* indicating that it is probably not present in domestic animals or rodents in the Manawatu.

PART E : SEROLOGICAL SURVEY OF WELL KEPT CITY AND TOWN DOGS PREDOMINANTLY FROM THE LOWER NORTH ISLAND.

Introduction

As a comparison with the survey of rural dogs recorded in Part D, a serological survey of well kept city dogs was conducted using sera collected from a wide variety of clinical cases which attended the Massey University Small Animal Clinic over the years 1975-79. None of these dogs was presented as a clinical caseof leptospirosis. Some had been referred to Massey from cities or towns other than Palmerston North.

Results

A total of 51 dog sera were available for the survey but four seronegative samples were excluded because they were from working dogs living on farms. Of the 47 city dogs, four (8%) had leptospiral titres of 1:24 or greater. One was an eight year old male dachshund from Auckland with titres of 1:24 to both *hardjo* and *balcanica*, one was a four year old male Corgi

from a country town, Kaponga in South Taranaki, with a titre of 1:48 to *pomona*, and two were from Wellington. One of these was a four year old male Shetland Collie and the other was a five year old female poodle, and both had titres of 1:24 to *australia*. No titres to *copenhageni*, *canicola*, *tarassovi* or *ballum* were detected in any of the sera.

Discussion

The most outstanding feature of this survey of city dogs compared with similar surveys in the U.K.(Michna, 1967) and the U.S.A.(Alexander *et al*, 1957) is the complete absence of titres to *canicola* and *copenhageni*. Salisbury (1954) also failed to detect any titres to these serovars in a survey of dogs from the lower North Island. These results support the hypothesis that *canicola* is not present in dogs in this country. Watson *et al* (1976b) also failed to detect *canicola* titres in dogs from the Sydney area of Australia and came to a similar conclusion. The dogs in the present survey were predominantly from the lower North Island and the lack of *copenhageni* titres can be explained by the absence of *copenhageni* infections in rodents in this area (Blakelock and Allen, 1956; Hathaway, 1978). The single *pomona* titre was detected in a dog living in a rural town in South Taranaki where it is more likely to have been exposed to infected pig effluent than a dog from a large city. There was no history of exposure of the Dachshund to a farming environment and its Hebdomadis serogroup titre may have been due to infection with either *hardjo* or *balcanica*. The two titres to *australis* in dogs living in the Wellington district are intriguing. Unfortunately there is no information available on the origin of these dogs and they may have been imported from Australia where *australis* is endemic in rodents. However, if they acquired their *australis* titres in the Wellington district or some other district in N.Z. it points to a hitherto undetected reservoir of

australis infection in N.Z. A serological survey of rats from the Wellington district in the mid 1950s (Blakelock and Allen, 1956) failed to show any evidence of *australis* infection, but it may have been introduced subsequently. The only confirmed isolation of *australis* in N.Z. was from a beef cattle farmer suffering from clinical leptospirosis in the North Auckland area in 1978 (Thompson,1980). No reservoir of infection has been reported in this country. An alternative explanation for the low *australis* titres is that they are nonspecific reactions or crossreactions to a serovar other than those used in the test. Recent studies in sheep suggest that these animals have nonspecific serological titres of 1:24 to *australis* (Blackmore, pers.comm.).

The absence of *ballum* titres suggests that well kept city dogs are not exposed to infected rodents to any great extent. If a similar group of stray city dogs had been available for sampling they would have made an interesting comparison as they would perhaps have had more contact with rodents. However, a recent survey of cats showed a similarly low serological prevalence of titres to *ballum* (Shophet, 1979).

GENERAL DISCUSSION

On the basis of criteria suggested by Hathaway (1978) and Blackmore and Hathaway (1980), for the dog to be a maintenance host for a particular serovar, the organism must be of low pathogenicity and high infectivity for dogs, while leptospiruria must be of long duration and infection in a dog population must be maintained by dog-to-dog transmission. It is widely accepted that the dog is a maintenance host for *canicola* (Broom and Joshua, 1949; van der Hoeden,1955; Willcox, 1976). This serovar is of low pathogenicity for adult dogs (Hubbert & Shotts,1966; Taylor *et al*,

1970), infected dogs remain leptospiruric for long periods (Meyer and Brunner, 1949) and infection is maintained in dog populations by dog-to-dog transmission (Broom and Joshua, 1949; Hubbert & Shotts, 1966). Some investigators (Alexander *et al*, 1957; Torten, 1979) have noted that males are two to five times more frequently infected with *canicola* than females, an observation attributed to the male dog's behaviour of sniffing the genital organs of other dogs and their tendency to roam more extensively than females. However, this difference of sex specific attack rates has not been recorded with Icterohaemorrhagiae serogroup infections in dogs, indicating that both sexes are equally exposed to a common source. *Copenhageni* and *icterohaemorrhagiae* are both maintained by *Rattus norvegicus* in most parts of the world (Anon, 1966). These serovars appear to be more pathogenic for dogs than *canicola* (Michna, 1970), and dog-to-dog transmission does not appear to occur readily (Gray, 1942). In the investigations described in Part A, the epidemic spread through the majority of hounds kept in close contact with each other, but no Icterohaemorrhagiae serogroup organisms could be isolated from the hounds subsequent to the epidemic, indicating that long term leptospiruria did not occur. Thus it appears that the dog is not likely to act as a maintenance host for either *copenhageni* or *icterohaemorrhagiae* although sporadic infections may occur. Similarly, *pomona*, *tarassovi*, *ballum* and *hardjo* appear to cause only sporadic infections and although long term kidney infection with *tarassovi* in dogs has been demonstrated in Parts A and B and with *pomona* by other workers (Cholvin *et al*, 1959; Menges *et al*, 1960), it is doubtful that significant dog-to-dog transmission normally occurs. However, when the population density of dogs is high, as in the hound kennels described in Part A, limited dog-to-dog transmission probably occurs, resulting in a self limiting epidemic. This fundamental difference between the ability of serovars to be passed readily from dog-to-dog may depend on the intensity and duration of leptospiruria and the ability of the

serovar to survive in dog urine, which has a high osmolarity and low pH. Studies of these aspects may provide further information on the attributes necessary for a particular species of animal to be a maintenance host for a particular serovar.

The serological surveys demonstrated that rural dogs are more exposed to an environment contaminated with leptospires than well kept city dogs. *Canicola* which in overseas countries is normally maintained at a high endemic level in city dogs, especially strays (Michna, 1967; Alexander *et al*, 1955), does not appear to be present in N.Z. and thus city dogs in this country are probably only exposed to *ballum* and *copenhageni* infections from rodents. *Ballum* appears to have a nationwide distribution whereas *copenhageni* appears to be restricted to the upper North Island and possibly Dunedin. Dogs living in rural towns are more likely to be exposed to *hardjo*, *pomona* and *tarassovi*, maintained by domestic animals, and *balcanica*, maintained by possums, than city dogs. The rural dogs sampled in the survey described in Part D of this chapter, all lived and worked on dairy farms and were probably more at risk than dogs on sheep farms due to the lower prevalence of leptospiral infections in sheep. Thus the risk to dogs appears to parallel the risk to humans and titres in both reflect the prevalence of infection in dairy cattle.

The detection of *australis* titres in the two city dogs from Wellington was unexpected, and if they in fact represent *australis* infection they might be explained by the importation of dogs from Australia where *australis* is present in rodents, and dogs have been shown to have titres to this serovar (Watson *et al*, 1976b). Large numbers of dogs are imported into N.Z. annually. For example, in 1975, 1147 and 375 dogs were imported into N.Z. from Australia and the U.K. respectively (Anon, 1976a). However, dogs imported into N.Z. from Australia are not required to undergo a serological

test for leptospiral antibodies whereas dogs imported from the U.K. must not have an MAT titre of 1:200 or greater to *canicola* or *copenhageni* (M.A.F. Import Regulations, 1980). Therefore it is quite possible that leptospiruric dogs have been imported into N.Z., especially from Australia. Fortunately it appears from previous surveys that *canicola* is not endemic in Australia (Forbes and Lawrence, 1952; Boon, 1952; Watson *et al*, 1976b).

The isolation of *pomona* and *tarassovi* from dogs and the serological evidence of canine infections with *copenhageni*, *ballum*, *hardjo*, and possibly *balcanica* and *australis*, raises the question of the significance of infected dogs as public health risks in this country. Overseas, the dog has been recognised for many years as a major source of *canicola* infections in humans, especially for pet owners, veterinarians and other people handling dogs. Two out of 68 human cases of leptospirosis reported in the U.K. in 1978 were attributed to dog contact and both were due to *canicola* (Coghlan, 1979), however it has also been shown that humans can contract *canicola* from pigs (Coghlan *et al*, 1957). In the U.S.A., between 1971 and 1976 (Anon,1971-76) dogs were implicated as "the most probable" source of infection in 14-39% of human cases annually. Canicola serogroup titres were reported in 58% of these cases, Icterohaemorrhagiae in 22%, Autumnalis in 9%, Hebdomadis in 4%, Grippotyphosa in 4%, Australis in 3% and Pomona in 1%. Many of these cases had multiple animal exposure and therefore the associations with dogs were not proven. However, in the U.S.A. the average number of notifications for that period was only 100 cases annually from a population of 220 million people. A third of these cases were attributed to dog contact, and this is equivalent to only 0.015 cases/100,000 per year, more than half of which would have been due to *canicola*. This emphasises the point made by Feigin and Anderson (1975) in their review of human leptospirosis that despite the high prevalence of infection in dog populations the morbidity rate in

humans in close contact with dogs is very low. Torten (1979) suggested that the dog is proportionately less hazardous than other species of domestic animals as a public health risk due to the low pH of their urine, which prevents the survival of leptospires for more than a "few minutes". In N.Z., owners of pet dogs avoid contact with dog urine and are encouraged to wash their hands after handling dogs, whereas people working in a rural environment or in an abattoir frequently come in contact with cattle and pig urine. In N.Z. the average annual attack rate of leptospirosis in humans is 16/100,000 and Brockie (1976) reported that between 1971 and 1974 over 99% of these cases were due to *hardjo* and *pomona*. Over 90% of human cases in this country are occupationally associated with working with cattle and pigs on farms or in abattoirs, and it is most unlikely that dogs represent a significant public health risk compared to other domestic livestock. Similarly, it is unlikely that dogs represent significant sources of *hardjo*, *pomona* or *tarassovi* infections on dairy farms compared with cattle and pigs. Despite the demonstration of long term kidney infections with *pomona* and *tarassovi* in dogs, the lack of dog-to-dog transmission indicates that the leptospiruria is either of low intensity or the leptospires do not remain viable for sufficiently long in dog urine to cause significant environmental contamination. This is quite different from the situation demonstrated in cattle and pigs for these serovars (Doherty, 1967c; Hellstrom, 1978; Ryan, 1978).

SUMMARY

1. An outbreak of clinical leptospirosis due to *copenhageni* in a pack of hounds in the South Auckland district was investigated. Over a 14 week period, 26 of the 36 hounds were infected, four showed clinical symptoms and two died. At the time of infection a peak titre of 1:3072 was recorded. Five to eight months after infection, the GMT of the infected hounds had declined to

1:41 (range < 1:24 to 1:192). The infection was presumed to have originated from rats. During the investigation of this outbreak several subclinical infections with *tarassovi* were also detected. A total of nine hounds had low titres to *tarassovi*. This serovar was isolated from four hounds and one of these hounds was leptospiruric for at least four months although the intensity of leptospiruria was low.

2. The experimental inoculation of five dogs with *tarassovi* caused a subclinical infection resulting in kidney colonisation and low grade leptospiruria which persisted until the dogs were destroyed eight to ten months pi. Peak titres of 1:768 occurred 10 to 21 days pi in all dogs, and within five months one dog's titre had declined to less than 1:24 although the animal was still leptospiruric. The titres of the other four dogs had declined to 1:48 when they were destroyed 232 to 295 days pi. No serological crossreactions to *pomona, hardjo, ballum* or *copenhageni* were detected throughout the experiment.

3. Despite the long duration of kidney colonisation in dogs infected with *tarassovi*, the intensity of leptospiruria and the poor survival of this serovar in the urine of normal dogs appeared to mitigate against significant dog-to-dog transmission. It is suggested that the infectivity of *tarassovi* is lower than that of *canicola* for dogs. Therefore, dogs are unlikely to act as maintenance hosts for *tarassovi* in this country and infected dogs are unlikely to present a significant public health risk or to act as primary sources of infection on dairy farms. It is assumed that *pomona, hardjo* and *ballum* infections in dogs are similarly unimportant on dairy farms.

4. *Pomona* infection was detected in seven dogs from three dairy farms which

had concurrent epidemics of *pomona* infection in dairy cows or calves. All seven titres to *pomona* ranged from 1:192 to 1:3072 and *pomona* was isolated from the urine of four out of five of these dogs. All seven dogs were treated with penicillin/streptomycin at their owners' request. On the three properties the epidemics in the cattle appeared to have been initiated by exposure to contaminated pig effluent or by the introduction of infected stock. It appeared that the dogs became infected from the cattle and were not associated with the epidemiology of the infection in the cattle.

5. A serological survey of 64 cattle dogs from 37 dairy farms in the Manawatu showed that 29 (45.3%) had leptospiral titres $\geq$ 1:24. Of these dogs, 18 had titres to *hardjo*, 16 to *balcanica*, 6 to *ballum*, 4 to *pomona*, 2 to *copenhageni*, 1 to *tarassovi* and 1 to *canicola*. All dual titres to *hardjo* and *balcanica* appeared to be crossreactions. Apart from these, five dogs had titres to two or more serovars. The *canicola* titre, of 1:24, was either a nonspecific reaction or a crossreaction as it occurred in conjunction with a titre of 1:24 to *copenhageni*, 1:24 to *hardjo* and 1:24 to *balcanica*. There was a significant association between titres to *hardjo* in the milker and his dog.

6. A serological survey of 47 well kept city dogs, predominantly from the lower North Island, showed that four (8%) had leptospiral titres $\geq$ 1:24. These comprised one dual reaction to *hardjo/balcanica* in a dog from Auckland, a titre to *pomona* in a dog from a country town in South Taranaki, and titres to *australis* in two dogs from Wellington. No titres to *copenhageni*, *canicola*, *ballum* or *tarassovi* were detected.

7. *Canicola* infection did not appear to be present in dogs in N.Z. and

copenhageni infection of dogs appeared to be restricted to the upper North Island.

8. The high prevalence of titres to *hardjo*, *ballum* and *pomona* in country dogs compared with town dogs reflected their greater exposure to infected cattle, rodents and pigs, and paralleled the prevalence of occupationally associated leptospirosis in humans exposed to cattle and pigs.

9. The titres to *australis* detected in two dogs from Wellington were probably due to nonspecific reactions but it is possible that they were due to previous infection with *australis* in dogs imported from Australia.

CHAPTER SEVEN

EXPERIMENTAL INFECTION OF SHEEP AND CATTLE WITH *LEPTOSPIRA INTERROGANS* SEROVAR *BALCANICA*.

INTRODUCTION

Control programmes for any anthropozoonosis should be based on identifying the major animal sources of infection, and the control of leptospirosis is no exception. In N.Z. the majority of cases of leptospirosis in humans have Hebdomadis serogroup titres which, in the past, have all been assumed to be due to serovar *hardjo* (Brockie, 1976). However, there are two serovars in this country which belong to the Hebdomadis serogroup : *hardjo* and *balcanica*. Because of their antigenic similarity it is not possible to differentiate *hardjo* and *balcanica* infections by routine serological methods. Although *hardjo* has been isolated from humans on a number of occasions (Christmas *et al*, 1974a ; Brockie, 1976), there are no published reports of the isolation of *balcanica* from humans in N.Z. and therefore it is not known if such infections occur, and if so, how frequently. Dairy farm workers are the occupational group most frequently infected with Hebdomadis serogroup organisms (Brockie, 1976) and the work reported in Chapter Five shows that the prevalence of infections in milkers is associated with a high prevalence of Hebdomadis serogroup titres in the dairy cattle to which they are exposed. *Hardjo* has been isolated from cattle in N.Z. on a number of occasions (Lake,1973; Brockie, 1976; Hellstrom,1978) and because cattle have been shown to be maintenance hosts for this serovar (Hellstrom,1978) it has therefore been assumed that *hardjo* is responsible for the majority of the Hebdomadis serogroup titres found in dairy cattle and dairy farm workers. However, some workers (Hathaway, 1978; Hellstrom,1978; Durfee and Presidente,1979c) have suggested that cattle and sheep may be susceptible to infection with

balcanica. If natural infection of cattle with this serovar does occur then a proportion of the Hebdomadis serogroup titres detected in cattle and dairy farm workers may be due to *balcanica*. Therefore it is important to determine the susceptibility of cattle to infection with *balcanica* and the possible degree and duration of leptospiruria.

Balcanica has been isolated from possums (*Trichosurus vulpecula*) in N.Z. (Marshall *et al*, 1976) and Australia (Durfee and Presidente,1977) and studies by a number of workers (Hathaway, 1978; Hathaway *et al*,1978; Durfee and Presidente, 1979a,b) have shown that this serovar is endemic in the possum populations of both countries. These isolates from possums were typed* as serovar *balcanica* because of their close antigenic similarity to the type strain of *balcanica* (1627 Burgas), which was first isolated from a man in Bulgaria (Babudieri and Mateeve,1961). *Balcanica* has also been isolated from cattle and pig kidneys in the U.S.S.R. (Semenova *et al*,1965). Durfee and Presidente (1979c) suggested that *balcanica* may have been introduced into Australia by sheep imported from Europe and then became established in the possum population. However, it has been shown(Robinson *et al*, in press) that the 1627 Burgas strain has a distinctly different restriction endonuclease DNA fragment pattern from the N.Z.and Australian isolates of *balcanica*. Therefore the Australasian and European strains must be considered to be different. Nevertheless, for the purposes of this thesis the official nomenclature, serovar *balcanica*, will be retained for these isolates from N.Z.possums.

A number of experimental infections of sheep and cattle with *balcanica* have been conducted to determine their susceptibility to infection with this serovar. Hellstrom (1978) inoculated three, 5 to 6 month old calves, and detected leptospiruria in only one calf on one occasion, 18 days pi Durfee and Presidente (1979c) found calves "rather resistant" to infection

* CDC. Centre for Disease Control,Atlanta,Georgia,USA.

with *balcanica* but readily established kidney infection and leptospiruria in sheep, and therefore suggested that sheep might act as maintenance hosts for this serovar. On the other hand, Hathaway and Marshall (1979) were able to demonstrate only transient renal infection with this serovar in one of three sheep. They therefore considered that sheep are unlikely to be maintenance hosts for *balcanica*.

Because of these inconsistent findings it was decided to repeat the experiments on the infectivity of *balcanica* for sheep and cattle and to assess their ability to act as maintenance hosts for this serovar.

MATERIALS AND METHODS

Inocula :

The two inocula used, originated from a single possum isolate, E32, (Hathaway,1978) typed by CDC as serovar *balcanica*, which had been passaged through a possum and reisolated from its kidneys. Inoculum A was a liquid culture containing 1.5×10^8 leptospires/ml which had undergone four subcultures from this possum kidney reisolate. Inoculum B was a homogenate of the livers and kidneys from ten hamsters, half of which had been inoculated five days previously and the other half 14 days previously, with a subculture of the possum kidney reisolate (50gm of organs in 500 ml Stuarts Medium*).

To test their viability and infectivity direct cultures were made of Inocula A and B and, in addition, 0.5 ml of each was injected intraperitoneally (i p) into two young adult hamsters which were killed 21 days after inoculation when their kidneys were cultured.

* Difco Laboratories, Detroit, Michigan, U.S.A.

Animals :

Twelve yearling heifers and 12, one year old sheep serologically negative to the six leptospiral serovars, *hardjo, balcanica, ballum, copenhageni, pomona* and *tarassovi*, were used in this experiment. The heifers and sheep were grazed separately on areas that had not been stocked for the previous six weeks. After four weeks in these paddocks, just prior to the commencement of the experiment, the animals were again bled to confirm that they were still seronegative. All 12 heifers were grazed together and were kept separate from the sheep, which were also grazed together. None of the heifers or sheep had contact with other stock.

On day 0 of the experiment the 12 heifers and 12 sheep were each divided randomly into three groups of four animals.

Treatment Group A : Four sheep (1S, 2S, 3S, 4S) and four heifers (1H, 2H, 3H, 4H) each received 10 ml of Inoculum A (5 ml intramuscular (im) and 5 ml ip).

Treatment Group B : Four sheep (5S, 6S, 7S, 8S) and four heifers (5H, 6H, 7H, 8H) each received 10 ml of Inoculum B (5 ml im and 5 ml ip).

Control Group C : One sheep (9S) and one heifer (9H) received 10 ml of uninoculated culture medium. One sheep (10S) and one heifer (10H) received 10 ml of uninfected hamster kidney and liver homogenate. The remaining two sheep (11S, 12S) and two heifers (11H, 12H) served as in-contact uninoculated controls.

Blood, Urine and Kidney Cultures :

Heparinised blood samples from all animals were cultured from day 1 to day 12 pi.. Midstream urine samples were collected from each animal

daily from day 1 to 23 pi, then on days 26,29,36,42 from all animals and on days 49 and 56 from the heifers. The urine samples were cultured within 90 minutes of collection. All urine samples were also examined for the presence of leptospires by dfm at 150X magnification.

At the termination of the experiment, 50 days pi in the case of the sheep and 56 days pi for the heifers, the animals were killed and their kidneys immediately removed. Within two hours the kidneys were aseptically removed from their capsules and a 25 gm portion was homogenised and cultured. Blood, urine and kidney samples were cultured as described in Chapter Three.

The isolates were provisionally typed within the Hebdomadis serogroup using the following antisera : cross-absorbed *hardjo* and *balcanica* rabbit antisera, homologous anti-*balcanica* cattle and sheep sera from the experiment, anti-*hardjo* cattle serum, anti-*mini* and anti-*wolfii* rabbit sera.

Serological Examinations :

Serum samples were collected daily for the first three weeks and thereafter at weekly intervals until the termination of the experiment. The *hardjo* antigen was *hardjo-prajitno* supplied by National Health Institute, Department of Health, Wellington, and the *balcanica* antigen was possum isolate T78 (Hathaway, 1978). Serological examinations were performed as described in Chapter Three.

Pathological Examinations :

From days 0 to 22 pi all urine samples were tested with test strips* for nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood

* BM test : Combur 8 test : Boehringer, Mannheim.

and haemoglobin and a refractometer* was used to measure the specific gravity.

From days 0 to 14 pi EDTA blood samples were examined by Ms. J.C. Thompson**to determine the cell volume, haemoglobin concentration, total white cell count, white cell differential and total protein.

At the termination of the experiment the sheep were killed by cervical dislocation and exsanguination, while the heifers were stunned with a captive bolt pistol prior to being bled out. The carcases and viscera were examined for gross lesions and samples of liver, kidney, and in the case of the sheep, brain, were taken for histological examination. Urine from the bladder and kidney samples were taken for culture. Paraffin embedded sections were cut and either stained by haemotoxylin and eosin or by a modified Warthin and Starry method for demonstrating spirochaetes.

RESULTS

The control animals, four sheep and four heifers, remained clinically normal and had no serological or cultural evidence of leptospiral infection throughout the experiment.

Clinical signs

One sheep (4S) displayed signs of pneumonia, with dyspnoea and an elevated temperature , at the time of inoculation and therefore its subsequent signs of respiratory disease were regarded as unassociated with the experimental infection. It had recovered clinically, without treatment, by day 14 pi.

* "Atago" Urine S.G. Refractometer. Atago Optical Works Co.Ltd.
**Department of Veterinary Pathology & Public Health,Massey University.

None of the other sheep or cattle showed any abnormalities in behaviour or appetite. The control animals had a wide range of temperatures (cattle 37.6 to 39.3°C, sheep 37.6 to 39.7°C) which varied according to the prevailing environmental conditions. Therefore the mean temperatures of the control animals ± two standard deviations were chosen as the normal ranges and temperatures occurring outside these ranges were considered abnormal. Using this criterion, transient temperature rises were observed in five of the eight inoculated sheep and six of the eight heifers (see Table 7.1).

Cultural Findings

Balcanica was readily isolated from the original inocula and from kidney cultures of the hamsters injected with these. Table 7.2 summarises the leptospiral isolations from blood, urine and kidneys of the inoculated cattle and sheep. Leptospiraemia was detected in two sheep (2S, 3S) and three heifers (1H, 2H, 3H) all of which received inoculum A. Leptospiruria was detected on only one occasion from one sheep (2S) 18 days pi. This was the only sheep that yielded a *balcanica* isolate from kidney cultures 50 days pi. Leptospiruria was detected in five of the eight heifers (3H which received inoculum A and 5H, 6 H, 7H and 8H which received inoculum B) 42 - 56 days pi. Kidney cultures from three of these heifers (3H, 6H and 7H) yielded *balcanica* isolates. All isolates were serologically identical to the original E32 strain and a laboratory strain of *balcanica*. All urine samples examined by darkfield microscopy were negative for leptospires throughout the experiment.

Serological Findings

The serological responses are summarised in Tables 7.3 and 7.4

Sheep : Titres to *balcanica* were first noted on days 3 and 4 pi

TABLE 7.1 : ANIMALS WITH SIGNIFICANTLY* ELEVATED TEMPERATURES

	Days post inoculation															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sheep Identification (temp. ^{o}C)				2S(A) (39.5)	7S(B) (39.6)			2S(A) (39.3)	1S(A) (39.6)						3S(A) (39.4)	3S(A) (39.4)
				3S(A) (40.4)												
				6S(B) (39.3)												
				7S(B) (39.4)												
Cattle Identification (temp. ^{o}C)		4H(A) (39.2)		1H(A) (40.8)	2H(A) (39.3)											
				3H(A) (40.3)					8H(B) (39.2)	7H(B) (39.9)						

* outside the range given by the mean of the four control animals in each group ± 2 standard deviations.

(A) = inoculum A
(B) = inoculum B

TABLE 7.2 : ISOLATIONS OF *BALCANICA* FROM BLOOD, URINE AND KIDNEY

Species	Inoculum	Animal Identification	Days post inoculation on which isolations were made		
			blood	urine	kidney
SHEEP	A	1S	-	-	-
		2S	1	18	50
		3S	1,2	-	-
		4S	-	-	-
	B	5S	-	-	-
		6S	-	-	-
		7S	-	-	-
		8S	-	-	-
CATTLE	A	1H	1	-	-
		2H	2	-	-
		3H	2	56	56
		4H	-	-	-
	B	5H	-	56	-
		6H	-	56	56
		7H	-	42,56	56
		8H	-	42,56	-

TABLE 7.3 : TITRES TO *BALCANICA* IN INOCULATED SHEEP

Inoculum	Animal Identification	Days post inoculation																		
		0	1	2	3	4	5	6	7	8	9	10	12	14	16	22	29	36	42	50
A	1S					96	192	384	1536	768	768	384	384	384	384	384	96	96	96	96
	2S				*48	384	768	1536	1536	1536	1536	768	768	768	768	384	192	96	96	96
	3S					192	384	1536	1536	768	1536	768	384	192	192	192	48	48	24	ND
	4S				96	1536	3072	3072	3072	3072	3072	768	768	768	768	384	96	192	96	ND
B	5S											24	96	96	96	96	48	48	48	48
	6S									24	192	192	768	768	768	768	192	96	48	48
	7S												48	192	192	192	48	48	24	24
	8S													48	192	96	48	48	48	24

* reciprocal titres

ND = not done

TABLE 7.4 : TITRES TO *BALCANICA* IN INOCULATED CATTLE

Inoculum	Animal Identification	Days post inoculation 0	1	2	3	4	5	6	7	8	9	10	11	13	15	22	29	36	42	49	56
A	1H					*96	768	384	768	768	768	768	1536	1536	768	768	768	384	384	192	192
	2H							24	48	96	384	768	768	768	768	768	192	192	192	96	96
	3H					24	96	192	384	768	768	1536	1536	1536	768	384	384	384	384	192	96
	4H						24	48	192	384	768	768	768	1536	768	768	768	384	384	192	192
B	5H											24	96	384	768	384	384	192	96	96	48
	6H										96	192	384	768	768	768	384	192	96	96	48
	7H												24	192	384	384	96	96	96	96	48
	8H											24	96	384	768	768	384	192	96	96	48

* reciprocal titres

in the four Group A sheep, compared with days 8 to 14 pi in the four Group B sheep, The geometric mean titre (GMT) of the maximum titres of the sheep in each group was 1:1826 for Group A sheep, and 1:384 for Group B sheep. Shortly after reaching their maximum level, the titres of Group B animals declined more rapidly than those of Group A. The GMT of Group A animals at 42 days pi was 1:68 compared with 1:40 for those in Group B. Initially *hardjo* titres of Group A sheep were only one or two dilutions lower than the *balcanica* titres but from the 19th day pi the *hardjo* titres declined more rapidly, and by the 36th day pi they were all less than 1:24. The *hardjo* titres of Group B sheep remained very low throughout the experiment and, except for 242, they were all less than 1:24 by day 16 pi.

Heifers : Positive *balcanica* titres were first recorded from days 4 to 6 pi in Group A and days 9 to 11 in Group B. The GMT of the maximum titres of heifers in Group A was 1:1290 compared to 1:768 for those in Group B. By day 56 pi the GMT for Group A had declined to 1:136 and Group B to 1:48. The *hardjo* titres of Group A heifers closely followed the *balcanica* titres and were generally one dilution lower throughout the experiment. In Group B animals the *hardjo* titres were lower than the *balcanica* titres by nearly two dilutions.

Pathological Examinations

The sheep (4S) which had clinical evidence of pneumonia at the start of the experiment, concurrently showed a raised while cell count, with a peak of 26×10^9 cells/litre, a neutrophilia and a raised total protein level with a peak of 96gm/dl. However, these values had returned to within the normal range by day 14 pi. All other haematological and biochemical parameters of both sheep and heifers were within normal ranges throughout the experiment.

The only gross lesions seen at necropsy in any of the animals were in the lungs of sheep 4S. These were pale areas of antero-ventral consolidation involving approximately half of the apical and intermediate lobes, indicative of a chronic non-progressive pneumonia.

On histological examination no abnormalities were detected in the kidney sections from the control animals, apart from one heifer (9H). In this animal there were a few foci of subacute interstitial nephritis characterised by aggregates of macrophages, lymphocytes and occasional neutrophils.

Of the inoculated sheep only two showed histopathological changes in the kidneys and these were very mild. There were a few cellular casts in the collecting tubules of 3S and 8S, and in addition, 8S showed a few cortical interstitial aggregates of lymphocytes. There were no abnormalities detected in brain sections of the sheep.

The histopathological changes in the inoculated heifers were more severe than in the inoculated sheep. In heifers 1H, 2H, 3H, 6H and 8H there was some degeneration and necrosis of tubular epithelial cells. Several foci of chronic interstitial nephritis were seen in the cortex and cortico-medullary zone of heifers 3H, 4H, 5H, 6H and 8H.

No leptospires were seen in any of the kidney sections stained by the Warthin and Starry method.

DISCUSSION

The mild febrile response to infection with *balcanica* seen in the cattle and sheep in this experiment is similar to that seen in previous experimental infections with this serovar (Hathaway, 1978; Hellstrom, 1978;

Durfee and Presidente, 1979c) and appears typical of most Hebdomadis serogroup infections of cattle and sheep (Hanson and Brodie , 1967; Sullivan, 1970 a,b; Sullivan and Callan, 1970; Farina *et al*,1972; Sullivan,1972; Ellis and Michna, 1977) although two serovars *sejroe* and *szwajizak* have been shown to produce moderately severe but non-fatal infections in young calves (Ristic *et al*,1957; Fennestad,1963; Nervig *et al*, 1978). The present experiment failed to demonstrate any of the signs related to the involvement of the central nervous system that had been shown to occur in hamsters infected with *balcanica* (Hathaway 1978). The fact that leptospiruria could not be detected by darkfield microscopy indicates that, when it occurred, it was at a low level, i.e. less than 10^3 - 10^4 leptospires/ml (Turner, 1970), and this finding supports previous observations that urine culture is more sensitive than dfm (Hathaway, 1978; Ris and Hamel, 1978).

The isolation of *balcanica* from the kidney of only one of the eight inoculated sheep at seven weeks pi agrees with the finding of Hathaway and Marshall (1979) that the colonisation of the kidney with *balcanica* does not readily occur in sheep. This is apparently in contrast to the situation with both natural and experimental *hardjo* infection in sheep (Hathaway and Marshall, 1979; Bahaman *et al*,1980). These findings differ from those of Durfee and Presidente (1979c) who inoculated four sheep with *balcanica* and observed leptospiruria in all four cases, and in one for up to 25 days pi. Unfortunately kidney and urine samples were not cultured at the termination of their experiment to determine the duration of leptospiruria or kidney infection, or to confirm the identity of the infecting serovar.

Cattle appear to be more susceptible than sheep to infection with *balcanica*. At the termination of the experiment, 8 weeks pi, *balcanica*

was isolated from the urine of five of the eight inoculated cattle. However, the low number of urine isolates and the fact that all the df examinations of urine were negative indicates that the degree of leptospiruria was not as great as that seen in *hardjo* infections in cattle (Farina *et al*,1972; Hellstrom, 1978; Mackintosh *et al*, 1980c). These results differ from those of Durfee and Presidente (1979c) who found that calves were "rather resistant" to *balcanica* infection and a transient leptospiruria was observed in only one of the four inoculated calves on days 12 and 13 pi. However, two of the four calves in their experiment had received colostrum and leptospiruria was only monitored by dfm which is a relatively insensitive method. It is also possible that the strain of *balcanica* used by those workers differed from the N.Z. isolate of *balcanica* used here. It has been shown(Robinson *et al*, in press) that there are minor differences in the restriction endonuclease DNA patterns between N.Z. possum isolates and some Australian possum isolates of serovar *balcanica*. These differences may also be associated with changes in infectivity. Using a recent N.Z. possum isolate of *balcanica* Hellstrom (1978) inoculated three calves aged five months and only detected leptospiruria in one calf on one occasion 17 days pi by dfm and failed to isolate *balcanica* from kidney cultures at the termination of the experiment 160 days pi. However, three other calves of the same age were inoculated with *hardjo* and leptospiruria was not detected in any of these. Hellstrom suggested that residual colostral protection interfered with the establishment of infection because three of the four calves challenged at eight months of age with the same number of organisms of the same strain of *hardjo* developed leptospiruria. In the present experiment the use of one year old animals should have ensured that there was no colostral interference.

There was an apparent correlation between the time taken for

seroconversion and the number of organisms in the inoculum. Animals receiving inoculum A (1.5×10^9) had all seroconverted by day 4 pi as did calves and sheep which received similar inoculations in previous experiments (Hellstrom,1978; Hathaway and Marshall,1979). Animals receiving inoculum B did not seroconvert until 9 to 14 days pi. As well as differences in the degree of serological response there were also differences seen in the clinical and cultural results between Group A and B animals. All Group A heifers had elevated temperatures on days 1 to 4 pi and positive blood cultures were obtained from three of the four heifers in this group on day 1 or 2 pi. Only two of the Group B heifers had elevated temperatures (days 8 and 9 pi) and all blood cultures from this group were negative indicating that the leptospiraemic phase was mild or transient. It is possible that the number of organisms in inoculum B was smaller than in inoculum A, although it was more effective in establishing kidney colonisation. The rapid, high serological response seen in Group A heifers may have reduced the length of time of the leptospiraemic phase and reduced the degree of kidney colonisation. An alternative explanation for the higher rate of kidney infection in the Group B heifers is that the leptospires in this organ homogenate were tissue adapted and therefore better able to colonise the kidney than the organisms grown in artificial media.

In contrast to the situation in cattle there did not appear to be a clear-cut association between the febrile response in the sheep and the type of inoculum used. The biphasic rise in temperature seen in sheep 4S occurred two days prior to the single isolation from the urine and this was the only sheep to yield positive kidney cultures at 50 days pi. Several authors (Fennestad, 1963; Doherty,1967a; Sullivan,1970a; Ellis and Michna, 1977) have observed recurrent febrile responses in infected animals and suggested that these temperature elevations may coincide with kidney

infection. However, biphasic temperature responses were not recorded from the heifers in this experiment.

The eight control animals, four sheep and four cattle, remained serologically and culturally negative throughout the experiment despite being exposed to leptospiruric animals under natural pasture conditions. This differs from the rapid spread of *hardjo* infection in heifers under similar conditions reported previously (Hellstrom, 1978; Mackintosh *et al*, 1980c). In the present situation, although the control heifers would have been exposed to leptospiruric cattle for at least two weeks the degree of exposure of the sheep would have been very small because only one positive urine sample was detected over the seven week period. This lack of transmission from heifer to heifer and sheep to sheep indicates that these animals are unlikely to act as maintenance hosts for *balcanica*. Hellstrom (1978), who detected leptospiruria in one of three calves inoculated with *balcanica*, also failed to demonstrate natural transmission to other calves grazed under natural conditions.

The histopathological changes seen in the kidneys of the inoculated cattle were mild and those seen in the kidneys of the inoculated sheep were even milder. These lesions were similar to those described by Durfee and Presidente (1979c).

It has been shown that *balcanica* is endemic in possums in N.Z. (Hathaway, 1978; Hathaway *et al*, 1978). Because grass and clover can form a major component of a possum's diet (Gilmore, 1965; Quinn, 1968; Harvey, 1973), cattle may be exposed to pasture and water contaminated with infected possum urine. However, cattle are recognised as the maintenance hosts for *hardjo* which is assumed to be the serovar responsible for the majority of the Hebdomadis titres found in over 60% of cattle and in over 80% of

herds in N.Z. (Hellstrom, 1978). Both serovars have similar antigenic properties as shown by their high degree of cross-reactivity in the MAT and it would seem likely that these *hardjo* antibodies would provide at least some degree of cross protection against *balcanica* infection and more evidence for this hypothesis will be presented in Chapter Ten. Therefore it is unlikely that *balcanica* infects cattle on farms where *hardjo* is endemic. However, on properties where *hardjo* is not present the cattle may be susceptible to *balcanica* infection and sporadic cases may occur. The present study shows that cattle can become infected if they receive a sufficient challenge and may be leptospiruric for at least 56 days pi. However, the low level of excretion and the apparent lack of cow to cow transmission under natural conditions indicate that *balcanica* is unlikely to become endemic in a herd. Therefore cattle are not likely to be a significant source of *balcanica* infection for man.

This experiment indicates that sheep are less susceptible than cattle to infection with *balcanica*, and leptospiruria, if it occurs, is of low intensity and short duration. Although sporadic infections may occur *balcanica* is unlikely to become endemic in sheep flocks.

SUMMARY

1. Eight one-year old ewes and eight yearling heifers were inoculated with *Leptospira interrogans* serovar *balcanica* which had been isolated recently from a possum *(Trichosurus vulpecula)*.

2. All 16 animals had seroconverted by 14 days pi.

3. Mild transient temperature elevations were detected in five of the

eight sheep and six of the eight cattle between one and nine days pi. No other clinical abnormalities due to the infection were observed.

4. Leptospiruria was detected in one of the eight sheep 18 days pi and *balcanica* was isolated from the kidney of this sheep 50 days pi.

5. Five of the eight heifers were leptospiruric between 42 and 56 days pi and *balcanica* was isolated from the kidneys of three of these heifers 56 days pi.

6. Focal chronic interstitial nephritis and/or tubular degeneration were seen in seven of the eight inoculated heifers. Only two of the eight inoculated sheep showed similar but milder histopathological changes in their kidneys.

7. Leptospiruria was not detected by dark field examination of urine samples from either sheep or cattle and was, therefore, of low intensity (less than 10^3-10^4 leptospires/ml).

8. There was no natural transmission of *balcanica* infection to the control animals grazed with the infected animals under spring pasture conditions.

9. It is concluded from the results of this experiment that cattle and sheep do not appear to be maintenance hosts for *balcanica* and, although sporadic infections may occur, this serovar is unlikely to be maintained endemically by these species.

CHAPTER EIGHT

THE INVESTIGATION OF A SPORADIC OUTBREAK OF *BALCANICA* INFECTION IN A DAIRY HERD.

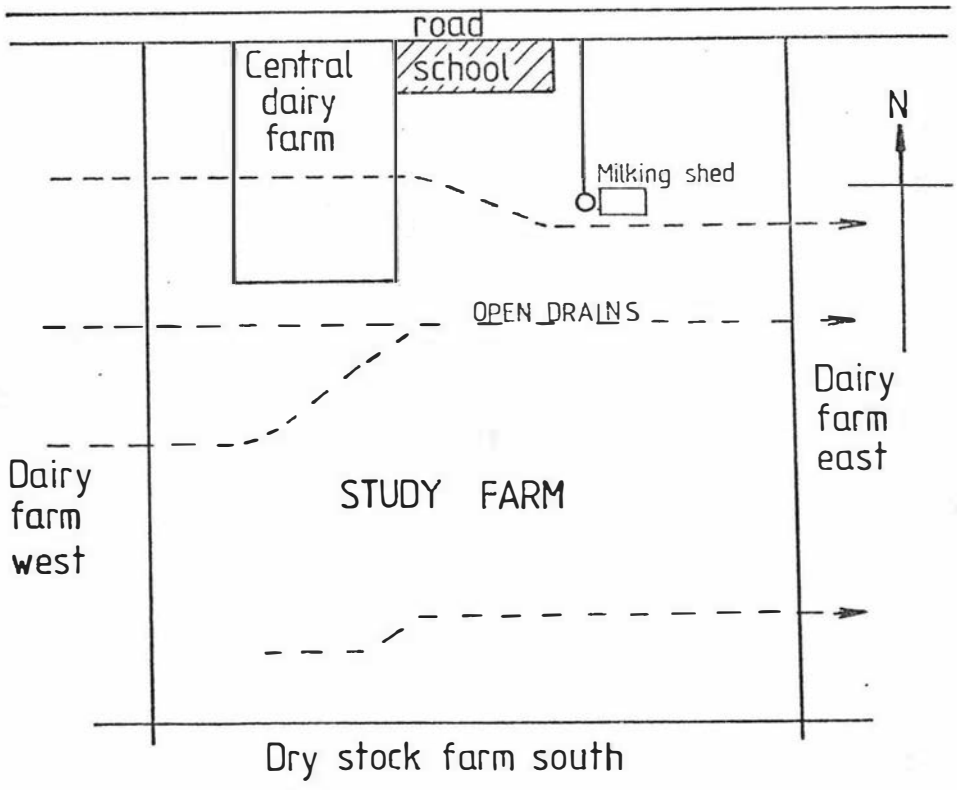
INTRODUCTION

Prior to the commencement of this study, a farmer,with an interest in leptospirosis, offered his farm to the Department of Veterinary Pathology and Public Health, Massey University, for use in any appropriate studies of leptospirosis. Twenty of the two and three year old cows randomly selected from this farm were all serologically negative to *hardjo* and *pomona* while similar samples from adjoining farms showed evidence of endemic *hardjo* infection in the cattle. These farms were only separated by a wire fence and drainage from these adjoining properties crossed the study farm in open ditches to which the cattle had access. It was decided that an epidemiological investigation of the study farm should be carried out to establish whether or not any of the other cattle had evidence of *hardjo* infection, and if so to investigate why the two and three year old cattle had not become infected. If there was no evidence of infection in the other animals, the herd was to be monitored periodically to determine if or when *hardjo* was introduced to the herd from the neighbouring properties. Hellstrom (1978) contended that the cow-to-cow transmission of *hardjo* infection usually occurred by close contact between susceptible and leptospiruric cattle. Although outbreaks of *pomona* in cattle have been attributed to access to infected water (Shield, 1974) and pig effluent (Anon, 1974a), it has not been shown that the transmission of *hardjo* to cattle can occur by such indirect methods. A knowledge of such factors is important in the control of *hardjo* and the maintenance of herds free from infection, and it was hoped that this study would throw some light on the subject.

HERD HISTORY AND MANAGEMENT

The study farm was a commercial factory supply dairy farm situated 13 kilometres inland from the coast at Himatangi in the Manawatu district and comprised 128 hectares of sandy undulating land which included areas of drained flax swamp and two natural ponds. Adjoining properties included dairy farms on both eastern and western boundaries, a dry stock unit on the southern boundary and a small dairy farm in the centre of the property (see Fig. 8.1). A road formed the northern boundary. Drainage ran from west to east between adjoining properties and took the form of open drains to which the stock had access.

FIG. 8.1 : PLAN OF STUDY FARM AND ADJOINING PROPERTIES



The property had been a dairy farm since 1920. Artificial insemination had been used in the milking herd for the last eight years and two beef-cross bulls were run with the yearling heifers. Calves and yearlings were grazed separately and were rotated approximately six weeks ahead of the cows. Yearlings and cows which did not conceive were held over and run with the following season's yearlings. Cows which had ceased milking early in the season, usually in late summer or early autumn, were also run with the yearlings. These yearling cattle usually joined the main herd in late June and calving, which started in July, was usually completed by the end of September.

The farm livestock usually comprised 200-220 cows, 40-50 heifers, 50-60 calves and two bulls. Periodically, two weaner pigs, 6-12 weeks of age, were bought in to fatten for the farmer's own consumption. They were kept in a small sty approximately 100 metres from the cowshed.

General disease status of the herd : Apart from Johnes disease, there had been no particular disease problems in the milking herd. The calves were regularly drenched with anthelmintic to which selenium had been added. The Johnes problem became apparent in the late 1960s when approximately 12 clinical cases occurred each year. Under the supervision of Ministry of Agriculture and Fisheries veterinarians, a Johnes vaccination programme was instituted and since the spring of 1973 all calves were vaccinated. By the 1976/77 season the annual incidence of clinical cases of Johnes disease had declined to five per year and Johnes vaccinated animals comprised 51% of the herd. In the 1980/81 season 86% of the herd had been vaccinated and there had been no clinical cases midway through the season. When the problem was at its worst in the late 1960s and early 1970s it was necessary to buy replacements

annually. The need for this declined and the last cow or heifer replacements were bought in the spring of 1974, although eight young calves were bought and vaccinated against Johnes in 1977. The herd was then "closed" and all replacements were bred on the property.

There had been no evidence of clinical leptospiral infection in the herd or young stock and a leptospiral vaccine had never been used. There had been no other major changes in management or grazing policy in the last ten years.

MATERIALS AND METHODS

The calves, yearlings and milking cows were bled in April and May 1978, at the start of this investigation. All the cattle were allotted to age cohorts with a group number denoting the year of their birth. At each subsequent bleeding the results for each cohort could then be compared with the results from previous samplings. Each season a new cohort, representing the new crop of calves, was added to the result sheet (Table 8.1). During the two and a half years of this study the milking cows were bled on a total of four occasions. Ten months after the initial sampling, random samples of ten calves (Group 78) and ten yearlings (Group 77) were bled. Two of these ten yearlings were seropositive and all 51 yearlings were bled two months later. The following December these animals had entered the milking herd and were rebled together with the older cows. Calves in Group 78 were all bled 14 months after their first sampling and the following year they were also included as members of the herd. All blood samples were serologically tested using the method described in Chapter Three.

Because it is not possible to distinguish between *balcanica* and *hardjo*

infections in cattle by normal serological methods, either serovar can be used as the antigen in the MAT. In this study *hardjo* was used as it was easier to maintain in the laboratory than *balcanica*.

At the initial sampling one of the 40 yearlings in Group 76, number H58, had a Hebdomadis serogroup titre. An attempt was made to estimate how recently this infection had occurred by fractionating the serum using column separation (Hellstrom,1978). The fractions were then individually tested by the MAT to determine the ratio of specific antileptospiral activity in the IgM and IgG antibody components.

Urine samples were obtained from the seropositive heifer,H58 on seven occasions over a period of three weeks shortly after the study commenced. Urine samples were also obtained from two seropositive heifers and ten other heifers chosen at random from Group 77 when a number of these were also found to have Hebdomadis serogroup titres. Urine samples were collected again from these heifers, ten months later, to determine if any of the heifers that had been leptospiruric on the first occasion were still shedding leptospires. All urine samples were examined by dfm and cultured by the method described in Chapter Three.

Various species of wildlife on the property were trapped using mouse, rat and possum cages described by Hathaway (1978). Serological examinations and kidney cultures were performed by the methods described in Chapter Three.

RESULTS

A summary of the Hebdomadis serogroup titres found in the cattle on this property during the study is shown in Table 8.1. At the initial test,

TABLE 8.1 : PREVALENCES OF TITRES TO *HARDJO* IN ALL AGE COHORTS OF CATTLE SAMPLED ON A NUMBER OF OCCASIONS OVER A THIRTY THREE MONTH PERIOD.

Dairy season	Blood sampling dates	Group 80	Group 79	Group 78	Group 77	Group 76	MILKING COWS								PREVALENCE RATES	
							Group 75	Group 74	Group 73	Group 72	Group 71	Group 70	Group 69	Group 68-	Groups 68-73 Milking herd	
July 1977					Calves	Yearlings	1st calvers	2nd calvers	3rd calvers	4th calvers	5th calvers	6th calvers	7th calvers	8th+ calvers		
Dec																
Jan 1978	7/3/78 14/4/78				52 seroneg	1/40 seropos	31 seroneg	4/28 seropos	16/23 seropos	13/23 seropos	12/15 seropos	10/12 seropos	4/5 seropos	13/26 seropos	68/102 (67%)	72/161 (45%)
June																
July 1978				Calves	Yearlings	1st calvers	2nd calvers	3rd calvers	4th calvers	5th calvers	6th calvers	7th calvers	8th calvers	9th+ calvers		
Dec																
Jan 1979	27/2/79 25/4/79			10 seroneg	2/10 seropos 9/51 seropos	*	*	*	*	*	*	*	*	*	*	*
June																
July 1979			Calves	Yearlings	1st calvers	2nd calvers	3rd calvers	4th calvers	5th calvers	6th calvers	7th calvers	8th calvers	9th calvers	10th+ calvers		
Dec	6/12/79				10/48 seropos	3/36 seropos	2/28 seropos	5/27 seropos	14/21 seropos	9/14 seropos	8/10 seropos	5/6 seropos	5/5 seropos	10/11 seropos	51/67 (76%)	70/205 (34%)
Jan 1980																
June	22/4/80		1/40 seropos	1/48 seropos	10/47 seropos	2/33 seropos	1/25 seropos	4/25 seropos	14/18 seropos	9/14 seropos	7/9 seropos	5/6 seropos	4/4 seropos	6/7 seropos	45/58 (78%)	62/188 (33%)
July 1980		Calves	Yearlings	1st calvers	2nd calvers	3rd calvers	4th calvers	5th calvers	6th calvers	7th calvers	8th calvers	9th calvers	10th calvers	11th+ calvers		
Dec	18/12/80	*	*	1/44 seropos	9/42 seropos	2/32 seropos	1/23 seropos	4/25 seropos	13/18 seropos	5/11 seropos	4/7 seropos	3/4 seropos	4/4 seropos	4/5 seropos	33/49 (67%)	50/215 (23%)
Jan 1981																

* not examined

the Group 77 calves were all serologically negative. One of the Group 76 yearling heifers, H58, had a titre of 1:96 to both *hardjo* and *balcanica* antigens but no leptospiruria could be detected in this animal. Column separation of this animal's serum showed that 73% of the agglutinating activity was in the IgG fraction and 27% in the IgM fraction.

At the initial test all 31 cows comprising Group 75, which were in their first milking season, were serologically negative and only four of the 28 cows in Group 74 had titres to *hardjo*. The other age cohorts in the herd, Groups 73 to 68, all had similar serological prevalences of titres to *hardjo*, ranging from 50-83% with an average of 67%. Of these 102 cattle, one had a titre of 1:768, six had titres of 1:192, 61 had titres between 1:96 and 1:24 and 44 were seronegative (see Table 8.2). Because of the very low prevalence (7%) of titres in the two and three year old cattle (Groups 75 and 74) and the small number of high titres in the older cattle it was concluded that a Hebdomadis serogroup infection, presumed to be *hardjo*, had either been cycling endemically in the herd or the yearlings until two or three years previously and then died out, or had infected most of the herd during an epidemic three years previously and had failed to become endemic.

Ten calves, chosen at random from Group 78, were bled in February 1970 and all were seronegative, while two of the ten yearling heifers in Group 77, numbers 221 and 258, had titres of 1:48 and 1:384 to *hardjo* respectively (see Table 8.3). When all the heifers in Group 77 were bled eight weeks later, nine of the 51 heifers had titres to *hardjo*. Leptospires were also isolated from the urine of two of these heifers, numbers 221 and 236 (see Table 8.3). It was initially assumed that an outbreak of *hardjo* had occurred in this group of yearling heifers and that it had probably been

TABLE 8.2 : DISTRIBUTION OF TITRES TO *HARDJO* ACCORDING TO AGE GROUP AT PERIODIC BLOOD SAMPLINGS

Sampling date	Titres to *hardjo*	No. of cattle in each age group											
		79*	78	77	76	75	74	73	72	71	70	69	68
	0			52	39	31	24	7	8	3	2	1	13
	24**						1	9	6	6	4	2	7
7/3/78	48						2	3	4	3	1		2
14/4/78	96				1		1	1	2	2	4	1	4
	192							3	1		1	1	
	384												
	768									1			
	0			42									
	24			1									
	48			5									
25/4/79	96			3									
	192												
	384												
	768												
	0			38	33	26	22	7	5	2	1	0	1
	24			2	2		1	1	4	2	2	1	7
	48			3	1		4	7	4	3	2	2	3
6/12/79	96			4		1		5		1	1	2	
	192							1	1	2			
	384			1									
	768					1							
	0	44	47	37	31	24	21	4	5	2	1	0	1
	24			2	1		1	5	3	3	3	1	5
	48		1	6	1		2	6	5	2	1	1	1
22/4/80	96	1		2			1	3	1	1	1	2	
	192									1			
	384					1							
	0		43	33	30	22	21	5	6	3	1	0	1
	24			7	2		2	10	2	1	3	2	4
	48		1	2		1	1	3	3	3		2	
18/12/80	96						1						
	192												

* denotes year of birth
**reciprocal titres

TABLE 8.3 : SEROLOGICAL AND CULTURAL RESULTS FROM PERIODIC MONITORING OF GROUP 77 CATTLE

Cattle Identification	Titres to *hardjo* 7/3/78	27/2/79*	25/4/79	6/12/79	22/4/80	18/12/80	Urine culture 25/4/79	26/2/80
13	0	NT	0	0	0	0	-	-
26	0	0	0	0	0	0	-	NT
29	0	NT	48	24	24	24	NT	-
31	0	0	0	384	96	24	NT	-
42	0	NT	48	Culled			NT	NT
51	0	0	0	0	0	0	- (c)	- (c)
55	0	NT	0	0	0	0	-	- (c)
74	0	0	0	0	Culled		NT	
166	0	NT	48	96	48	24	NT	NT
182	0	0	0	0	0	0	NT	NT
183	0	NT	0	0	0	0	-	NT
187	0	NT	96	96	48	48	-	- (c)
221	0	48**	48	24	48	24	+	-
224	0	NT	0	48	96	48	NT	-
231	0	NT	24	48	48	24	NT	-
236	0	NT	48	96	48	24	+	-
237	0	0	0	0	0	0	NT	NT
241	0	0	0	0	0	0	NT	NT
245	0	NT	384	48	24	24	NT	-
252	0	NT	0	0	0	0	- (c)	NT
258	0	384	96	96	48	Culled	-	-
261	0	0	0	0	0	Culled	- (c)	NT
273	0	NT	0	0	0	Culled	-	NT
***								
Serological Prevalence	0/52	2/10	9/51	10/48	10/47	9/42		
Number of new cases			9	2	0	0		
Cultural prevalence							2/12	0/12

NT Not Tested
* Random sample of 10 animals
** Reciprocal titre
*** Plus 28 other cattle from this group that were seronegative throughout the study

(c) Cultures contaminated
\+ Isolation of *balcanica*

introduced from a neighbour's property. Over a period of some months these isolates were subcultured to produce sufficient growth for identification and were confirmed as belonging to the Hebdomadis serogroup using serogroup specific antisera. The isolates, together with specific rabbit antisera, were sent to the WHO Reference Laboratory in Brisbane for definitive typing. Meanwhile the restriction endonuclease analysis technique had been perfected for use with leptospires (Marshall *et al*, 1981). This test showed that the DNA fragment patterns for both 221 and 236 were identical to that produced by a possum isolate of *balcanica* (Plate 8.1). Subsequently the Queensland laboratory confirmed both isolates as being serovar *balcanica*.

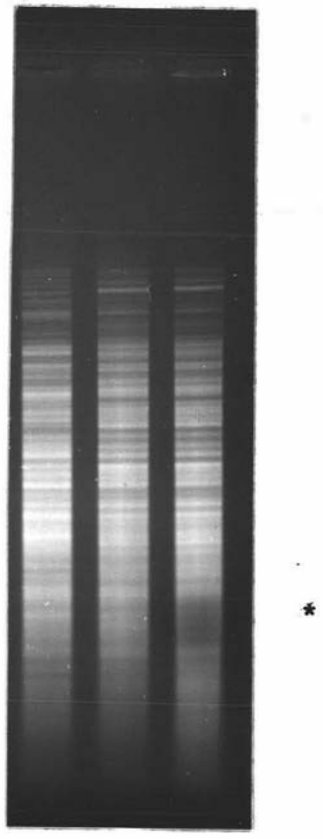
The next test bleeding of the milking herd took place in December 1979 and it showed that, in the intervening eight months, two more of the Group 77 animals, which had now entered the milking herd, had seroconverted. Two new seroconversions had also occurred in each of the Group 76, 75 and 74 cattle. No new seroconversion had occurred in the rest of the herd.

In February 1980, ten months after *balcanica* isolates were obtained from heifers 221 and 236, no evidence of leptospiruria was detected in samples collected from 12 of the Group 77 cattle (see Table 8.3).

In April 1980, all calves, yearlings and milking cows were bled. One of the 40 calves in Group 79 and one of the 48 yearlings in Group 78 had Hebdomadis serogroup titres while no new titres had arisen in the other animals since the previous test four months earlier. Eight months later, in December 1980, the herd was bled again and no new seroconversions were detected.

By examining the serological results of each cohort it can be seen

PLATE 8.1 : RESTRICTION ENDONUCLEASE DNA FRAGMENT PATTERNS OF THREE LEPTOSPIRAL ISOLATES : *HARDJO* ISOLATED FROM A COW, *BALCANICA* ISOLATED FROM HEIFER 221 AND *BALCANICA* ISOLATED FROM A POSSUM.



a b c

* dark patch was due to a marker dye and is incidental to the pattern.

a. *hardjo* isolated from a cow during the course of a vaccine trial reported in Chapter Ten. This pattern is identical to that from all other N.Z. field isolates of *hardjo* examined in this laboratory.

b. *balcanica* isolated from heifer 221. This pattern is identical to c.

c. *balcanica* isolated from a possum on the study farm. This pattern is identical to that from all other N.Z. field isolates of *balcanica* examined in this laboratory.

that the majority of new cases of Hebdomadis serogroup infection occurred in the Group 77 animals. Between 7/3/78 and 25/4/79 nine new cases occurred and *balcanica* was isolated from two of these. In the following eight months, only two further cases were detected. Altogether, 11 of the 52 animals in Group 77 became infected, presumably all with *balcanica*, in a 20 month period while no new cases arose in the following 12 month period. Over the 33 months of the study no new cases were detected in the older animals in the herd (Groups 73 to 68) and the serological prevalence of titres in these groups remained relatively constant, although the overall milking herd prevalence declined due to the increasing proportion of younger seronegative animals and the culling of older animals.

A total of six possums *(Trichosurus vulpecula)*, two hedgehogs *(Erinaceus europaeus)*, three mice *(Mus musculus)* and one rabbit *(Oryctolagus cuniculus)* were trapped on the farm and the only leptospiral isolate, identified as serovar *balcanica*, was cultured from the kidneys of an adult male possum. None of these wild animals had titres to the five test antigens. Two porker pigs (4-5 months old) that had been kept on the property during the winter of 1978 were slaughtered at the local abattoir. Both were seronegative and no leptospires were isolated from their kidneys.

The contamination rate of urine cultures was 25% and this may have reduced the number of isolates obtained. There was negligible contamination of the kidney cultures of the wild animals.

DISCUSSION

This study, which was planned as an epidemiological investigation of *hardjo* infection in cattle on a commercial factory supply dairy farm, fortuitously resulted in the first recorded isolation of *balcanica* from

naturally infected cattle in Australasia. Prior to this isolation it was not known if cattle were susceptible to natural infection with the strain of *balcanica* isolated from possums in N.Z. It has been shown (Chapter Seven) that cattle can be infected experimentally with *balcanica*, but no cow-to-cow transmission was demonstrated and the low intensity of leptospiruria indicated that cattle were unlikely to act as true maintenance hosts as defined by Hathaway (1978). The results of this present study tend to support these experimental findings. Although the outbreak occurred in a group of yearling heifers, only 20% of this group became infected and when they were introduced into the milking herd only a further six cows in the herd sero-converted. Although cow-to-cow transmission probably occurred, *balcanica* infection did not become permanently established in the herd. As with the experimental infections, there were no clinical signs of disease in those cattle naturally infected with *balcanica*.

Apart from this minor epidemic of *balcanica* infection in the Group 77 animals, a small number of isolated sporadic Hebdomadis serogroup infections occurred in other groups. These were probably due to *balcanica* rather than to *hardjo* because other studies (Hellstrom, 1978; Marshall *et al*, 1979b) have shown that, under similar natural conditions, *hardjo* infection spreads rapidly in susceptible cattle and infects the majority of them.

The yearling heifer, H58, was the only animal in Group 76 to become infected prior to the initial test, and 32 months later only two new cases in this cohort had occurred. At the time of the initial test H58 had a titre of 1:96 to both *hardjo* and *balcanica*, and the ratio of IgM to IgG was 27:23. The results of studies of IgM:IgG ratios by Hellstrom (1978) suggest that this heifer was probably infected less than six months previously. This animal was not leptospiruric during the month following the initial bleeding and no

other animals in this cohort appeared to have been infected at the time of the first test. It has been shown (see Chapter Ten) that the majority of heifers infected with *hardjo* remain leptospiruric for at least 12 months after infection, and the attack rate of infections with *hardjo* in a susceptible group of heifers is up to 100%. Thus, the Hebdomadis serogroup titre in heifer H58 was probably due to infection with *balcanica* rather than *hardjo* and this animal failed to transmit *balcanica* to the rest of the cattle in its cohort.

It is interesting to note that most of the sporadic *balcanica* infections occurred in the young replacement stock. Possible explanations for this include (a) young stock may have been more susceptible to infection, (b) they were grazed in different areas from the main herd and may have been more exposed to a source of *balcanica* infection, (c) the older stock which had experienced *hardjo* infection were more likely to have a degree of cross-protection to *balcanica*. This last hypothesis seems the most likely and is supported by the findings reported in Chapter Ten and by the previous work of Hellstrom (1978). In this latter work it was shown that three calves experimentally infected with *balcanica* were resistant to infection with *hardjo* but were fully susceptible to *pomona*, a serovar in a different serogroup.

The source of the *balcanica* infection on the farm was assumed to have been infected possums. It has been shown (Hathaway, 1978; Hathaway *et al*, 1978) that the possum is the maintenance host for *balcanica* in N.Z. and that there is a high serological and cultural prevalence of *balcanica* in possums in rural areas. *Balcanica* was isolated from one of the possums on the study farm confirming that it was present on the property and there were a number of stands of trees on the study farm and adjoining farms that were known to harbour possums. As stated in Chapter Seven, grass and clover constitute

a large proportion of the possum's diet. Possums can range up to 1.6km from their nesting site (Tynedale-Briscoe, 1955) and therefore widespread pasture contamination with infected possum urine could have easily occurred on this property.

Prior to commencing this investigation, a sample of 20 two and three year old animals from this farm were found to be serologically negative. However, when the whole herd was bled for the first time a high serological prevalence of Hebdomadis serogroup titres was found in the older cattle (Groups 73-68). Although it is possible that these titres were due to widespread *balcanica* infection, they are more likely to have been due to a previously endemic infection with *hardjo* which subsequently died out. There is good evidence, as reported in Chapter Five and by Hellstrom (1980), that on most dairy farms endemic *hardjo* infection cycles in either the yearling replacement cattle or in the two and three year olds in the herd. The majority of cattle infected with *hardjo* are leptospiruric for at least a year (Hellstrom,1978) and thus infection persists from season to season by the mixing of leptospiruric cattle with susceptible animals. If *hardjo* had still been present in the milking herd at the time of the first sampling the majority of two and three year old animals would have had a high serological prevalence of titres and all subsequent cohorts entering the herd would also have seroconverted. The reason for the apparently spontaneous elimination of *hardjo* infection from the herd was not determined. There was no history of unusual environmental conditions, marked changes in management or the mass treatment of animals with antibiotics in the 1975-76 or 1976-77 dairy seasons. If *hardjo* had been cycling in the yearling heifers, rather than in the milking herd, infection would have been maintained from one year to the next by the grazing of infected non-pregnant heifers or cows with the following season's yearlings. The results of Chapter Ten show that cattle can remain leptospiruric for an

average of 53 weeks after infection, with a range of 0 - 82 weeks. Thus, if only a small number of non-pregnant heifers or cows infected in the previous season were retained with the yearlings it is possible that they all could have ceased shedding before contact with the yearling heifers. This seems the most likely explanation for the disappearance of *hardjo* from this property after a period of apparent endemicity.

Acid-fast micro-organisms, especially tubercule bacilli, have been shown to stimulate cell mediated immunity non-specifically (Thompson,1976), and the possibility that Johnes vaccination of the calves may have increased their immunity non-specifically and reduced their susceptibility to infection with leptospires was considered. However, the first group of calves to be vaccinated were Group 73 animals and their serological prevalence of *hardjo* titres was the same as previous groups. Nevertheless, to investigate this possibility further a small experiment was conducted (Appendix V) in which mice were inoculated with BCG vaccine and challenged with *Leptospira interrogans* serovar *ballum*, and it was shown that there was no difference in the rate of infection between vaccinated and control mice. Ryan (1978) also showed that immunity to leptospiral infection is more likely to be due to specific humoral antibody than to cell mediated immunity.

The distribution of Hebdomadis serogroup titres on this farm is not typical of the pattern seen on properties where *hardjo* is apparently endemic as described in Chapter Five and by other workers (Hellstrom,1978). However, the situation investigated on this dairy farm may not be unique in this country, and some of the atypical patterns of *hardjo* titres observed during a survey of 37 Taranaki herds (Hellstrom, 1980) may represent sporadic *balcanica* infections similar to the one studied here.

The 60% prevalence of titres to *hardjo* in N.Z. cattle reported by Hellstrom (1978) may include a small proportion of animals which had been infected with *balcanica*.

Having established the fact that *hardjo* was not endemic on the property the herd was monitored periodically in order to determine whether or not *hardjo* was introduced from the adjoining properties. Over the 33 month period of the study there was no evidence of such an occurrence. During this time the yearling animals on the property to the west of the study farm were re-bled and it was shown that 90% had titres to *hardjo*, and a sample of ten milking cows on the central property surrounded by the study farm had a serological prevalence of 70%. These results indicate that *hardjo* was still endemic on these two properties and add weight to the suggestion that close contact between leptospiruric and susceptible cattle is necessary for transmission to occur under normal pasture conditions, and that indirect transmission either from the environment or via contact with other species does not readily occur. These observations are important in relation to the control of *hardjo* on dairy farms. If *hardjo* can be eradicated by management or vaccination procedures, as described in Chapter Ten, then the maintenance of a closed herd and the control of stock movement should prevent the reintroduction of infection. However, these conclusions are based on observations of only one farm, and further studies of the survival of *hardjo* in the environment and possible modes of indirect transmission between cattle are required.

SUMMARY

1. A commercial factory supply dairy farm, with approximately 220 milking cows and nearly 100 young stock, was monitored periodically over a 33 month period to investigate the epidemiology of leptospirosis occurring on that property.

2. The initial serological survey showed that 67% of the older milking cows had titres to *hardjo* while only 3% of the two and three year old milking cows, yearlings and calves had titres to *hardjo*.

3. In the following year serovar *balcanica* was isolated from two heifers during an outbreak of asymptomatic infection involving 11 of the 51 yearlings (21.6%). This was the first reported isolation of this serovar from naturally infected cattle in Australasia.

4. *Balcanica* infection in these yearling heifers resulted in leptospiruria of less than 10 months duration and there appeared to be little heifer-to-heifer transmission as only 21.6 % of this group became infected.

5. From the limited observations of this natural outbreak it appears that *balcanica* infections in cattle are sporadic in nature and that cattle are unlikely to act as maintenance hosts for this serovar. Consequently cattle are unlikely to be a significant source of *balcanica* infection for dairy farm workers.

6. The source of the *balcanica* infection on the farm was assumed to have been possums which were known to live in trees on the property. One of six possums trapped on the property was shown to be infected with *balcanica*.

7. It appeared that *hardjo* had been endemic on the farm until the mid 1970s but had then apparently died out spontaneously. This occurrence could not be associated with any obvious climatic or management factors. A possible explanation is that *hardjo* infection, which previously may have been cycling in the yearling heifers, may not have passed onto the next season's yearlings.

8. In spite of the presence of active *hardjo* infection on neighbouring farms and the drainage of water from them across the study farm, there was no evidence of the transmission of *hardjo* infection to the cattle on the study farm.

9. The apparent lack of indirect transmission of *hardjo* between cattle on neighbouring properties is important in relation to the control of *hardjo* on dairy farms.

CHAPTER NINE.

THE EFFECT OF VACCINATING CATTLE DURING AN EPIDEMIC OF *POMONA* ABORTIONS.

INTRODUCTION

Infections with serovar *pomona* in New Zealand livestock were first recognised in the early 1950s and an outbreak of bovine abortions caused by *pomona* was first reported by Te Punga and Bishop (1953). Similar abortion "storms" have been reported in Australia (Knott and Dadswell,1970) and in the U.S.A. (Stoenner *et al*,1956). Despite the availability of effective *pomona* vaccines for use in cattle since the mid 1950s (see Chapter One) *pomona* infection was still one of the most commonly diagnosed causes of abortion in cattle in the 1970s (Anon,1975a; Anon,1977c).

As stated in Chapter One in the review of the epidemiology of leptospirosis in N.Z., Ryan (1978) and Hellstrom (1978) have presented convincing evidence that, in this country, pigs represent the major reservoir of *pomona* infection for cattle . Although cattle can readily become infected they are only short term carriers and an outbreak within a herd is self-limiting. The rate of spread of *pomona* within a herd depends largely on stocking densities and environmental conditions (Stoenner *et al*, 1956; Doherty, 1967a; Hellstrom,1978).

In an excellent review of the literature on the pathogenesis of *pomona* abortions in cattle, Murphy and Jensen (1969) summarised the main features of bovine leptospiral abortions. They suggested that abortions, although not a constant sequel to leptospirosis in pregnant cows, occur during the latter half of pregnancy and are accompanied by a serological response in the infected cows. Clinical signs of pyrexia and malaise may occur at the time of leptospiraemia, but often abortion is the only sign

of infection observed and aborted foetuses usually appear to have died 24 hours or more before expulsion. From the results of their studies on the pathogenesis of bovine abortions due to *pomona* they suggest that after leptospiraemia the leptospires localise in the kidney tubules of the cow and the placenta. They report that spontaneous degenerative changes often occur in the bovine placentomes in the latter half of pregnancy and leptospires may localise in such areas and thence gain access to the foetal circulation. Individual variation in the occurrence of such degenerate areas may explain why some apparently susceptible cattle infected in the second half of gestation fail to abort. The death of the foetus is apparently associated with the lysis of foetal erythrocytes by leptospiral haemolysins. Leptospires do not appear to survive in the dead foetus and, therefore, are rarely recovered from aborted material. Experimental and natural *pomona* infections in cattle have been studied by a number of workers (Ringen *et al*, 1955; Morse and McNutt, 1956; Ferguson *et al*, 1957; Hamdy and Ferguson, 1957; Gillespie and Kenzy, 1958; Morse, 1960; Doberty, 1967c; Murphy and Jensen, 1969) and they found that infection was followed by a febrile period of 1-2 days duration, 3 to 8 days pi. Leptospiruria first occurred 11 to 26 days pi with an average of 15 days pi and the greatest intensity of leptospiruria occurred between 25 and 35 days pi. The longest recorded duration of leptospiruria in cattle naturally infected with *pomona* was 118 days. Abortions usually occurred from 18 to 32 days pi with an average of 22 days although Ferguson *et al* (1957) also reported the expulsion of a mummified foetus 47 days pi. This knowledge of the course of infection is useful when investigating natural outbreaks in order to estimate the time at which the epidemic began and to determine thereby the possible source of infection. It may also be used to assess whether or not vaccination alters the course of the outbreak.

Abortion rates during outbreaks of infection in herds of pregnant cows

have been reported to range from less than 5% to greater than 40% (Stoenner *et al*, 1956). These abortion rates are greatest in herds in which the cows are all in the last trimester of pregnancy. However, information on the proportion of susceptible animals in a herd which actually becomes infected during an outbreak of *pomona* is difficult to obtain. In many studies of natural outbreaks of abortion, measures have been instigated which may have interfered with the natural course of the disease. These measures include treatment, isolation of infected animals and vaccination. In some cases serological tests may have been conducted before the epidemic had terminated. Te Punga *et al* (1953) reported an abortion rate of 24% in a herd of 88 cows in their second half of pregnancy and at the time of sampling 83% had titres to *pomona*. Hellstrom (1978) investigated a natural outbreak of *pomona* infection in a group of heifers which were all presumed to be in the second half of pregnancy, and found that four of the 17 heifers had aborted (an abortion rate of 24%), and 11 out of 12 heifers tested had developed titres to *pomona* (an infection rate of 92%). In both these cases the investigation may have taken place before the epidemic had completed its natural course. Doherty (1967a) observed a natural epidemic in two herds of cattle initiated by the introduction of three experimentally infected steers. Over a six month period under wet environmental conditions 20 out of 21 heifers (95%) and 26 out of 26 steers (100%) seroconverted. It has therefore been shown that under suitable natural conditions an epidemic of *pomona* in a herd can lead to infection rates of up to 100% if it is allowed to run its course.

Vaccination of cattle with killed *pomona* bacterins usually promotes a protective antibody response in six to ten days (Kenzy *et al*, 1958; Phillips, 1958; Kenzy *et al*, 1961). As abortions usually occur 18 to 32 days after pregnant cows have become infected, it would be expected that vaccination of cattle at risk during an epidemic would have no effect on

the expected abortion rate for three weeks and abortions could continue for a further two weeks but at a lower rate. However, there are reports in the literature of outbreaks of abortions terminating in one to three weeks after vaccination with *pomona* bacterins (Kenzy *et al*,1960; Kenzy *et al*, 1961). South and Stoenner (1975) also claimed that the simultaneous injection of one dose of dihydro-streptomycin (DHS) and *pomona* vaccination terminated three *pomona* storms immediately. However, these last three trials were uncontrolled and the exact proportions of the herds that were infected prior to treatment were not known. There are also conflicting opinions (Clark, 1977; Ryley, 1956) as to whether or not the level of DHS in the foetal circulation can reach bactericidal levels, and eliminate foetal infection.

During the course of this study an opportunity arose to investigate an outbreak of *pomona* abortions in a large dairy herd. This chapter records the results of this investigation and, attempts to trace the source of the infection as well as assessing the effects of vaccination of the herd on the course of the epidemic.

CASE HISTORY

The study farm was a factory supply dairy farm in the Wairarapa district of the North Island. The home property comprised 76 hectares of flat land on two levels separated by a 10m bank and sandhill. Drainage was by mole and tile drains leading into open drains. Most of the drains originated on the property and there was little drainage from neighbouring farms to the property. Another farm, comprising 48 hectares of flat well-drained land, five miles from the home farm, was used as a "run-off" to graze non-milking stock in the winter. The majority of the herd were mated in October or November and were due to calve in July or August. Prior to the outbreak of abortions the whole herd was rotationally grazed

at the home farm until the 8th May when they had all ceased to lactate. At this time, 40 two year old cows were removed from the main herd and grazed separately, having no further contact wtih any other stock. From the 8th May to the 5th June the main herd were grazed at the home farm mainly on flat pastureland, but were put onto a sandy hill paddock on six occasions during periods of heavy rain, between the 12th and the 23rd May. At this time all the cows were 5½ to 7½ months pregnant. Effluent from a small piggery traversed this paddock in a tile drain which overflowed onto the pasture during these periods of heavy rain (Fig.9.1). The two year old cows did not have access to this paddock. On the 6th June both groups of cows were taken up to the "run-off" by truck, but the two year old cows were still kept separate from the main herd. At the "run-off" the 189 cows in the main herd were further separated into two approximately equal groups of Friesian-cross and Jersey-cross cows. These three groups of cows, which were now all 6-8 months pregnant, were grazed in three paddocks as shown in Fig. 9.2. The three paddocks were strip-grazed and all had access to an open watercourse on the western side. The first abortion was noticed in the Friesian-cross group on the 16th June, 12 days after arriving at the "run-off" (see Fig. 9.3). From the 16th to the 26th June, 11 cows aborted (7 Friesian and 4 Jersey-cross cows) and on the 27th June the results of serological tests conducted by the Ministry of Agriculture and Fisheries confirmed the diagnosis of abortions due to *pomona*. On 29th June all animals at the "run-off" were vaccinated with a commercial *pomona/ icterohaemorrhagiae* vaccine*. From the 27th June to the 2nd July there were no abortions. From the 3rd to the 26th July there were 25 abortions with the peak of this secondary epidemic occurring on the 19th and 20th of July. Cows which had aborted were identified and taken back to the home farm by truck and kept in isolation. Those that were beginning to lactate were milked, and the others were sent for slaughter after being held on the farm

* Leptovax - ICI Tasman, Upper Hutt, New Zealand.

FIG.9.1 : PLAN OF HOME FARM PADDOCK WHERE CATTLE WERE EXPOSED TO PIG EFFLUENT.

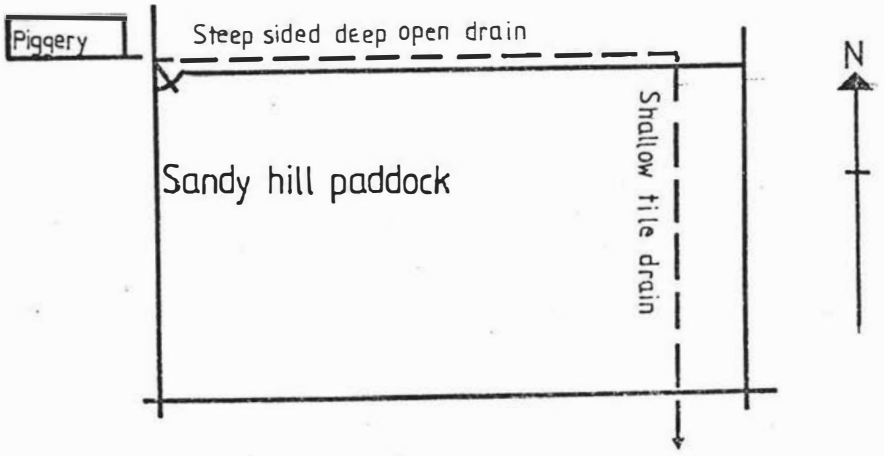


FIG.9.2 : PLAN OF PADDOCKS AT "RUN OFF" WHERE ABORTIONS OCCURRED.

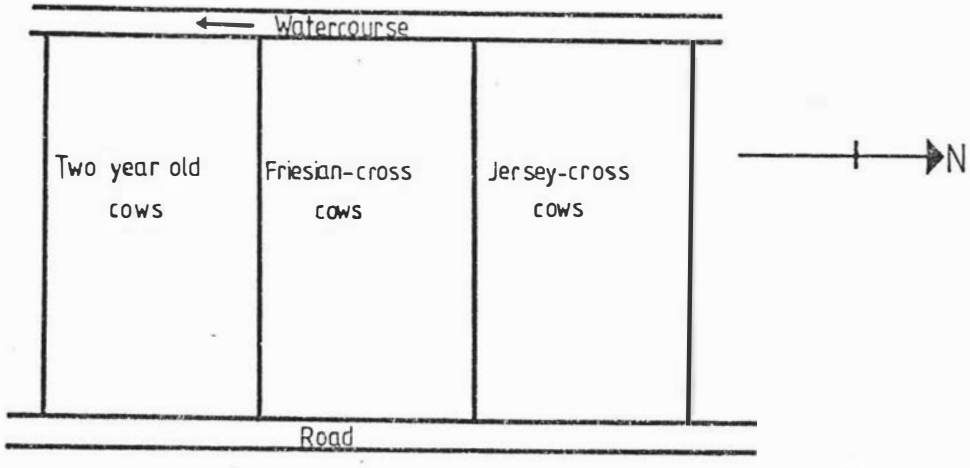
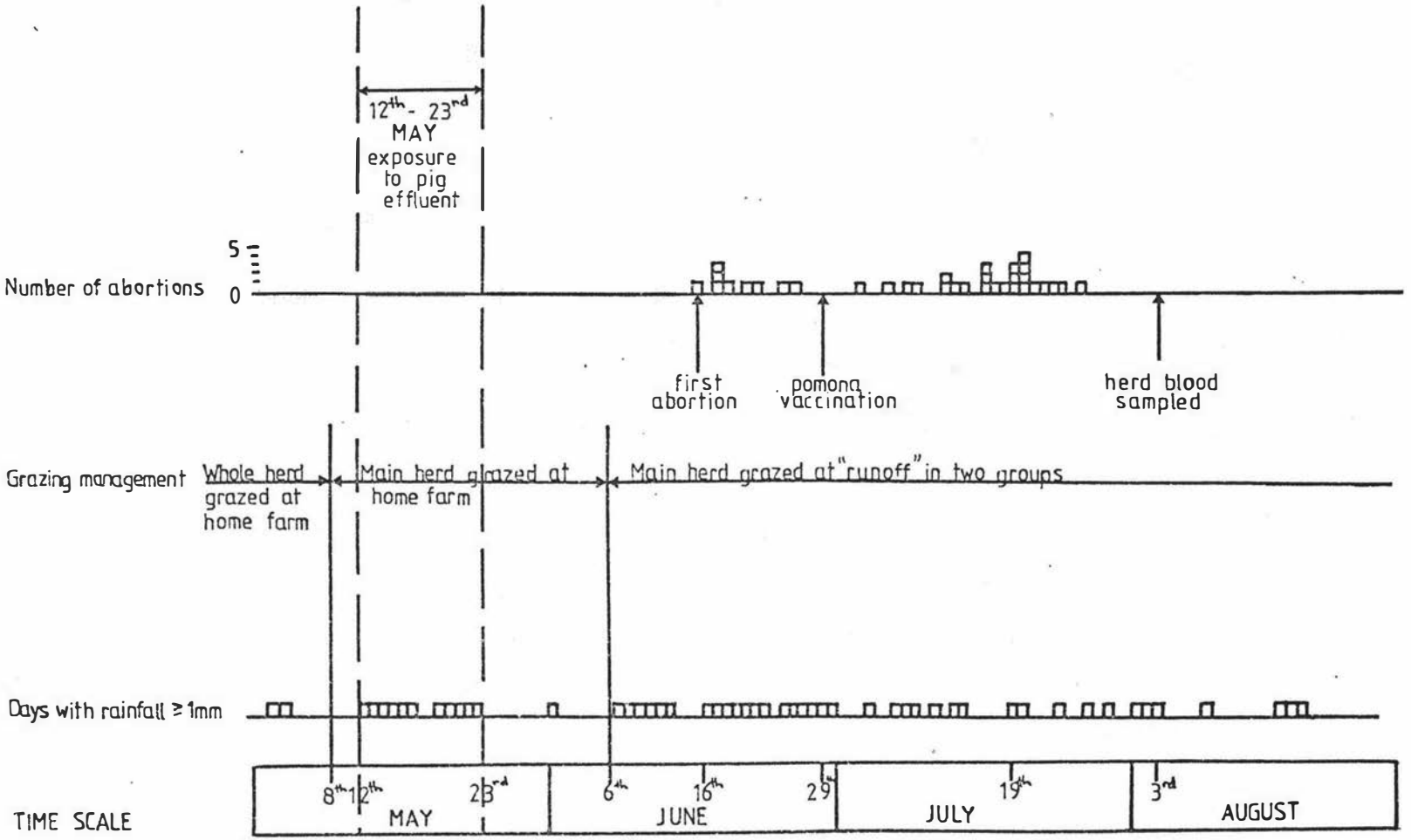


FIG.9.3 : TEMPORAL DISTRIBUTION OF RAINFALL, BOVINE ABORTIONS AND GRAZING MANAGEMENT.



for 30 days. On the 3rd August the whole herd was bled and received a second dose of vaccine. Two weeks later three cows produced mummified calves. At the end of August, parturition was induced in a number of cows by the parenteral administration of corticosteroids and four cows produced mummified or macerated foetuses, and six cows which had been diagnosed as pregnant prior to the outbreak were found not to be pregnant. These last 13 cows were all considered to have had *pomona* and were subsequently found to have high *pomona* titres.

During the period of the heavy rain, 12th-23rd May, there had been two "bacon" pigs in the piggery. These had been bought 4-5 months previously as 12-15 week old weaners from a neighbour who kept a breeding sow. These two "baconers" and the sow had been slaughtered just prior to the investigation and their disease status could not be determined. Cultures of water samples taken from around the piggery at the time of the investigation were all contaminated, and thus it was not possible to determine whether or not leptospires were present.

The farmer stated that none of the cattle on the farm had come into direct contact with any pigs or sheep and no stock had been bought or introduced onto the property that year. The only other animals on the property with which the cows had contact were three cattle dogs which were kept in kennels at the home farm unless working. Rainfall for May, June and July, recorded by the Tauherenikau Meteorological Station nearby is displayed graphically in Fig.9.3. Over this period, the number of wet days (more than 1mm rain) for May, June and July were 12, 19 and 12 respectively and the total rainfall for each month was 78.5, 319.4 and 154 mm respectively.

MATERIALS AND METHODS

Blood samples were collected on the 3rd August from all the cows in the main herd and from 11 of the 40 two year old cows including one two year old that had aborted on the 28th June. Blood samples were also taken from the three dogs. Serological examinations were conducted as described in Chapter Three.

Urine samples were collected from four of the cows that had aborted and were examined by darkfield microscopy and cultured as described in Chapter Three.

One month later urine samples were obtained from the three dogs by bladder paracentesis and these were cultured.

The age and mating dates of each cow were recorded.

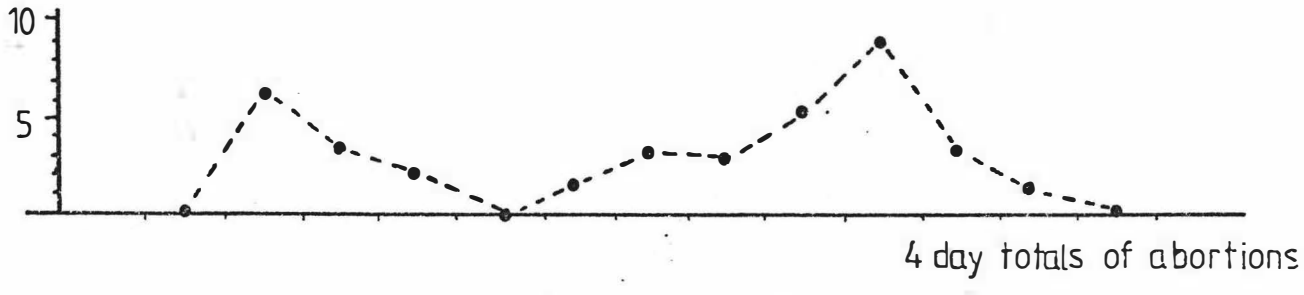
RESULTS

Although most of the animals in the herd had low titres to *hardjo*, only the titres to *pomona* were considered relevant to this outbreak of abortions and these results are summarised in Table 9.1. None of the 11 two year olds, including the one that aborted, had titres to *pomona*. Of the 189 cows in the main herd 43 were seronegative, eight had a titre of 1:24 and 138 (73%) had a titre of 1:48 or greater to *pomona*. The herd had been vaccinated with a *pomona* vaccine five weeks prior to being bled and, as discussed later, it was decided that the majority of titres of 1:48 or greater represented a reaction to *pomona* infection while the majority of titres of 1:24 or less represented vaccinal titres. Using these criteria, the attack rate of infection with *pomona* for the 189 cows in the main herd was 73% while the apparent abortion rate was 25.9%. However, two of the older cows which aborted were seronegative and were regarded as unassociated

with the outbreak under investigation. Therefore the overall abortion rate due to *pomona* in the whole of the main herd was 24.8% compared with zero % in the two year old cows. However, the abortion rate for the 138 actually infected cows was 34%. During the period that the abortions occurred all the cows were between six and nine months pregnant. The cows that aborted were from six and a half months pregnant to full term at the time of their abortion (or delivery of a full term dead foetus).

The titres of infected cows showed a bimodal distribution according to whether or not they had aborted (Table 9.1, Fig. 9.4). The non-aborting infected cows had a GMT of 1:375 (range 1:48 to 1:6144) while the aborting cows (excluding the two that were seronegative) had a GMT of 1:4126 (range 1:384 to 1:98304), a difference which was significant at the 1% level. As discussed later the GMT of non-aborting cows with titres 1:384 or greater was 1:1016, which is still significantly lower than the GMT of the aborting cows ($P < 0.05$).

FIG. 9.5 : TWO CLUSTERS OF ABORTIONS



The abortions occurred in two clusters as shown in Fig. 9.3 and 9.5. The first cluster was from the 16th to 26th June or 38 to 48 days before the animals were bled, and the seven seropositive animals in this group had a

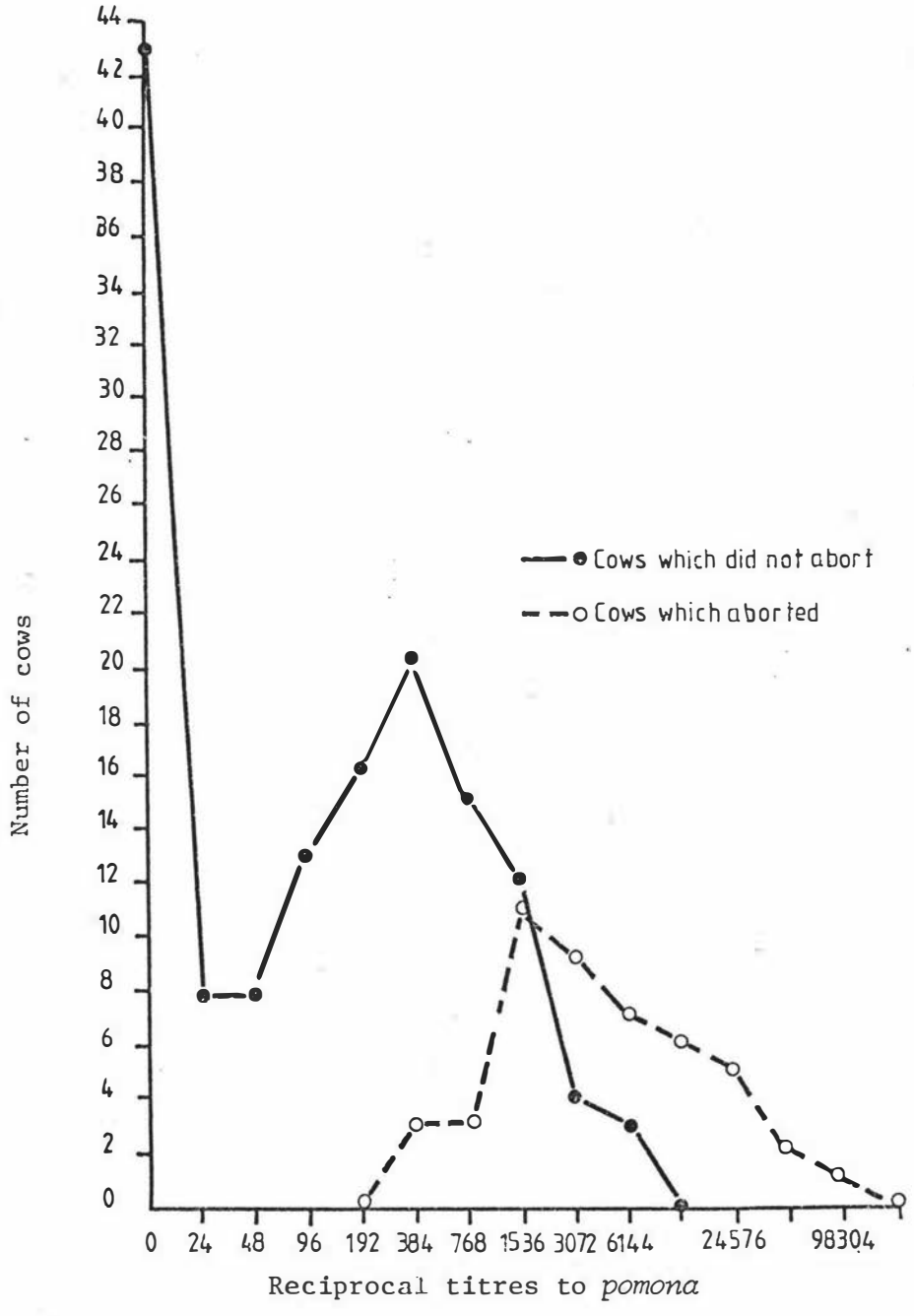
TABLE 9.1 : DISTRIBUTION OF TITRES TO *POMONA* IN ABORTING AND NON-ABORTING COWS*

** Titres to *pomona*	Cows Non-Aborting	Cows classified as aborting				All Cows
		True Abortions	Full-term Dead Foetus	Non-pregnant on induction	Total (%)	
0	41	2			2	43
24	8					8
48	8					8
96	13					13
192	16					16
384	20	2	1		3	23
768	15	3			3	18
1536	12	10		1	11	23
3072	4	8	1		9	13
6144	3	4	1	2	7	10
12288		4		2	6	6
24576		4	1		5	5
49152		1		1	2	2
98304		1			1	1
	140	39	4	6	49 (25.9)	189
No.cows with titres ≥ 1:48	91	37	4	6	47	138
(%)	(48)	(19.6)	(2.1)	(3.2)	(24.8)	(73)
No.cows with titres ≥ 1:192	70	37	4	6	47	117
(%)	(37)	(19.6)	(2.1)	(3.2)	(24.8)	(62)
No.cows with titres ≥ 1:384	54	37	4	6	47	101
(%)	(28.6)	(19.6)	(2.1)	(3.2)	(24.8)	(53.4)
Abortion rate of cows with titres ≥ 1:48 %	0	27	3	4	34	

* Main herd only (cows ≥ 3 year of age)

** Reciprocal titres.

FIG.9.4 : DISTRIBUTION OF TITRES TO *POMONA* IN ABORTING AND NON-ABORTING COWS.



GMT of 1:2444. The second cluster was from the 3rd to the 26th July, 7 to 30 days before the animals were bled, and the 25 cows in this group had a GMT of 1:4656. The difference between the GMT of these two groups is less than one doubling dilution and is not signficant.

Comparison of the distribution of titres and abortions in each age group (Table 9.2) showed that there were no significant differences in the attack rates of infection or abortion between any of the age groups in the main herd.

Of the 39 cows which aborted between June and August, 19 were Jersey-cross cows and 20 were Friesian-cross. Information was not available on the breed of the other ten cows which had dead calves or were found to be nonpregnant.

The three dogs had titres to *pomona* of 1:192, 1:384 and 1:1536.

Culture

Isolates were obtained from three of the four bovine urine samples and from two of the three urine samples from dogs. These isolates all gave patterns of agglutination to 12 C.D.C.* antisera identical to a typed laboratory strain of *pomona*. Darkfield examination of the urine samples revealed leptospires in only three of the four bovine urine samples.

DISCUSSION

Vaccination trials of cattle, as described in Chapter Ten and by authors (Hanson *et al*, 1964; Ris, 1977; Ris and Hamel, 1979) have shown that a single dose of a leptospiral bacterin produced a minimal serological reaction which had usually diminished to a level of 1:24 or less four weeks afte

* Center for Disease Control, Atlanta, Georgia, USA.

TABLE 9.2 : DISTRIBUTION OF TITRES TO *POMONA* AND A COMPARISON OF THE AGE SPECIFIC INFECTION RATES, ABORTION RATES AND GMTs OF COWS IN THE MAIN HERD.

Age (years)	3		4		5		6		7+		Total	
Titres	Whole group	Aborted cows	Whole group	Aborted cows	Whole group	Aborted cows	Whole group	Aborted cows	Whole group	Aborted cows	All cows	Aborted cows
0	10		12	1	12	1	1		5		40	2
24*	0		2		2		1		1		6	0
48	2		1		3		2				8	0
96	4		2		3		3		1		13	0
192	1		3		5		3		4		16	0
384	3		6	2	6		3	1	5		23	3
768	6	1	3	2	5		3		1		18	3
1536	5	4	7	3	2	2	5	1	4	1	23	11
3072	2	1	4	3	4	3	3	2			13	9
6144	2	2	4	2	2	1	1	1	1	1	10	7
12288	0	0	0	0	3	3	1	1	2	2	6	6
24576	0	0	1	1	1	1	1	1	2	2	5	5
49152	0	0					1	1	1	1	2	2
98304	1	1									1	1
Totals	36	9	45	14	48	11	28	8	27	7	184**	49
No. of cows with titres ≥ 1:48	26 (72%)		31 (69%)		34 (71%)		26 (93%)		21 (78%)		138 (75%)	
No. of aborted cows with titres ≥ 1:48	9 (25%)		13 (29%)		10 (21%)		8 (29%)		7 (26%)		47 (25.5%)	
Abortion rate for cows with titres ≥ 1:48	34.6%		38.2%		29%		30.7%		33.3%		34.3%	
GMT of titres ≥ 1:48 for each group	1:709		1:960		1:708		1:832		1:1260		1:853	

* reciprocal titres
** excluding five cows of unknown age.

vaccination, although a small percentage of titres of 1:48 and 1:96 still persisted. The distribution of titres found in the main herd was indicative of two populations of cattle with different serological responses. The majority of animals with titres of 1:48 or greater were considered to be cows naturally infected with *pomona* and the majority of animals with titres of 1:24 or less were considered to be uninfected cattle which had been vaccinated. However, all the cattle that aborted had titres 1:384 or greater, whereas 37 non-aborting cows had titres of 1:48 to 1:192. Previous work has shown that natural *pomona* infection in unvaccinated cattle usually resulted in a titre of 1:300 or greater (Doherty, 1967b ,c). Therefore, it is possible that some of these low titres (1:48 to 1:192) represent infection in vaccinated cattle which were developing vaccinal immunity and a few may have been due to high vaccinal titres. If vaccinal immunity developed while the animal was incubating the disease then in some cases it may have reduced the animal's immune response to active infection, or the natural infection may have boosted the vaccinal response.

It has been shown (Doherty, 1967a) that natural outbreaks of *pomona* infection in cattle can infect up to 100% of animals in a herd if allowed to run their course under suitable environmental conditions. The outbreak in the present study occurred over a relatively short period of time in midwinter at a time when rainfall was comparatively high and the cattle were grazed at high stocking densities thus creating ideal environmental conditions for the transmission of infection (Doherty, 1967a; Hellstrom, 1978). Under these conditions up to 100% of susceptible cows might have become infected if the epidemic had been allowed to run its course. In a similar outbreak on another property (Mackintosh,unpub.) a total of 12 abortions occurred in a herd of 240 cows between late March and mid April when the animals in the herd were 5-6 months pregnant. However, a random sample of 20 cases, which were bled in late April, all had titres to *pomona*

of 1:48 to 1:3072 with a GMT of 1:457. These cattle had had contact with pig effluent in January and it is hypothesised that an epidemic of *pomona* had spread through the herd undetected during January, February and March when the herd were in their first half of pregnancy and only the last few animals infected in March and April were in the second half of pregnancy and therefore susceptible to abortion. These results demonstrate that the infection rate of an uninterrupted *pomona* epidemic in a herd of cows can approach 100%, although the abortion rate depends on the gestational status of the herd.

If it is assumed that in this outbreak all the cattle were susceptible to infection and that an infection rate of 100% would have occurred, and that a titre of 1:48 or greater indicated recent infection with *pomona*, then vaccination during this abortion epidemic prevented 27% of animals from becoming infected, a third of which might have aborted.

In this case-study, 25 abortions occurred up to 28 days *post* vaccination (pv) and seven mummified or macerated foetuses were expelled up to 53 days pv. These observations agree with the expected duration of continuing abortions after vaccination and are similar to those of other abortion epidemics which have been reported in the literature (see Introduction). This investigation also emphasises the importance of vaccinating as early as possible in an epidemic. If this herd had been vaccinated a week earlier it might have reduced the size of the second cluster of abortions. However, this second cluster occurred 21-22 days pv and this may have resulted from an increased spread of infection as a result of yarding the cattle for the purposes of vaccination as suggested by previous workers (Te Punga *et al*, 1953; Doherty, 1967a). Obviously, vaccinating animals during an abortion epidemic is a poor alternative to the prophylactic vaccination of cattle.

The bimodal distribution of titres of 1:48 or greater is interesting (Table 9.1, Fig. 9.4). The majority of titres in the first peak are those of infected cows which did not abort while the second peak mainly represents the titres of cows which aborted. The distributions of titres and the GMTs of these two groups are significantly different. It might be suggested that the lower GMT of the non-aborting cows was due to previous infection with *pomona*. However, there was no history of *pomona* infection occurring on the property previously, no cattle had been bought in for some years and also there were similar attack rates of both infection and abortion for all the age groups (Table 9.2). One would expect a lower rate of infection in the older cows if these animals had previously been infected with *pomona* and were therefore no longer susceptible. It is unlikely that the lower titres of the non-aborting group represent infections which took place earlier in the outbreak because the GMT of the first seven cows which aborted were still significantly higher ($P<0.05$). Also, the average gestational period was the same for both aborting and non-aborting groups. As mentioned previously, it is possible that a partial vaccinal immunity may have interfered with the development of a natural immunity in animals infected after vaccination. Alternatively, heavy natural challenge may have boosted the vaccinal titre of some animals. One explanation for the higher titres of the aborting cows is that they may have received greater antigenic stimulation associated with leptospires localising and multiplying in the placentae. It is also possible that those animals experiencing the most intense or prolonged leptospiraemia produced the highest titre and were also the most likely to abort.

The source of *pomona* on this property was not conclusively determined but it appears likely that the two bacon pigs in the sty had been leptospiruric. It appears that the milking herd became infected after the two year olds were separated from the main herd on the 8th May, because this latter group

remained uninfected. It also appears that the infection was introduced before the herd was split into the two groups at the "run-off", as they were both equally affected. There were two clusters of abortions suggestive of primary and secondary attacks as part of a typical propogating epidemic. The primary attack of abortions involved animals which had been exposed either to the primary source of infection or to a small number of cattle which became infected 2-3 weeks previously, but were not seen to be clinically affected. If it is accepted that the pig effluent was the original source of infection then the cattle were exposed to this between the 12th and 23rd May on six occasions. A group of cattle exposed to this effluent towards the end of this period may have aborted 24-34 days after exposure resulting in the first cluster of abortions. These animals would have become leptospiruric approximately 15 days after exposure with a peak intensity of leptospiruria in 25-35 days pi and resulting in a large number of infected cattle which would have started to abort three to four weeks later. Alternatively, the primary outbreak could have involved a small number of cattle, possibly even one, which had been exposed to the pig effluent on the 12th or 13th May and became leptospiruric in approximately 15 days but failed to abort and was therefore not detected. They would have infected a second group of cattle, some of which would have aborted approximately three weeks later causing the first cluster of abortions.

The three dogs were probably infected during the outbreak after exposure to leptospiruric cows and infected foetal membranes. They were probably "dead end" hosts in this outbreak and it is unlikely that they were responsible for initiating it. The significance of leptospirosis in farm dogs is discussed further in Chapter Six.

This investigation confirms previous observations that *pomona* abortions in cattle can occur during an outbreak for at least four weeks after vaccination of susceptible cattle. It suggests that losses may be reduced by vaccinating as early as possible in an outbreak, although the yarding of cattle may increase the risk of spread of infection at the time of vaccination. It is also evident that vaccination in the face of an outbreak of abortion is a very poor alternative to routine vaccination to prevent an outbreak occurring.

SUMMARY

1. An epidemic of abortions due to *pomona* in a commercial dairy herd was investigated. Two weeks after the first abortion had occurred all animals in the herd were vaccinated with a *pomona/icterohaemorrhagiae* bacterin. Attempts were made to assess the effects of this intervention on the course of the epidemic.

2. Approximately 73% of cattle developed titres to *pomona* of 1:48 or greater and the distribution of these titres indicated that the majority of these animals had been recently infected.

3. Of these infected cattle 27% aborted, 3% had mummified or macerated foetuses and 4% were found not to be pregnant.

4. The majority of abortions occurred up to four weeks after vaccination. A small number of mummified or macerated foetuses were expelled 50-60 days after vaccination when parturition was induced with corticosteroids.

5. Cows which aborted had a higher GMT (1:4126) to *pomona* than infected cows which did not abort (1:375).

6. If it is assumed that in this outbreak an infection rate of 100% could have been expected and that a titre of 1:48 or greater indicated recent infection with *pomona*, then vaccination during this abortion epidemic prevented 27% of animals from becoming infected, a third of which might have aborted.

7. Yarding the cattle for the purpose of vaccination may have increased the spread of *pomona* and resulted in the cluster of abortions which occurred 21-22 days after vaccination.

8. The most likely source of infection was pig effluent.

9. Three farm dogs were probably infected by the cattle during the outbreak but they appeared to be "dead end" hosts.

CHAPTER TEN

VACCINATION TRIALS

INTRODUCTION

As stated in Chapters One and Four, N.Z. has one of the highest incidence rates of human leptospirosis reported in the world and dairy farm workers comprise the occupational group most at risk. *Hardjo* and *pomona*, which are maintained by cattle and pigs in this country, account for 99% of the human infections reported to District Offices of the Department of Health (Brockie, 1976). The dramatic rise in the number of cases of human leptospirosis reported annually during the last three decades may be due to increased public awareness, but it may also be due to changes in dairy farming practices as discussed in Chapter Four. Hellstrom (1978) presented some evidence to suggest that the prevalence of *hardjo* infections in cattle may have increased over the last 30 years while the relative importance of *pomona* in cattle has decreased over this period. The survey of Manawatu dairy farmers reported in Chapter Five showed that the prevalence of titres to *hardjo* and *pomona* in dairy farm residents was related to the milking of cows, the sex of the milker, milking large herds in herringbone sheds, the time spent in the milking shed each day and the prevalence of *hardjo* in the milking herd. The conclusion drawn was that the risk to dairy farmers could be reduced by controlling leptospirosis in the dairy cattle. The prevention of outbreaks of *pomona* on dairy farms would also reduce the incidence of leptospirosis in milkers, and the control of *pomona* infection in pigs is important in this respect.

In addition to these public health aspects, the economic losses due to *pomona* abortion epidemics (see Chapter Nine) and leptospiral "redwater" in calves provide further reasons for the control of *pomona* infection in

cattle. There is also some evidence that *hardjo* might be responsible for sporadic abortions in dairy cattle (Holroyd and Smith, 1976; Ellis and Michna, 1976 a, b; 1977; Little *et al*,1980) and *hardjo* has been reported to cause "mastitis" or agalactia in milking cows (Sullivan and Callan, 1970; Hoare and Claxton, 1972; Lake, 1975; Ellis *et al*,1976; Ellis, 1978). Thus the elimination of *hardjo* from a herd may also have some direct economic benefit.

Hellstrom (1978),in a study of the epidemiology of *hardjo* infection in cattle, suggested that "control and eradication" of this serovar could be achieved "through management practices supplemented with vaccination". However, in a recent government publication, Hellstrom (1980) advocated the exposure of young replacement cattle to infected cows so that by the time these replacements entered the herd they would have ceased shedding leptospires in their urine, thereby eliminating the risk to the milker. This theoretical proposition has a number of flaws. It would be difficult to ensure that infection was transmitted to all the young replacement heifers every year, and even if these replacements became infected as yearlings they would still be leptospiruric when they entered the milking herd, since leptospiruria persists for 12 to 14 months (Hellstrom, 1978). Such a scheme would result in the maintenance of *hardjo* infection endemically on the property and, at best, might only reduce the risk to the milker. The most effective means of eliminating the risk to the milker is to eradicate *hardjo* from the dairy cattle on the property and to prevent their reinfection.

The most practical means of achieving eradication of *hardjo* and protection against *pomona* infections in cattle appears to be the use

of an efficient *hardjo/pomona* vaccine. Previous trials have shown *pomona* bacterins to be effective under N.Z. conditions (Webster and Reynolds,1955; McDonald and Rudge, 1957; Hodges, 1977; Ris and Hamel, 1979). An experimental *hardjo/pomona* vaccine*, which satisfied the potency standards recommended by the U.S. Department of Agriculture using hamster protection tests, was made available for field trials in N.Z. to test its efficacy in cattle. Preliminary trials testing the *hardjo* component (Marshall *et al*, 1979 a, b) were encouraging, but in the first trial two vaccinated heifers apparently became infected, possibly as a result of colostral interference as the calves were less than six months of age at the time of vaccination. Nevertheless, leptospiruria was not detected in either of these vaccinates whereas it was present in six out of ten naturally infected control heifers. In the second trial, where vaccinated sheep were artificially challenged with 6×10^8 *hardjo* organisms, isolates were obtained from two of the ten vaccinates, when their kidneys were cultured three weeks after challenge, compared with ten out of ten unvaccinated control sheep. Because of these inconclusive results it was decided to conduct further trials on this *hardjo/pomona* bacterin.

In a vaccine trial it is important to test the ability of the vaccine to promote an immune response capable of protecting the animal from natural challenge and the ultimate aim is to prevent kidney colonisation and leptospiruria. Experimentally it is difficult to simulate natural infection and kidney colonisation. A number of authors have reported difficulty in consistently producing leptospiruria in cattle by artificial challenge with *hardjo* (Strother, 1975; Tripathy *et al*, 1976; Hellstrom, 1978). Thus it can be difficult to test the ability of a *hardjo* vaccine to prevent or significantly reduce the degree or duration of leptospiruria when using artificial challenge of vaccinated and control cattle. This has

* prepared at the Wellcome Research Laboratories, Langley Court, Beckenham, Kent, England.

also been a problem in *pomona* vaccine trials. Killinger *et al* (1970) found that only two out of 15 unvaccinated animals inoculated with *pomona* developed leptospiruria and when killed 28 days pi no isolates were obtained from cultures of their kidneys. However, by chance a natural outbreak of *hardjo* occurred in his experimental animals and although leptospiruria was not detected, 93% of the unvaccinated cattle yielded *hardjo* isolates from cultures of their kidneys compared to 43% of the 15 cattle which had received a single dose of a *hardjo/pomona* vaccine 12 months previously.

In a preliminary trial of the vaccine used in this study (Marshall *et al*, 1979b) natural challenge by *hardjo* resulted in the infection of all ten control cattle and leptospiruria was detected in six of them. Therefore, in the present trials it was decided to use natural *hardjo* challenge by conducting these trials on farms where endemic infection of cattle with *hardjo* was known to be present.

Four trials were conducted. The aim of the first two trials was to test the ability of the *hardjo* component of the *hardjo/pomona* bacterin to prevent infection and the development of leptospiruria in 10 to 12 month old heifers exposed to natural challenge with *hardjo*. It was also hoped to obtain additional information from the control animals in these trials on the duration of leptospiruria and the serological response of cattle to natural infection with *hardjo*.

The aim of the third trial was to test the hypothesis that vaccination of all the milking cows, bulls, yearlings and calves older than six months on a property would eradicate *hardjo* from a commercial dairy herd where it was known to be endemic prior to vaccination.

Hellstrom (1978) suggested that "the introduction of *balcanica* to the national herd might have significant economic and public health implications". It has been shown in this present study that experimental (see Chapter Seven) and natural (see Chapter Eight) infections of cattle with this serovar were not associated with any clinical signs of disease and it was suggested that its natural occurrence is likely to be sporadic. Nevertheless the short term leptospiruria seen in cattle infected with *balcanica* could be a potential public health risk, although probably not of great significance. If a *hardjo* bacterin cross-protected cattle against infection with *balcanica* it would increase the potential usefulness of the vaccine in dairy cattle. Therefore a fourth trial was conducted to determine whether or not bovine antiserum produced in response to the *hardjo/pomona* bacterin would passively crossprotect hamsters against infection with *balcanica*.

GENERAL MATERIALS AND METHODS

Vaccine

The vaccine used in Trials A, B and C was a commercially prepared *hardjo/pomona* bacterin, described in Appendix VI , and 2 ml were administered by subcutaneous injection in the neck. Although the batches of vaccine used in 1978 and 1979 were experimental batches and the vaccine used in 1980 was the product marketed in N.Z. as Leptavoid*, their composition was reputed to be identical.

Serological and Cultural Examinations

These procedures were carried out as described in Chapter Three.

* Wellcome N.Z.Ltd., Otahuhu, Auckland

For the sake of clarity the Materials and Methods and the Results for each of the four trials will be described separately. However, all the findings will be discussed together in the General Discussion.

TRIAL A : HEIFER VACCINATION ON A TOWN SUPPLY FARM

Materials and Methods

Animals and Vaccination : Blood samples were taken from 22 ten month old heifers on a commercial town supply dairy farm and 11, chosen at random, were vaccinated with the first of two doses of the *hardjo/pomona* vaccine. Subsequent serological examinations showed that three of the vaccinated and one of the unvaccinated heifers already had titres to *hardjo*. Therefore, only the eight animals which were seronegative at the time of their first vaccination were included in the vaccinated group and revaccinated four weeks later. The ten unvaccinated heifers which were seronegative at the first sampling acted as controls.

Challenge : Challenge was by natural transmission from four heifers which had been shown to be infected with *hardjo* at the commencement of the trial, and a number of infected cattle which were periodically grazed with the experimental heifers during the trial. Further challenge to the vaccinated animals was provided by the unvaccinated controls which became infected during the course of the trial.

Sampling times : Blood samples were taken from the heifers at the time of the first and second vaccination and thereafter at approximately monthly intervals although management procedures prevented a number of the animals from being sampled during a three month period between the 26th and 38th week of the experiment. In the second and third years of the trial the animals

were sampled every two to three months.

A midstream urine sample was collected from each animal one week after the second vaccination and on each subsequent occasion when blood samples were taken.

Results

Cultural examinations : During the 28 months of the trial, leptospires were isolated from nine of the ten unvaccinated control animals, compared with two of the eight vaccinated animals ($P < 0.05$). During this time 52 of the 165 urine samples (31.5%) from unvaccinated heifers were culturally positive, whereas only two of the 135 urine samples (1.5%) from the vaccinates were positive ($P < 0.01$). Leptospires were cultured on an average of 5.8 occasions (range 3 to 9) from each of the infected controls and on only one occasion each from two of the vaccinates. Five weeks after the commencement of the trial three unvaccinated animals were shedding leptospires and two more were leptospiruric by the eighth week. Fifty one to 56 weeks after the commencement of the trial, nine of the unvaccinated animals were leptospiruric while none of the vaccinates were leptospiruric at that time (Table 10.1).

The duration of detectable leptospiruria in the naturally infected control heifers ranged from 7 to 82 weeks with an average of 53.1 weeks. The time from the first detectable *hardjo* titre to the last isolation ranged from 8 to 83 weeks with an average of 58.8 weeks. The three heifers which were seropositive at the time they were vaccinated were leptospiruric for 21 to 51 weeks after vaccination, and this was not significantly different from the nonvaccinated control heifers.

TABLE 10.1 : *HARDJO* ISOLATIONS FROM THE URINE OF VACCINATED AND UNVACCINATED HEIFERS OVER A PERIOD OF TWO AND A HALF YEARS

(TRIAL A)

Vaccinates	Weeks of the trial (sampling times) 0	4	5	8	12	15	21	26	31	38	42	47	51	56	64	69	78	87	100	109	124	Total isolations
21	V	V	–	–	–	–	–	NE	NE	–	–	–	–	–	–	–	–	–	–	–	–	0
26	V	V	–	–	–	–	–	NE	NE	–	–	–	–	–	–	–	–	NE	–	–	–	0
29	V	V	–	–	–	–	–	NE	NE	–	–	–	–	NE	–	–	–	–	–	–	–	0
32	V	V	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	1
35	V	V	–	–	–	–	NE	NE	NE	–	+	–	–	–	–	–	–	–	–	–	NE	1
37	V	V	–	–	–	–	–	–	NE	–	–	–	–	–	–	–	–	NE	–	–	NE	0
40	V	V	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0
43	V	V	–	–	–	–	–	NE	NE	–	–	–	–	–	–	–	–	–	–	–	–	0
Non Vaccinates																						2/135
18	NE	NE	–	+	–	+	+	NE	NE	–	–	–	–	+	–	–	+	–	–	–	–	5
20	NE	NE	–	+	+	+	–	–	+	–	+	–	+	+	–	–	+	NE	–	–	–	8
22	NE	NE	+	+	+	+	+	NE	NE	–	+	–	+	+	–	–	–	+	–	–	NE	9
28	NE	NE	–	–	+	+	–	+	+	–	–	–	+	+	–	–	+	NE	–	–	–	7
30	NE	NE	+	+	+	–	–	NE	NE	–	–	NE	–	–	–	–	–	NE	–	–	–	3
33	NE	NE	–	–	+	–	+	NE	NE	–	NE	–	+	+	+	–	–	NE	–	–	–	5
34	NE	NE	–	–	–	–	–	+	NE	–	+	–	+	+	–	–	+	NE	–	–	NE	5
36	NE	NE	–	–	–	–	–	–	+	–	–	+	+	+	+	–	–	NE	–	–	–	5
39	NE	NE	+	–	–	–	–	+	+	–	+	–	+	–	–	–	–	NE	–	NE	–	5
41	NE	NE	–	–	–	–	–	NE	NE	–	–	NE	–	–	–	–	–	–	–	–	–	0
																						52/166

1978			1979				1980			
WINTER	SPRING	SUMMER	AUTUMN	WINTER	SPRING	SUMMER	AUTUMN	WINTER	SPRING	

V = time of vaccination
– = urine culture negative
+ = confirmed *hardjo* isolation
NE = not examined
□ = in the drystock herd
▨ = in the milking herd

All urine isolates were identified as belonging to the Hebdomadis serogroup. Subsequently three of the isolates were shown to give restriction endonuclease patterns identical to previous field isolates of *hardjo*.

Serological examinations : The vaccinated heifers were serologically negative to *hardjo* four weeks after the first vaccination, but all had seroconverted after the second dose, with titres to *hardjo* ranging from 1:24 to 1:384 (Table 10.2). Three of the vaccinates had titres to *pomona* four weeks after the first dose and all had seroconverted four weeks after the second dose with titres ranging from 1:96 to 1:192 (Table 10.3). These vaccinal titres declined rapidly and all titres to *hardjo* and *pomona* were less than 1:96 by 12 weeks after the second vaccination. All titres to *hardjo* were less than 1:24 by 17 weeks after the second vaccination and *pomona* titres were less than 1:24 by 27 weeks.

Three of the unvaccinated, initially seronegative, control heifers had titres to *hardjo* four weeks after the trial commenced and by eight weeks all ten in this group had *hardjo* titres. The maximum titres recorded for each of these control heifers ranged from 1:96 to 1:384 with a GMT of 1:206, which is less than one dilution lower than the GMT of 1:266 reported by Hellstrom (1978) for a group of heifers five weeks after infection. This slightly lower GMT may reflect a reduction in antigen sensitivity or a difference in the reading of the endpoint of the MAT. By the end of the trial, 116 to 120 weeks after these animals became infected, 8 out of 10 still had titres of 1:24 or 1:48 to *hardjo*. However, the titre of heifer 34 had declined to less than 1:24 within three months of becoming infected although on four of the 14 subsequent tests it had a titre of 1:24. On the other occasions the level of agglutination was less than 50% at 1:24. Nevertheless

TABLE 10.2 : RECIPROCAL TITRES TO *HARDJO* OF VACCINATED AND UNVACCINATED HEIFERS (TRIAL A)

	Weeks of the trial (sampling times)																				
	0	4	5	8	12	15	21	26	31	38	42	47	51	56	64	69	78	87	100	109	124
21	0*	0	NE	24	0	0	0	NE	NE	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	NE	192	48	48	0	NE	NE	0	0	0	0	0	24	0	0	NE	0	0	0
29	0	0	NE	96	24	0	0	NE	NE	0	0	0	0	NE	0	0	0	NE	0	0	0
32	0	0	NE	192	96	48	0	0	0	0	0	0	0	0	24	24	0	0	0	0	0
35	0	0	NE	192	48	24	NE	NE	NE	0	0	0	0	0	0	0	0	0	0	0	NE
37	0	0	NE	384	48	24	0	0	NE	0	0	0	0	0	0	0	0	NE	0	0	NE
40	0	0	NE	192	48	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	NE	96	48	24	0	NE	NE	0	0	0	0	0	0	0	0	0	0	0	0
GMT**	0	0	NE	135	48	34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	NE	192	96	48	24	NE	NE	24	48	24	24	0	0	24	24	24	48	24	0
20	0	0	NE	384	192	192	192	96	96	48	96	96	96	96	48	48	96	NE	96	48	48
22	0	384	NE	192	192	96	24	NE	NE	24	48	48	48	24	24	24	48	48	48	48	NE
28	0	0	NE	384	384	384	384	192	192	96	192	96	192	192	96	192	96	48	96	48	48
30	0	96	NE	96	192	96	96	NE	NE	192	96	96	48	24	48	96	96	NE	24	48	48
33	0	0	NE	384	192	192	48	NE	NE	24	NE	48	48	24	48	48	48	NE	24	24	24
34	0	0	NE	96	24	0	0	0	NE	0	0	24	24	0	0	24	0	0	24	0	NE
36	0	0	NE	24	192	96	48	96	48	96	96	96	96	48	96	96	96	48	96	48	48
39	0	192	NE	96	48	48	24	24	24	48	48	48	96	96	48	48	48	NE	48	NE	24
41	0	0	NE	192	192	96	96	NE	NE	48	48	96	96	24	48	48	48	48	48	48	48

* 0 < 1:24
** GMT of titres ≥ 1:24
NE = not examined

in the drystock herd

in the milking herd

TABLE 10.3 : RECIPROCAL TITRES TO *POMONA* OF VACCINATED HEIFERS (TRIAL A)

Vaccinates	Weeks of the trial (sampling times)																				
	0	4	5	8	12	15	21	26	31	38	42	47	51	56	64	69	78	87	100	109	124
21	0*	0	NE	96	24	0	0	NE	NE	0	0	0	0	0	0	0	0	0	0	0	0
26	0	48**	NE	192	96	48	0	NE	NE	0	0	0	0	0	0	0	0	NE	0	0	0
29	0	0	NE	96	48	24	0	NE	NE	0	0	0	0	NE	0	0	0	NE	0	0	0
32	0	48	NE	192	96	48	48	24	0	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	NE	96	96	48	NE	NE	NE	0	0	0	0	0	0	0	0	0	0	0	NE
37	0	0	NE	192	48	24	0	0	NE	0	0	0	0	0	0	0	0	NE	0	0	NE
40	0	24	NE	96	96	48	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	NE	192	96	48	0	NE	NE	0	0	0	0	0	0	0	0	0	0	0	0
GMT**	0	38	NE	135	68	39	34	24	0	0	0	0	0	0	0	0	0	0	0	0	0

*0 < 1:24

** GMT of titres ≥ 1:24

NE = not examined

in the dry stock herd

in the milking herd

this animal was leptospiruric for at least 70 weeks after it had seroconverted. The two vaccinated heifers which were both shown to be leptospiruric on one occasion both had titres less than 1:24 to *hardjo* at the time of leptospiruria, although on two subsequent occasions heifer 32 had a titre of 1:24.

In order to compare post-vaccinal titres with titres arising as a result of infection, the GMT of each group of animals was calculated at each sampling for the first 56 weeks of the trial. For the unvaccinated group these have been related to the time of infection, which, for convenience, has been assumed to be four weeks prior to the first demonstration of agglutinating antibody. The first GMT was therefore calculated using each animal's first positive titre; the second GMT from each animal's second positive titre and so on (see Figure 10.1).

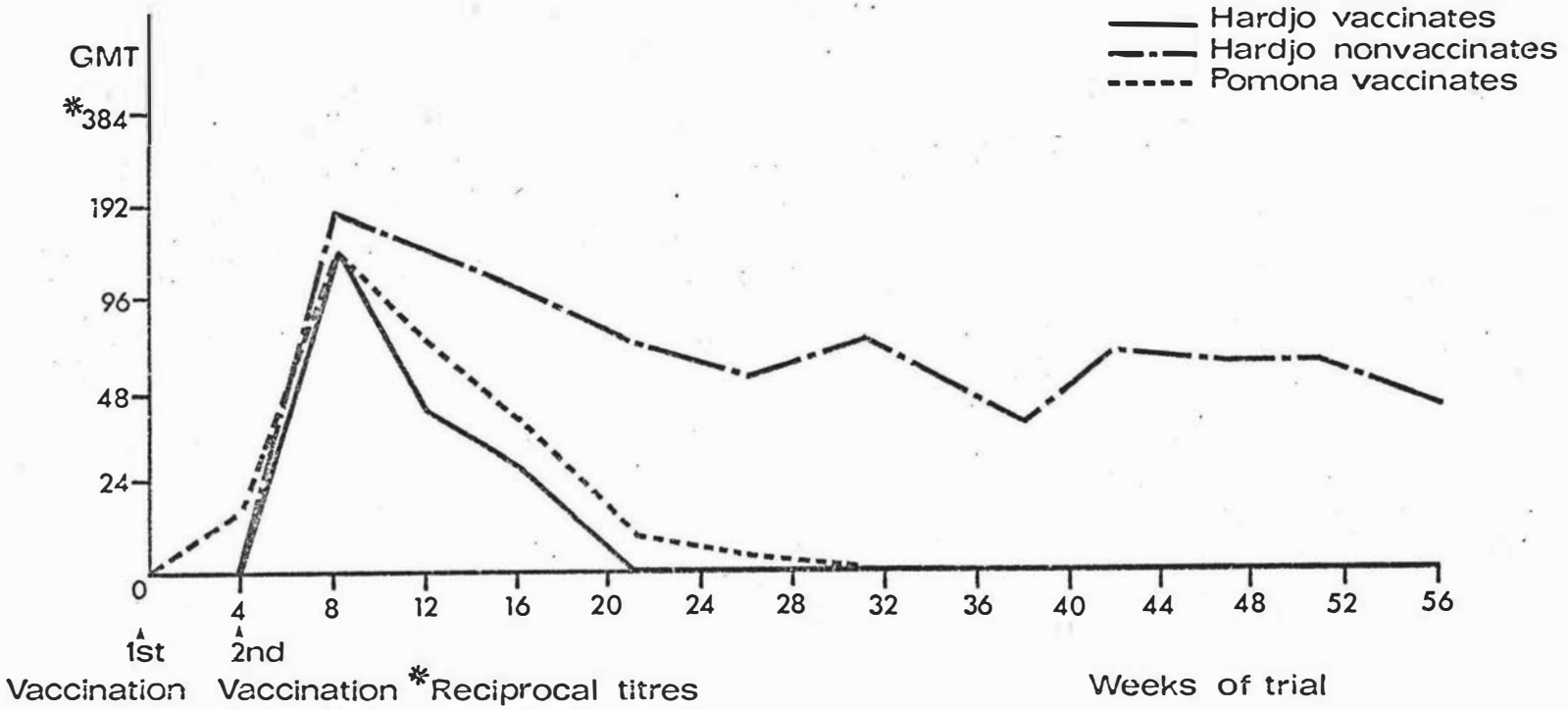
All unvaccinated heifers remained seronegative to *pomona* throughout the trial.

TRIAL B : HEIFER VACCINATION ON A FACTORY SUPPLY FARM

Materials and Methods

Animals and vaccinations : Twenty-three 11 month old heifers, including nine pairs of identical twins, on a factory supply farm at Massey University had blood samples taken and six of the nine pairs of twins were chosen at random and vaccinated with two doses, four weeks apart, of the *hardjo/pomona* vaccine. Unfortunately other experimental demands necessitated that both heifer twins in each pair be treated identically, otherwise one of each pair would have been vaccinated and the other used as an unvaccinated control. The 11 unvaccinated heifers were to act as controls throughout the experiment. Unfortunately, five of these control heifers were culled for management reasons halfway through the first year of the trial. However, two

FIG.10.1 : GEOMETRIC MEAN TITRES FOR VACCINATES AND NONVACCINATES (TRIAL A).



other unvaccinated heifers (15 and 16) and a number of dry cows were run with the group at various times and were monitored for evidence of infection.

Challenge: Just prior to the commencement of the trial a random sample of ten of the 11 month old heifers intended for the vaccine trial were all seronegative while nine out of ten 20-22 month old yearling heifers had titres to *hardjo*. It was assumed that the majority of these yearling heifers would have been leptospiruric. It was hoped that *hardjo* would have been transmitted to the group of trial heifers by a small number of the yearlings that had failed to become pregnant and were to be grazed with the calves during the following year.

Sampling times : Blood samples were taken at the time of the first and second vaccinations and then at four week intervals until the 20th week pi after which time the heifers were taken to a "runoff" for summer grazing and were unavailable for testing. They were returned to the property 44 weeks pi and were then sampled every two to three months until the end of the trial.

Urine samples were collected each time the animals were bled starting from four weeks after the second vaccination.

Results

Cultural and serological examinations : Confirmed *hardjo* isolates were obtained from four of the eight control heifers and from none of the vaccinated heifers during this 123 week trial (Table 10.4). The vaccinal titres to *hardjo* and *pomona* declined to 1:24, 16 to 20 weeks after vaccination (Tables 10.4 and 10.5). The first evidence of *hardjo* infection in the trial animals was detected 44 weeks after the trial started, on the

TABLE 10.4 : *HARDJO* ISOLATIONS AND RECIPROCAL TITRES TO *HARDJO* OF VACCINATED AND UNVACCINATED HEIFERS (TRIAL B)

Vaccinates	Week of the trial (sampling times)													Isolation
	0	4	8	12	16	20	44	52	60	67	75	84	93	123	
13	V 0	V-24	-96	-96	-24	- 0	NE	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
14	V 0	V-24	-192	-96	-24	-48	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
19	V 0	V- 0	-48	-48	-48	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
20	V 0	V- 0	-48	-24	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
79	V 0	V-24	-48	-48	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	C	-
80	V 0	V- 0	-48	-24	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	C	-
87	V 0	V- 0	-48	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
88	V 0	V- 0	-24	-24	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
101	V 0	V- 0	- 0	-48	- 0	NE	NE	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
102	V 0	V- 0	-96	-48	-24	NE	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
121	V 0	V- 0	-96	-48	- 0	- 0	NE	- 0	- 0	- 0	- 0	- 0	- 0	C	-
122	V 0	V- 0	-48	-48	-24	- 0	NE	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
Non-vaccinates															
15	NE	NE	- 0	- 0	NE	NE	NE	NE	NE	-192	-192	-48	-48	-48	-
16	NE	NE	- 0	- 0	NE	NE	NE	NE	NE	+192	+384	-96	-96	-48	+
45	0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
46	0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
81	0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	- 0	-
82	0	- 0	- 0	- 0	- 0	- 0	-96	+96	-192	-384	-192	-48	-96	-48	+
119	0	- 0	- 0	- 0	NE	NE	NE	NE	NE	-384	+384	-192	+192	-96	+
120	0	- 0	- 0	- 0	NE	NE	NE	NE	NE	-192	-192	- 96	+192	-96	+
32	0	- 0	- 0	- 0	- 0	- 0	C								
168	0	- 0	- 0	- 0	- 0	- 0	C								
171	0	- 0	- 0	- 0	- 0	- 0	C								
192	0	- 0	- 0	- 0	- 0	- 0	C								
68 *				-48	-96	+48									
10 *				-192	+192	-192									

{ = identical twin heifers
V = time of vaccination
0 < 1:24
NE = not examined

- = urine culture negative
+ = confirmed *hardjo* isolation
C = culled
□ = in dry stock herd

* two newly introduced cows grazed with the trial animals between the 12 &20th weeks of the trial
▨ = in milking herd

TABLE 10.5 : RECIPROCAL TITRES TO *POMONA* OF VACCINATED HEIFERS (TRIAL B)

Vaccinates	Weeks of the trial (sampling times)														
	0		4	8	12	16	20	44	52	60	67	75	84	93	123
13	V	0	V 24	768	192	96	24	NE	0	0	0	0	0	0	0
14	V	0	V 24	1536	384	96	96	0	24	0	0	0	0	0	0
19	V	0	V 96	768	192	192	96	48	48	24	0	0	0	0	0
20	V	0	V 48	384	96	48	48	0	0	0	0	0	0	0	0
79	V	0	V 0	96	96	48	48	0	0	0	0	0	0	0	C
80	V	0	V 0	192	48	48	24	24	24	0	0	0	0	0	C
87	V	0	V 0	768	96	48	48	0	24	0	0	0	0	0	0
88	V	0	V 0	48	96	96	24	0	0	0	0	0	0	0	0
101	V	0	V 24	192	96	48	NE	NE	0	0	0	0	0	0	0
102	V	0	V 96	768	192	192	NE	24	0	0	0	0	0	0	0
121	V	0	V 0	384	96	24	48	NE	0	0	0	0	0	0	C
122	V	0	V 0	192	48	96	48	NE	0	0	0	0	0	0	0

{ = identical twin heifers
V = time of vaccination
0 < 1:24
NE= not examined
C = culled
□ in dry stock herd
▨ in milking herd

first sampling after the animals had returned from the "runoff". This was despite their exposure to two leptospiruric non-lactating cows which were run with the trial animals for two months, from 12 to 20 weeks after vaccination. At the next sampling, eight weeks later, the first isolate was obtained from a urine sample from this animal. The following month, 11 of the 12 vaccinates and three of the eight control animals entered the milking herd where evidently there was no natural challenge as none of the control heifers became infected. However, infection was present in the group of non-lactating cattle as the remaining four control animals seroconverted and isolates were obtained from three of them. The vaccinated heifer in this non-lactating group remained serologically and culturally negative throughout this 30 week period. The following autumn, the herd ceased lactating and joined the non-lactating group. Two of the animals in this latter group, numbers 119 and 120, were shown to be still leptospiruric. However, neither the three remaining seronegative controls nor the vaccinates had seroconverted or had detectable leptospiruria by the end of the trial 40 weeks later.

TRIAL C : WHOLE HERD VACCINATION

Materials and Methods

Animals : A local Manawatu farmer allowed his commercial factory supply dairy herd to be used for a vaccine trial. This farm, comprising 87.8 hectares of gently rolling land, carried approximately 220 milking cows, 50 yearling heifers, 60 calves and five bulls. The farmer had contracted leptospirosis due to *hardjo* two years prior to the start of the trial. Six months prior to the start of this trial Hellstrom (1978) found titres to *hardjo* ranging from 1:48 to 1:192 in seven cows chosen at random from this herd.

One month prior to the start of the trial, eight two year old cows chosen at random were bled and all had titres to *hardjo* which ranged from 1:24 to 1:96. This high prevalence of titres to *hardjo* suggested that *hardjo* infection was endemic in the herd. It is likely that these animals had been infected the previous spring when they entered the herd and that the GMT of this group had declined to 1:78 at the time of the sampling which took place in winter. This apparent rate of titre decay is of the same order as that reported by Hellstrom (1978) who recorded a GMT of 1:96 in a group of 17 cattle ten months after infection, and the majority of these animals were leptospiruric for at least 12 months.

Vaccination : The trial commenced in June 1978 when all the calves, yearlings, cows and bulls on the property received their first dose of vaccine. This was followed four weeks later by a second vaccination. All these animals subsequently received an annual revaccination in May 1979 and 1980. Each new season's group of calves received their initial two doses of vaccine in May and June in 1979 and 1980, and their annual revaccination the following May.

Sampling times : All animals were bled at the time of their first vaccination, June 1978, to determine their immunological status. Subsequently all animals were bled in December of each year, six months after vaccination, and each season's calves and yearlings were also sampled at the time of their vaccination in May or June. The group of animals which were born in spring 1978 were also bled two weeks, four weeks, five months and eight months after receiving their first annual revaccination in order to measure their serological response to revaccination and the rate of decay of these titres.

Purchased animals : Halfway through the trial, in the winter of 1979, the farmer purchased 25 yearling heifers in three groups from local farmers. All these heifers were bled and vaccinated on arrival and were kept in isolation for ten days on the farm. The third group were known to have come from a farm which had recently experienced an epidemic of *pomona* infections and all six of these heifers received a single injection of streptomycin at a dose rate of 35mg/kg in addition to vaccination.

Urine sampling : Shortly after receiving their first annual revaccination the group of animals which were born in spring 1977 entered the milking herd. Six months after this vaccination these animals were found to have titres to *hardjo* up to 1:192 and to *pomona* up to 1:768. In order to investigate the possibility that these titres were due to infection rather than vaccination, ten of the 43 animals in this group were chosen at random and urine samples were taken for culture.

In November and December 1980, after the trial had been in progress for 30 months, urine samples were taken from ten animals, chosen at random, from each of the following four age groups of cattle : yearling heifers, two year old cows, three year old cows and cows aged four years and over.

Control animals : In an attempt to detect the possible presence of *hardjo* or *pomona* infection two seronegative yearling steers were run with the herd for three months towards the end of the trial, from September to November 1980. These animals were bled before introduction and at four weekly intervals throughout the three month period.

Management practices : Throughout the trial period, the calves which

were bucket reared on whole milk and weaned at 8-10 weeks, were grazed in small numbers in paddocks distributed around the farm during the milking season. The yearling heifers were grazed on another property from June 1st to May 31st the following year. While at that property they were grazed with two young bulls bred on the home property. On returning to the farm, they joined the milking herd. Over 75% of the herd were artificially inseminated each year and a home bred bull was used to serve the remainder. Calving commenced at the beginning of August and was completed by the end of September.

Results

Serological examination : The serological results for this trial are summarised according to age groups in Table 10.6. At the start of the trial, when the animals received their first vaccination, all of the 8 - 10 month old calves and 20 - 22 month old yearlings were seronegative. All age groups in the milking herd had evidence of past *hardjo* infection with the highest serological prevalence and the highest GMT in the two year old cows. Only 12 cows had titres to *pomona* and these were from a group of 18 four and five year cattle which had been bought a year earlier from a farm that had experienced an outbreak of *pomona* the previous year.

The groups of seronegative calves, which were vaccinated in three consecutive seasons during the 2½ year trial, all had similar serological prevalences to *hardjo* six to seven months after the initial two doses ranging from 2.3% to 4%. The prevalences of titres to *pomona* ranged from 39.4% to 72%. The seronegative yearlings vaccinated at the start of the trial had higher prevalences of titres to *hardjo* and *pomona* six months after vaccination, with 22.5% and 87.5% seropositive respectively, than did the calves.

TABLE 10.6 : THE PREVALENCE RATES AND GMTs OF TITRES TO *HARDJO* AND *POMONA* OF THE DIFFERENT AGE COHORTS THROUGHOUT THE TWO AND A HALF YEAR TRIAL (TRIAL C).

Time of sampling	Parameters	Age Cohorts: born in spring -													
		1979		1978		1977		1976		1975		1974		1973		1972 -	
		hardjo	pomona	hardjo	pomona	hardjo	pomona	hardjo	pomona	hardjo	pomona	hardjo	pomona	hardjo	pomona	hardjo	pomona
At the start of the trial	age					calves (10 mth)		yearlings (22m)		2 year olds		3 ear olds		4 year olds		5+ year olds	
	number					44		44		27		31		29		52	
	prevalence*					0%	0%	0%	0%	85.2%	0%	51.6%	0%	51.7%	14%	63.5%	15.4%
	GMT**					-	-	-	-	1:48	-	1:34	-	1:45	1:161	1:40	1:88
	range					-	-	-	-	1:24	-	1:24	-	1:24	1:96	1:24	1:48
										-1:192		-1:96		-1:192	-1:384	-1:96	-1:192
6mths after the initial double vaccination	age					yearlings(16m)		2 year olds		3 year olds		4 year olds		5 year olds		6+ year olds	
	number					43		40		24		30		28		52	
	prevalence					2.3%	39.5%	22.5%	87.5%	79.2%	58.3%	73.3%	36.7%	67.8%	57.1%	55.8%	50%
	GMT					1:24	1:33	1:35	1:43	1:41	1:37	1:32	1:31	1:35	1:44	1:36	1:48
	range					1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24
							-1:96	-1:48	-1:384	-1:96	-1:48	-1:48	-1:48	-1:96	-1:192	-1:96	-1:384
At the calves' 1st vaccin tion and the yearlings' 1st annual revaccination	age			calves (9mths)		yearlings(21m)		NE		NE		NE		NE		NE	
	number			56		42											
	prevalence			0%	0%	0%	21.4%										
	GMT			-	-	-	1:28										
	range			-	-	-	1:24										
							-1:48										
7mths after the calves' 1st vacc. and the herd's 1st annual revaccination	age			yearlings(16m)		2 year olds		3 year olds		4 year olds		5 year olds		6 year olds		7+ year olds	
	number			50		39		35		23		28		26		41	
	prevalence			4%	72%	79.5%	89.7%	94.2%	100%	100%	91.3%	100%	85.7%	100%	92.3%	95.1%	78%
	GMT			1:24	1:40	1:66	1:62	1:52	1:62	1;71	1:51	1:73	1:44	1:62	1:51	1:66	1:49
	range			1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24
					-1:192	-1:192	-1:768	-1:96	-1:768	-1:192	-1:192	-1:192	-1:192	-1:96	-1:192	-1:384	-1:384
At the calves' 1st vaccination the yearlings' 1st the herds 2nd annual revaccination	age	calves (9mths)		yearlings(20m)		2 year olds		NE		NE		NE		NE		NE	
	number	50		56		31											
	prevalence	0%	0%	4%	68%	83.9%	96.8%										
	GMT	-	-	1:24	1:39	1:47	1:59										
	range	-	-	1:24	1:24	1:24	1:24										
					-1:192	-1:192	-1:384										
7mths after the calves' 1st vacc. and the herd's 2nd annual revaccination	age	yearlings(16m)		2 year olds		3 year olds		4 year olds		5 year olds		6 year olds		7 year olds		8+ year olds	
	number	50		46		31		30		20		22		23		23	
	prevalence	4%	48%	45.6%	97.8%	83.9%	96.8%	80%	100%	100%	95%	100%	100%	100%	95.6%	91.3%	91.3%
	GMT	1:24	1:30	1:28	1:57	1:35	1:69	1:35	1:76	1:43	1:45	1:42	1:44	1:43	1:48	1:43	1:67
	range	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24	1:24
			-1:96	-1:48	-1:192	-1:96	-1:384	-1:96	-1:192	-1:96	-1:192	-1:96	-1:192	-1:96	-1:192	-1:96	-1:192

* titres $\geq$ 1:24
** geometric mean of titres $\geq$ 1:24
NE not examined

The GMT and prevalences of titres to *hardjo* and *pomona* for all groups were higher six months after the first annual revaccination than they were six months after the initial double vaccination. The group of yearlings monitored for eight months after their first annual revaccination showed a marked anamnestic response which was at its maximum two weeks post-vaccination (pv) (See Figures 10.2 and 10.3). At this time 100% of animals had titres to *hardjo* and *pomona* and these ranged from 1:48 to 1:384 with a GMT of 1:125 to *hardjo*, and 1:192 to 1:3072 with a GMT of 1:737 to *pomona*. However after eight months only 45.6% had titres to *hardjo* and the GMT of these had declined to 1:28 while the prevalence of titres to *pomona* remained close to 100%, although the GMT had fallen to 1:57 (range of titres 1:24 to 1:192).

The two groups of animals that were seronegative at the start of the trial had received four vaccinations by the end of the trial comprising the initial two doses and two annual revaccinations. Seven months after their second revaccination they had a serological prevalence of over 80% to *hardjo* and nearly 100% to *pomona*.

The animals in the milking herd had an overall serological prevalence of 62.6% to *hardjo* at the start of the trial. Six months after the initial double vaccination this had risen slightly to 66.4%. However, six months after the first annual revaccination it had risen to over 98% and remained at this level after the second annual revaccination (Table 10.7). The initial vaccination produced a seroprevalence of 50% to *pomona* and this rose to nearly 100% after the first annual revaccination.

The serological prevalence and GMT of titres of 1:24 or greater to *hardjo* and *pomona* for different age groups at the beginning and end of the trial are shown in Figures 10.4 and 10.5. This demonstrates the change

FIG.10.2 : THE SEROLOGICAL PREVALENCE AND THE RATE OF DECAY OF VACCINAL TITRES TO *HARDJO* OVER A PERIOD OF 18 MONTHS.

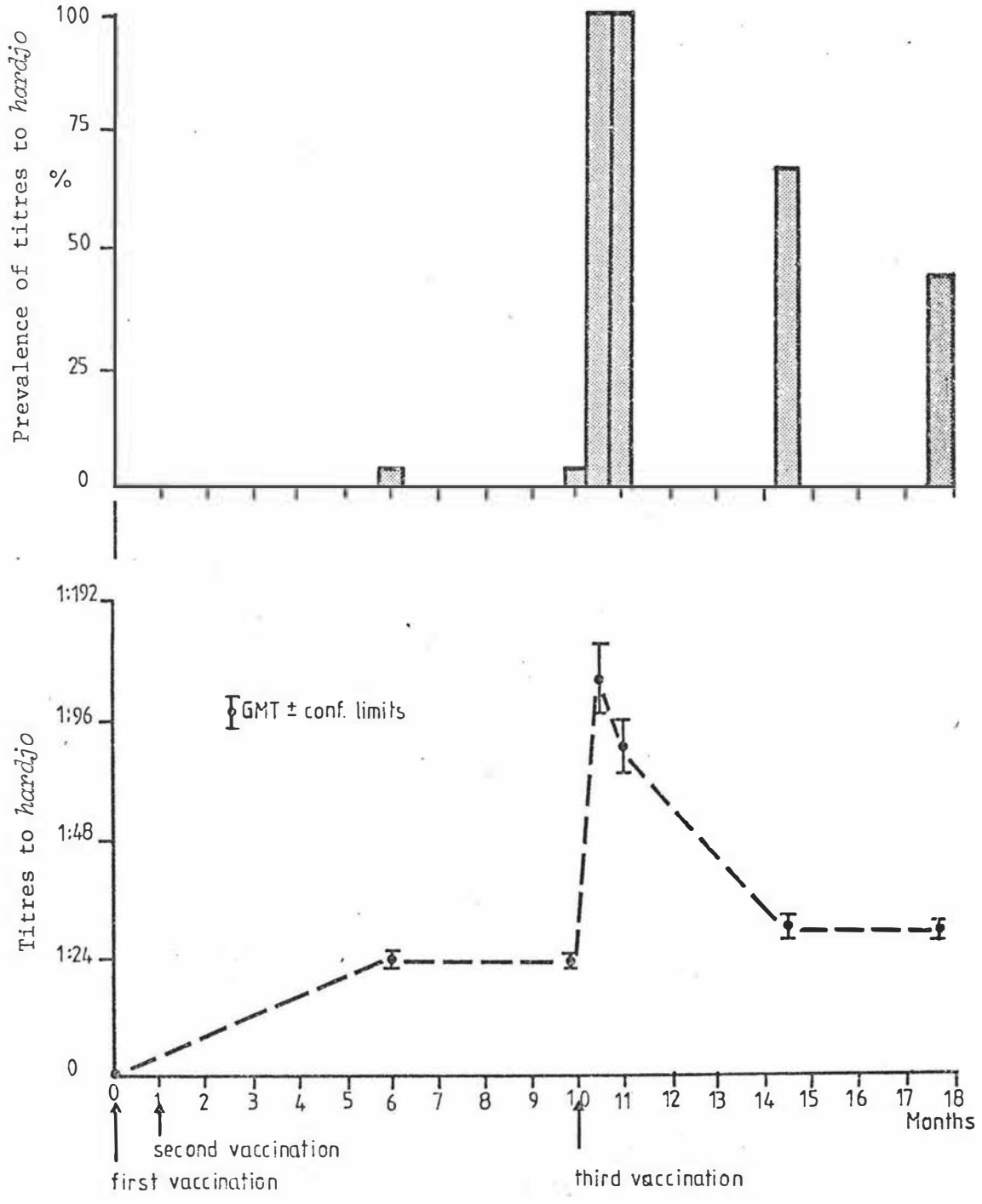


FIG.10.3 : THE SEROLOGICAL PREVALENCE AND THE RATE OF DECAY OF VACCINAL TITRES TO *POMONA* OVER A PERIOD OF 18 MONTHS.

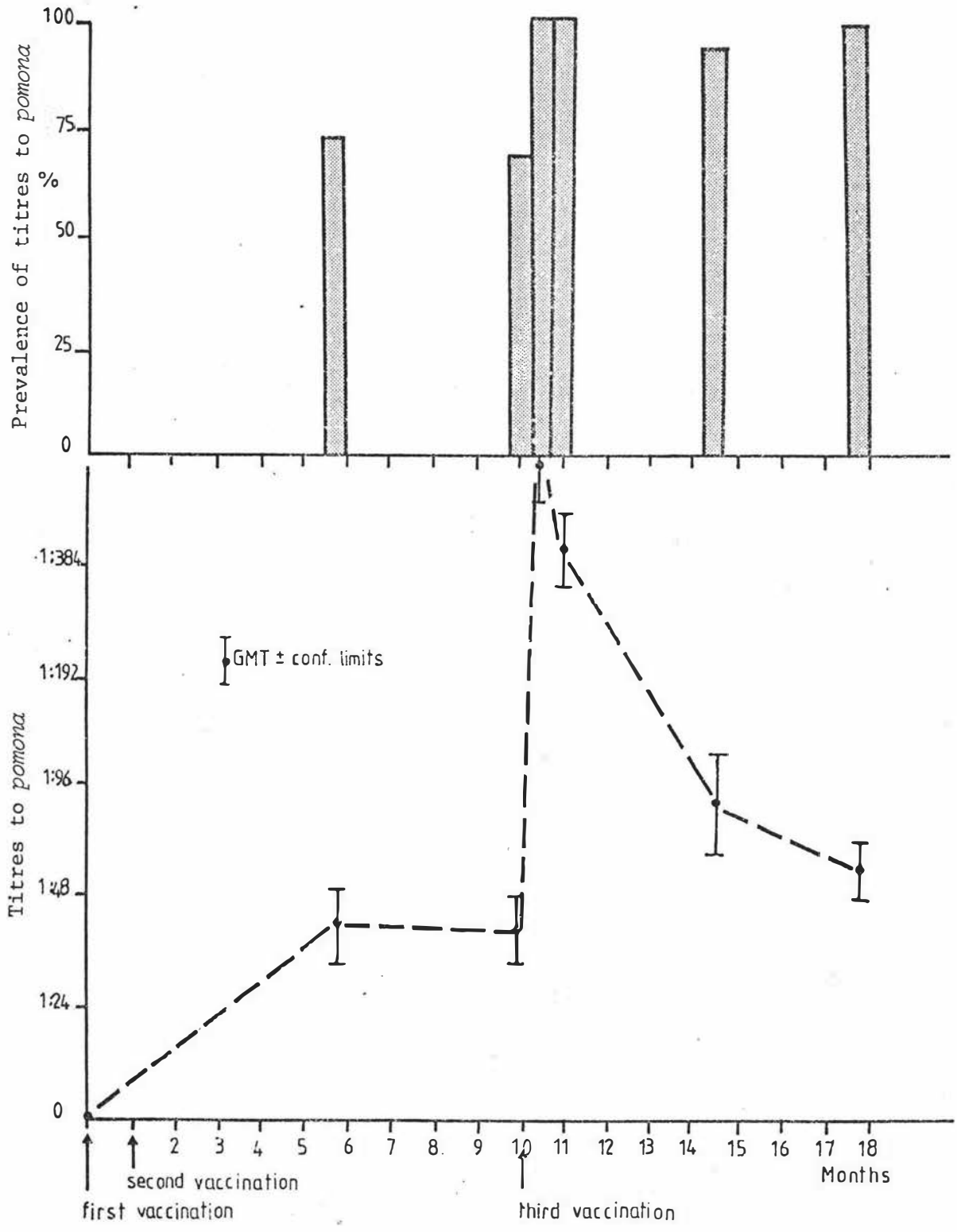


TABLE 10.7 : THE PREVALENCE RATES AND GMTs OF TITRES TO *HARDJO* AND *POMONA* AT THE START AND END OF THE TRIAL OF ALL THE CATTLE THAT WERE TWO YEARS OR OLDER AT THE START OF THE TRIAL (TRIAL C).

	Titres to *hardjo* ≥ 1:24				Titres to *pomona* ≥ 1:24			
	Prevalence		GMT	Range	Prevalence		GMT	Range
At the start of the trial	87/139	62.6%	1:41	1:24 - 1:192	12/139	8.6%	1:108	1:48 -1:38
6 months after initial double vaccination	89/134	66.4%	1:36	1:24 - 1:96	67/134	50.0%	1:42	1:24 -1:38
6 months after first annual revaccination	116/118	98.3%	1:68	1:24 - 1:192	101/118	85.6%	1:49	1:24 -1:38
6 months after second annual revaccination	86/88	97.7%	1:43	1:24 - 1:96	84/88	95.5%	1:50	1:24 -1:19

FIG.10.4 : THE GMT AND SEROLOGICAL PREVALENCE OF TITRES TO *HARDJO* IN DIFFERENT AGE GROUPS OF CATTLE AT THE START AND END OF THE 30 MONTH TRIAL (TRIAL C).

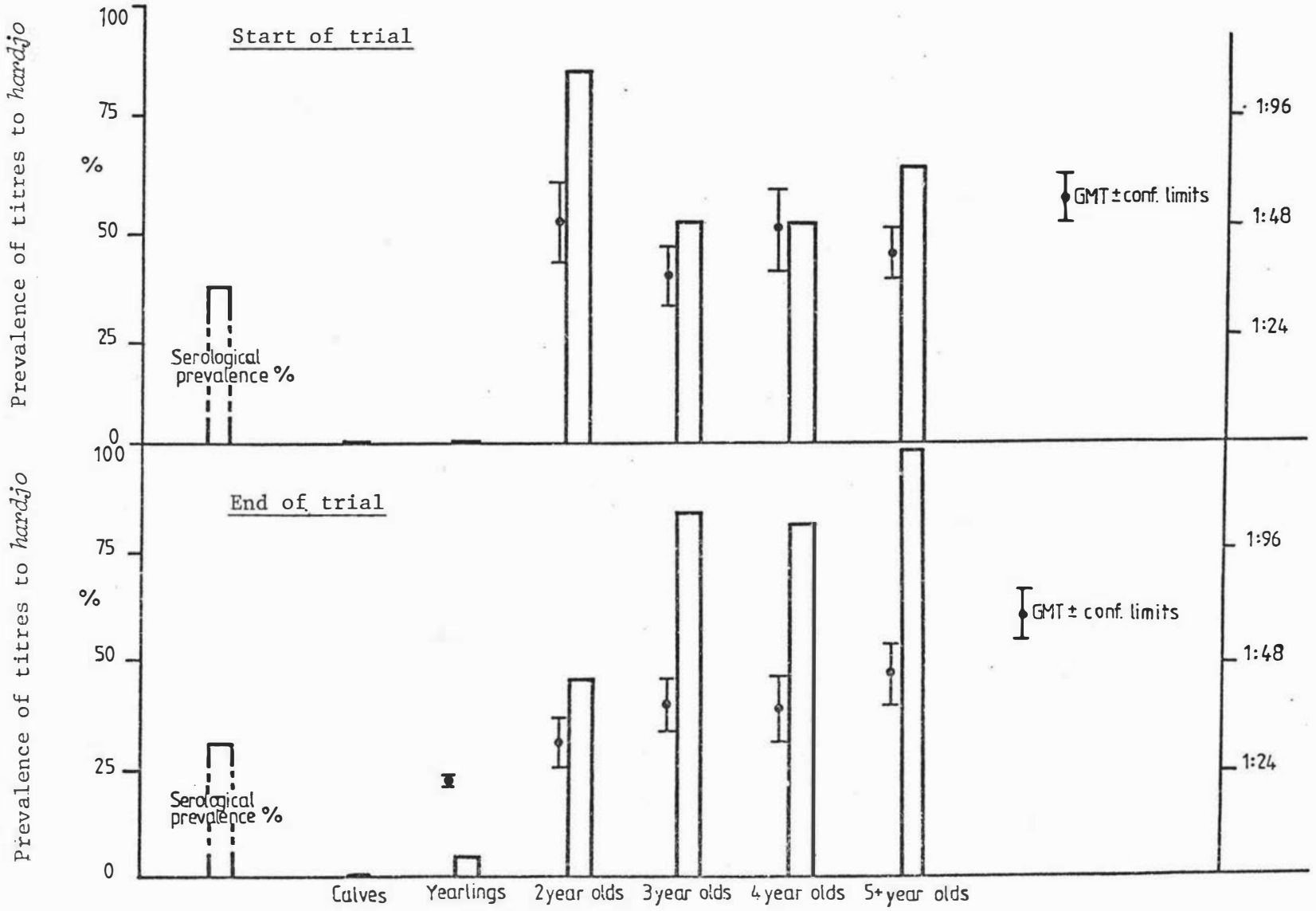
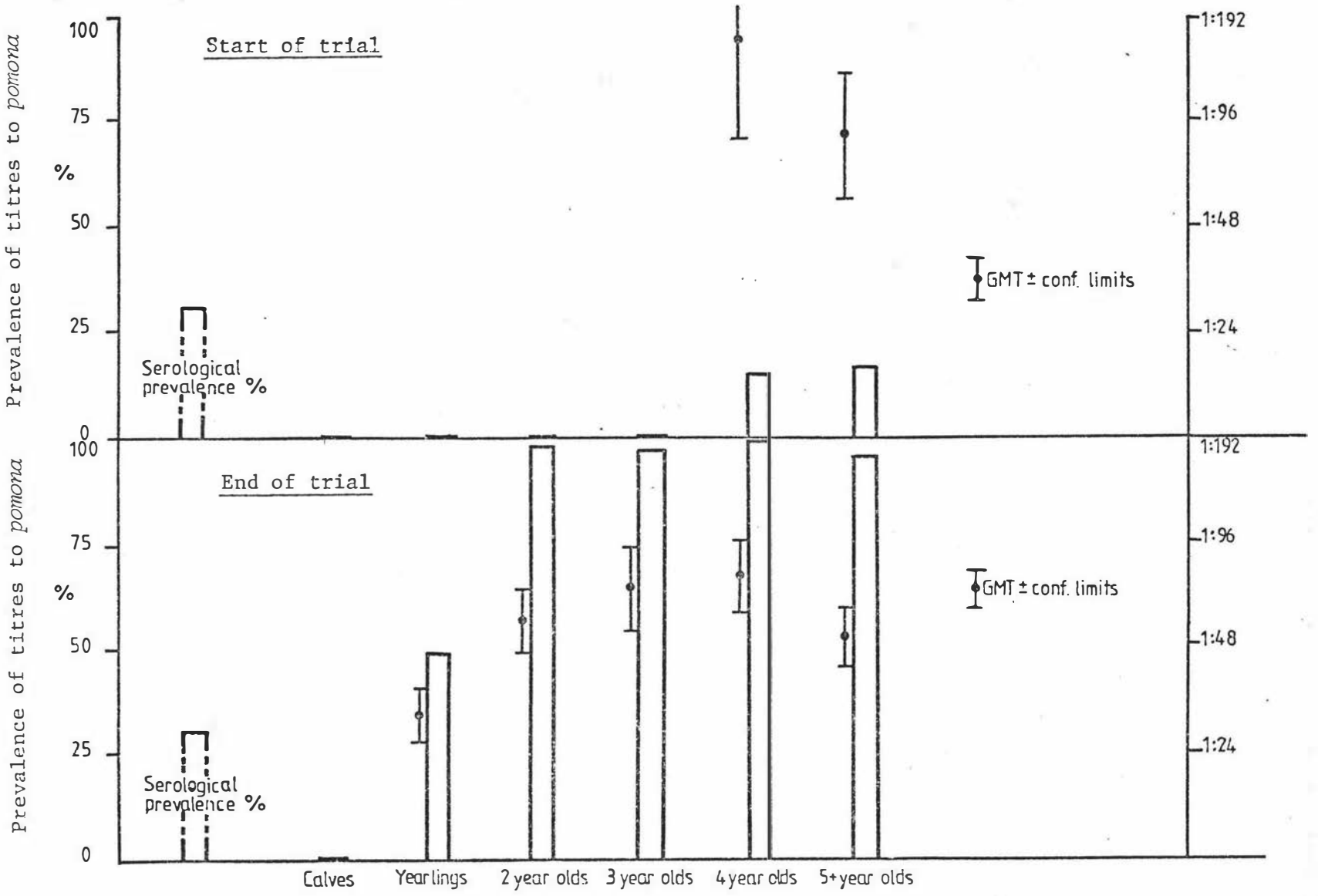


FIG.10.5 : THE GMT AND SEROLOGICAL PREVALENCE OF TITRES TO *POMONA* IN DIFFERENT AGE GROUPS OF CATTLE AT THE START AND END OF THE 30 MONTH TRIAL (TRIAL C).



in the pattern of titres in the different age groups before and after the vaccination programme.

Purchased cattle : On arrival, all nine of the first group of heifers were seronegative to both *hardjo* and *pomona*, seven out of ten of the second group had titres to *hardjo* ranging from 1:48 to 1:384 and one out of six of the third group had a titre of 1:384 to *pomona*. Six months after vaccination the titres of cattle which were originally seronegative were all 1:24 to *hardjo* and between 1:24 and 1:96 to *pomona*, while the titres of the previously infected animals ranged from 1:48 to 1:192. Six months after their annual revaccination 64% of these animals had titres to *hardjo* and 84% had titres to *pomona*.

Control animals : These remained seronegative throughout the three month monitoring period.

Culture : All urine samples were negative on dfm and culture. The contamination rate was 30%.

Clinical history : Throughout the trial there were no clinical signs of *hardjo* infection such as agalactia with a flaccid udder and discoloured milk, nor were there any clinical signs of *pomona* infection such as haemoglobinuria in calves or abortions. There were, however, six calves born two to three weeks premature shortly after the herd had access to macrocarpa trees (*Cupressus macrocarpa*) a known abortifacient (Whitten,1971). The calves were all born live but one subsequently died, and all cows retained their placentae. These cows had received their annual revaccination about eight weeks previously and their titres to *hardjo* ranged from 1:24 to 1:192 while their titres to *pomona* ranged from 1:24 to 1:768. There

was no known access to pigs or cattle infected with *pomona*.

Combined serological results from Trials A, B and C.

By combining the serological results of the three trials a composite picture of the serological response to primary vaccination and annual revaccination can be presented, together with the rate decay of titres (Figures 10.6 and 10.7). The animals were not bled until six months after receiving their fourth vaccination. However, it is likely that an anamnestic rise occurred in these animals soon after revaccination as occurred in animals which had received their third vaccination. This probable response is indicated as a broken line in Figures 10.6 and 10.7.

TRIAL D : PASSIVE HAMSTER PROTECTION TEST

Materials and Methods

Antisera : Twenty ml of serum were collected from a heifer which had been vaccinated two months previously with a *hardjo/pomona* bacterin during the course of Trial A. At the time of collection the heifer had a titre of 1:96 to *hardjo* and 1:96 to *balcanica*. Twenty ml of serum were also collected from a heifer which had been experimentally infected with *balcanica* two months previously (see Chapter Seven) and had a titre of 1:96 to *hardjo* and 1:192 to *balcanica*.

Animals : Forty-four weanling hamsters weighing 25-30 grams were divided into six groups of five animals (Groups A to F) and two groups of seven (Groups G and H). On day 0 of the trial, Group A, D and G hamsters each received 0.5 ml of anti-*hardjo* serum by intraperitoneal injection (I/P) and Group B, E and H each received 0.5 ml of anti-*balcanica* serum I/P. Group C and G hamsters did not receive any antiserum.

FIG. 10.6 : THE COMBINED SEROLOGICAL PREVALENCE AND RATE OF DECAY OF VACCINAL TITRES TO *HARDJO*

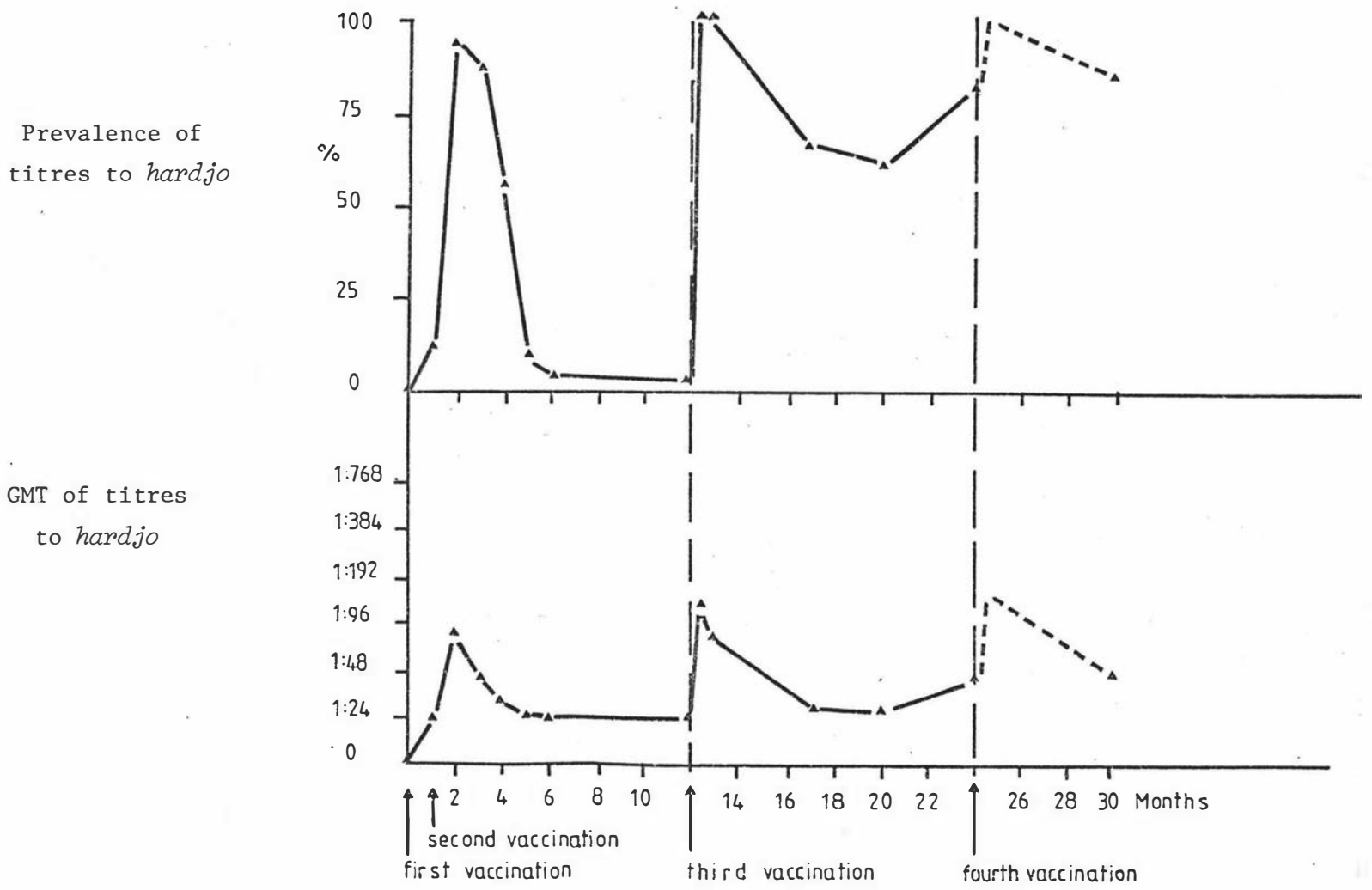
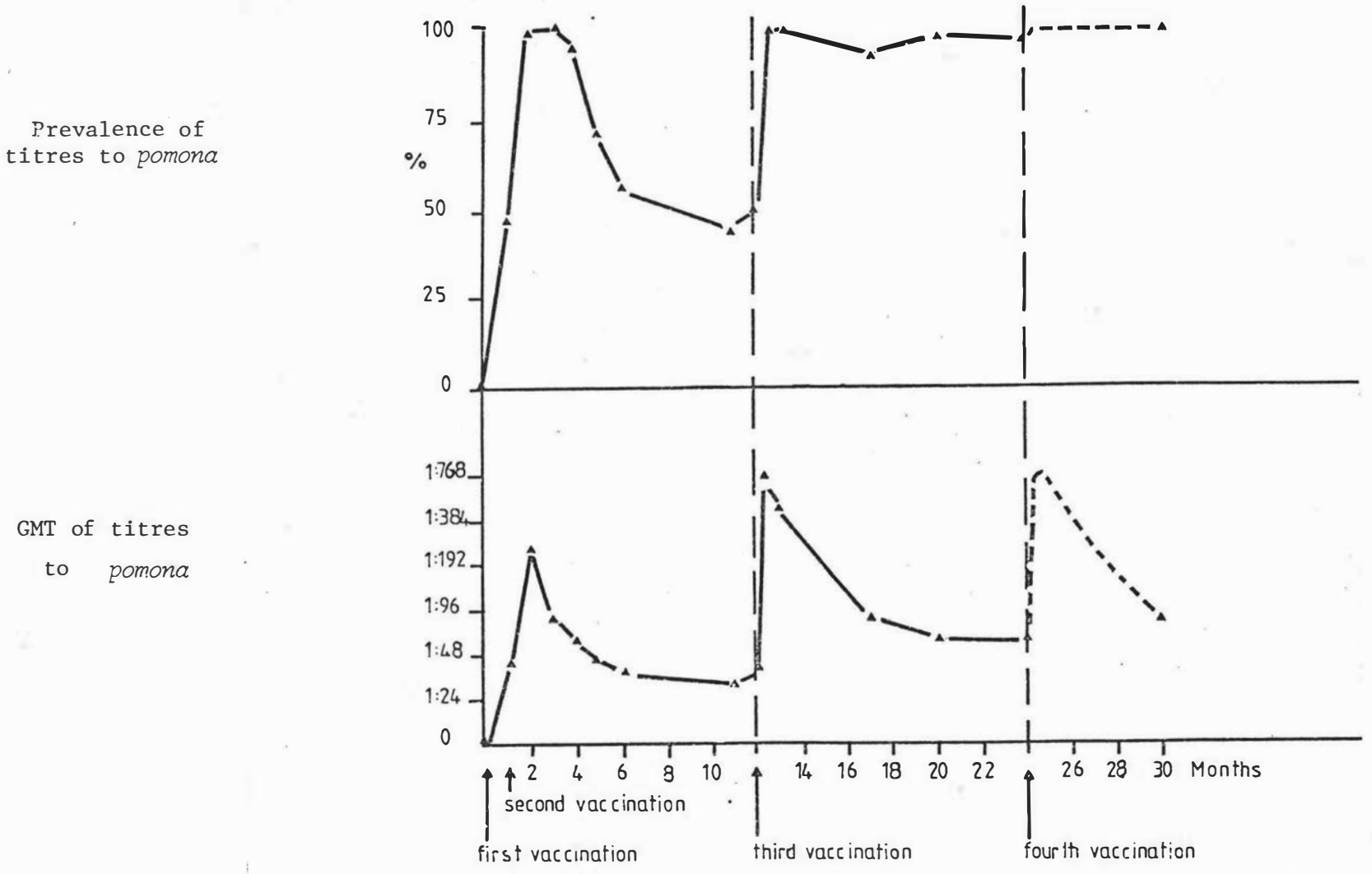


FIG.10.7 : THE COMBINED SEROLOGICAL PREVALENCE AND RATE OF DECAY OF VACCINAL TITRES TO *POMONA*



Challenge : A recent bovine isolate of *hardjo* from Trial A was passaged once through a hamster to prove its infectivity and was reisolated from the kidneys and subcultured in liquid media. A recent bovine isolate of *balcanica* (see Chapter Eight) was passaged in a hamster and reisolated. Both isolates were typed using the restriction endonuclease analysis technique described by Marshall *et al* (1980), and shown to be identical to the other field strains of *hardjo* and *balcanica* isolated in N.Z. (Plate 8.1). The number of organisms per ml was estimated for both inocula using a Petroff-Hauser counting chamber and they were diluted with Stuarts medium until both contained 4×10^7 leptospires/ml. On day 1, 24 hours after Groups A, B, D, E, G and H received injections of antisera, Groups A, B and C hamsters received 0.5 ml of *hardjo* culture I/P and Groups D, E and F hamsters received 0.5 ml of *balcanica* culture I/P. On day one, three hamsters from each of Groups G and H were killed and blood samples were taken. On day 19 of the trial all Group A to F hamsters were killed, blood samples were taken and their kidneys cultured. The remaining eight hamsters in Groups G and H were killed and blood samples were taken on day 19.

Results

Serological examination : The results are summarised in Table 10.8. None of the serum control hamsters in Group G and H had titres to *hardjo* or *balcanica* either one or 19 days after injection with *hardjo* or *balcanica* antiserum and therefore it was assumed that titres produced in the challenged hamsters were due to active infection. None of the hamsters injected with *hardjo* antiserum (Groups A and D) and challenged with *hardjo* or *balcanica* developed titres, while one hamster in each of Groups B and E, which received *balcanica* antiserum, developed titres after challenge with *hardjo* and *balcanica* respectively. All hamsters in Groups C and F

TABLE 10.8 : SEROLOGICAL AND CULTURAL RESULTS OF THE HAMSTER PASSIVE PROTECTION TEST (TRIAL D).

	Serum injected	Challenge	Killed	Titre to *balcanica*	Titre to *hardjo*	Kidney Culture Isolation
Group A	anti-*hardjo* day 0	*hardjo* day 1	day 19	-	-	-
				-	-	-
				-	-	-
				-	-	-
				-	-	-
Group B	anti-*balcanica* day 0	*hardjo* day 1	day 19	-	-	-
				-	-	-
				-	-	-
				1:96	1:96	+
				-	-	-
Group C	none	*hardjo* day 1	day 19	1:48	1:96	+
				1:48	1:48	+
				1:48	1:48	+
				1:24	1:48	+
				1:24	1:48	+
Group D	anti-*hardjo* day 0	*balcanica* day 1	day 19	-	-	-
				-	-	-
				-	-	-
				-	-	-
				-	-	-
Group E	anti-*balcanica* day 0	*balcanica* day 1	day 19	1:96	1:96	-
				-	-	-
				-	-	-
				-	-	-
				-	-	-
Group F	None	*balcanica* day 1	day 19	1:48	1:48	+
				1:24	-	-
				1:96	1:24	+
				1:192	1:48	+
				1:96	1:24	+
Group G	anti-*hardjo* day 0	None	day 1	-	-	
				-	-	
			day 19	-	-	
				-	-	
				-	-	
Group H	anti-*balcanica* day 0	None	day 1	-	-	
				-	-	
				-	-	
			day 19	-	-	
				-	-	

which did not receive antisera seroconverted after challenge.

Cultural examinations : Leptospires were cultured from the kidneys of all five hamsters in Group C, four out of five in Group F and from one hamster in Group B.

GENERAL DISCUSSION

The results of these trials showed that the vaccination of cattle with a *hardjo/pomona* bacterin stimulated the production of circulating antibody and this gave significant protection against natural infection with *hardjo*. The results also indicated that an intensive whole-herd vaccination programme eliminated *hardjo* infection from a commercial dairy herd.

In Trial A the rapid spread of infection in unvaccinated control heifers indicated that the vaccinated animals received a considerable natural challenge. At the commencement of the trial, four of the 22 heifers in the group had recently become infected and within eight weeks all ten of the unvaccinated controls had seroconverted. Thus, the trial demonstrated the efficacy of vaccination during a natural epidemic of *hardjo* infection. In addition, the vaccinated heifers continued to be exposed to leptospiruric cattle for at least 20 months.

In Trial B the degree of natural challenge was apparently not as great or as consistent as that seen in Trial A. The unvaccinated control cattle failed to become infected despite being exposed to a number of leptospiruric cattle between the 14th and 20th weeks of the trial, during late spring and early summer when conditions were apparently suitable for transmission. The reasons for this lack of transmission were unknown

but demonstrated that the exposure of susceptible cattle to a small number of leptospiruric cattle does not always lead to their infection. On their return from the "runoff", the following autumn, one of the heifers had a titre to *hardjo* and was shown to be leptospiruric. Four other unvaccinated control cattle run with the non-lactating cows became infected. Infection did not appear to be present in the milking herd as the unvaccinated controls in that group failed to become infected. The following winter all the animals were grazed together and two of the control heifers were shown to be leptospiruric. However, the degree of challenge must have been slight or the environmental conditions unsuitable for transmission, because the two remaining seronegative controls failed to become infected.

In Trial C there was good evidence that the two year old cattle in the milking herd had recently been infected with *hardjo* as this group had the highest prevalence of titres and the highest GMT at the start of the trial. It was shown in Trial A that cattle infected with *hardjo* can be leptospiruric for 20 months or more. Therefore, on the property described in Trial C, a number of cattle which had been infected during the previous season could have been leptospiruric for most of the following milking season. Consequently, the yearlings which entered the herd immediately after their first vaccination would have received a natural challenge in the spring, when environmental conditions have been shown to be suitable for transmission (Hellstrom, 1978). In fact, this group of two year olds, six months after vaccination, had a higher prevalence of titres and a higher GMT than the group of calves vaccinated at the same time. These two year old cattle joined the herd immediately after their first vaccination and it is possible that some of the titres in this group may represent *hardjo* infections which occurred shortly after vaccination, before the animals had time to develop protective antibodies. Alternatively, it may be that

yearlings produce a greater serological response to vaccination than calves. If a small number of these new season's two year olds were infected then some of these may have remained leptospiruric until the following season, when the next group of first calving cows entered the herd. However, these latter animals would have received three vaccinations by that stage and are unlikely to have become infected. If none of the new season's two year olds had been infected then the cycle would have been broken. Hellstrom (1978) suggested that the vaccinal protection "of only one or two successive cohorts against infection may be sufficient to break the cycle of infection and allow a herd to become free of *hardjo* infection". The results of the present trial appear to confirm this suggestion.

Some of the purchased seropositive yearlings introduced to the herd during the third trial may have been leptospiruric, as their titres indicated that they had been recently infected. However, when they entered the herd all in-contact animals had recently received an annual revaccination and appeared to have been resistent to natural challenge from these animals. The treatment of one heifer, which had a titre of 1:384 to *pomona*, with streptomycin may have reduced the degree and/or duration of leptospiruria in this animal (Ringen *et al*,1955; Stalheim, 1967, 1969; Hodges *et al*,1979b).

The prevention of leptospiruria is the ultimate aim of vaccination, as this will prevent the transmission of infection. In Trial A vaccination resulted in a significantly lower rate of leptospiruria in the vaccinated than the nonvaccinated animals,and in Trial B leptospires were only isolated from the urine of unvaccinated cattle. In Trial C there was no evidence of leptospiruria in any of the cattle at the end of the trial indicating that endemic infection had been eliminated. In similar trials Flint and Liardet (1980b) detected leptospiruria only in unvaccinated cattle after artificial

and natural challenge with *hardjo*.

The isolation of leptospires from two vaccinated heifers with titres less than 1:24 at the time of sampling in Trial A (see Table 10.1) was unexpected. Each of the two vaccinates showed a characteristic serological response to vaccination with no subsequent rise in titre prior to the isolation of leptospires. Consequently it was not possible to estimate when they became infected. The level of vaccinal antibody, which had decayed to less than 1:24, may not have prevented a mild leptospiraemia and kidney colonisation but may have prevented the normal serological response to infection which was seen in the control heifers. This possibility, that vaccinated animals may not give a good secondary response to natural *hardjo* infection, is supported by the finding by Hellstrom (1978) that animals which have been naturally infected with *hardjo* previously gave little or no anamnestic response to heavy experimental challenge. However, in Trial C, vaccinated cattle gave an anamnestic response to revaccination although the titre declined rapidly after four weeks.

The detection of long term leptospiruria in the two control heifers with titres of $<1:24$ demonstrated the importance of culturing urine samples to determine whether or not an animal has been infected. Ellis(pers.comm.), in an abattoir survey of unvaccinated cattle in the UK, found that approximately half of the isolates were from cattle with titres $<1:100$ and 16% of these sera had titres $<1:10$ when titrated against the homologous isolate.

These trials demonstrate that this bacterin and especially the *hardjo* component stimulated a poor immunological response as measured by the MAT. This was particularly apparent after a single dose of vaccine. Two doses,

four weeks apart, stimulated titres similar to those seen after infection but they decayed much more rapidly, falling to less than 1:24 within six months. Other workers (Huhn *et al*,1970; Killinger *et al*,1970; Negi *et al*, 1971; Tripathy *et al*,1973; Huhn *et al*,1975; Tripathy *et al*,1975; Tripathy *et al*,1976; Marshall *et al*,1979a, b; Ris and Hamel,1979; Tripathy *et al*,1980) have also shown that leptospiral bacterins stimulate a relatively poor immunological response as measured by the MAT, although they have demonstrated the presence of protective antibodies in sera by hamster protection tests, growth inhibition tests and natural or artificial challenge of vaccinated animals. In the present trials the vaccinated animals were protected against natural challenge with *hardjo* for some months after their MAT titres had disappeared. Some evidence of agglutination was seen in some of the vaccinated animals' sera but it was less than 50% at a final dilution of 1:24. If lower serum dilutions had been used these titres may have been quantified but this was not considered practical because of the increased likelihood of non-specific titres and cross reactions with other serovars.

It is interesting to note that in Trial B where identical twins were vaccinated with the same dose of bacterin the serological response varied between pairs of twins from 1:24 to 1:192 four weeks after the second dose, but both heifers within a pair gave very similar results (Tables 10.4 and 10.5). This indicates that the response to vaccination is due to host factors, some of which are probably genetically controlled, as well as agent factors.

The rapid anamnestic response seen after annual revaccination was greater than expected and does not appear to have been reported elsewhere. The artificial challenge by *pomona*, of animals which had been vaccinated with

a *pomona* bacterin, has been shown to produce a rise in titre in some but not all animals (Gillespie and Kenzy, 1958; Killinger *et al*,1970; Huhn *et al*, 1975). It has been suggested in Chapter Nine that natural challenge at the time of vaccination may increase a vaccinal response. Artificial challenge of *hardjo* vaccinated animals with *hardjo* has been reported to produce a rise in titre in one trial (Tripathy *et al*,1976) and not another (Flint and Liardet, 1980b). Hellstrom (1978) noted a small immediate rise in titre when he challenged seven previously infected cattle with large numbers of *hardjo* organisms but their titres had returned to their original level after three weeks. The variation seen here may be dependant on the time since vaccination,the level of circulating antibody present at the time of challenge and the number and infectivity of the leptospires used in the artificial challenge.

In Trial C, the vaccination of adult cattle,65% of which had natural titres to *hardjo*, did not markedly alter the seroprevalence or GMT of these animals. However, six months after their annual revaccination the prevalence of titres to *hardjo* was nearly 100%. This rise in prevalence may have been due either to a vaccinal response in previously uninfected cattle as seen in the younger groups, or to a slight elevation of titres of previously infected animals whose titres had declined to less than 1:24 at the start of the trial. It is possible that repeated antigenic stimulation, as would have occurred with the annual revaccination, may have resulted in a rise in the titres of previously infected animals. However, if the former explanation is correct it emphasises the necessity for vaccination of the whole herd as some of the older animals may not have become infected in previous seasons and may still have been fully susceptible to infection with *hardjo*.

The results of Trial D showed that an injection of bovine antiserum, produced in response to the *hardjo/pomona* bacterin, protected hamsters against kidney infection when artificially challenged with *hardjo*. Similar passive hamster protection tests against *hardjo*, *pomona* and *copenhageni* have been demonstrated by Flint and Liardet (1980b) when testing a triple vaccine containing the three serovars. They showed, as did Huhn *et al* (1975), that there was a correlation between the presence of circulating antibody in cattle and passive protection in hamsters injected with this serum. However, Trial D also showed that this anti-*hardjo* serum crossprotects hamsters against infection with *balcanica*. These results indicate that the vaccination of cattle with this *hardjo/pomona* bacterin and natural infection of cattle with *hardjo* will produce antibodies which will provide a degree of crossprotection against natural infection with *balcanica* which has been shown to occur in N.Z. (see Chapter Eight). Nervig *et al*(1977) found that a *hardjo* bacterin did not protect hamsters against infection with *szwajizak*, which is also a member of the Hebdomadis serogroup, although it reduced the mortality rate. However, it is possible that the N.Z. strain of *balcanica* is more closely related antigenically to the field strain of *hardjo* used in this experiment than is *szwajizak* and, therefore, a greater degree of crossprotection may exist. The trial also showed that bovine anti-*balcanica* serum protected hamsters against infection with *hardjo* and *balcanica* to the same degree, confirming the high degree of cross-protection conferred by these two serovars. It is interesting to note that after the injection of 0.5 ml of bovine serum, which had titres of 1:96 to 1:192 to *hardjo* and *balcanica*, none of the hamsters had detectable titres 24 hours later. However, there was still a high degree of protection against experimental infection. This tends to confirm the findings from the first two trials that animals with vaccinal titres of less than 1:24 may still be protected against infection.

Although these trials tested only the efficacy of the *hardjo* component of the vaccine, the *pomona* component was shown to produce a serological response higher and more persistent than that produced by the *hardjo* component. Other *pomona* vaccine trials (Huhn *et al*,1975; Ris and Hamel, 1979) have shown that similar *pomona* bacterins produced similar serological responses and protected cattle against challenge with *pomona* which concurrently produced kidney infection and leptospiruria in unvaccinated controls. The apparent success of such a vaccine in reducing the rate of abortions in an epidemic of *pomona* infections in cattle is reported in Chapter Nine. The annual revaccination of cattle in Trial C resulted in a prevalence of titres to *pomona* of up to 100% six months after vaccination and the majority of these titres persisted for over a year. It has been shown (McDonald and Rudge, 1957; Hellstrom, 1978) that antibody levels of this magnitude in cows will confer colostral immunity to calves and such passively acquired colostral titres have been shown to persist for up to six months (Hellstrom, 1978). As there is some evidence (Marshall *et al*, 1979b; Flint and Liardet, 1980b;Hellstrom, 1980) that colostral immunity may interfere with the development of vaccinal immunity in calves, it would appear that calves should not be vaccinated until they are at least six months of age.

SUMMARY

1. The vaccination of eight yearling heifers with two doses of a *hardjo/pomona* bacterin given four weeks apart gave significant protection against natural infection with *hardjo* during a 2½ year period. Within eight weeks of the start of the trial all ten of the unvaccinated heifers had become infected. During the course of the trial leptospiruria was detected an average of six

times from nine of the ten control heifers. The duration of detectable leptospiruria ranged from seven to 82 weeks with an average of 53.1 weeks, while the average time from the detection of the first titres to *hardjo* to the last urine isolate was 58.8 weeks. Leptospiruria was detected on only one occasion each from only two of the ten vaccinates, 26 and 42 weeks after vaccination. The vaccinated heifers all had titres to *hardjo* and *pomona* four weeks after the second vaccination ranging from 1:24 to 1:384 and 1:96 to 1:192 respectively. These titres all had declined to less than 1:24 by six months after vaccination, unlike the titres to *hardjo* in the infected controls which had a GMT of 1:39 at the end of the trial.

2. Trial B was not as satisfactory as Trial A because the degree of natural challenge was apparently not as high or consistent. Nevertheless, five out of eight unvaccinated heifers seroconverted and leptspiruria was detected in four of these during the 2½ year trial. As in Trial A, the vaccinates received two doses of the *hardjo/pomona* bacterin four weeks apart. Their vaccinal titres had declined to less than 1:24 by six months pv and none of these heifers showed a subsequent rise in titre and none had detectable leptospiruria.

3. The results of Trial C showed that the vaccination of all the bulls, cows, yearlings and calves over six months of age, with two doses of the *hardjo/pomona* vaccine given four weeks apart and annual revaccination for two years, apparently eliminated *hardjo* infection from a commercial dairy herd where it had been endemic prior to vaccination. It was shown that animals receiving their first annual revaccination produced an anamnestic response which reached a maximum after two weeks, with *hardjo* titres ranging from 1:48 to 1:384 and *pomona* titres 1:192 to 1:3072. These titres had declined markedly by

eight months pv when only 45% had titres to *hardjo*, and although 100% still had titres to *pomona*, their GMT had fallen to 1:57.

4. A passive hamster protection test indicated that the vaccination of cattle with a *hardjo/pomona* bacterin should give crossprotection against natural infection with *balcanica*.

5. The *pomona* component of the *hardjo/pomona* vaccine produced higher and more durable titres than the *hardjo* component which was shown to confer protection against natural *hardjo* infection. These results indicated that the *pomona* component of the dual vaccine should protect cattle against *pomona* infection.

CHAPTER ELEVEN

GENERAL DISCUSSION

Leptospirosis is one of the five most common notifiable infectious diseases in N.Z. There has been an average of 488 cases of human leptospirosis reported annually for the last ten years in this country which constitutes a crude attack rate of 16/100,000. By comparison approximately 100 cases were reported annually in the 1970s in the U.S.A. (Blackmore, 1979) which is a crude attack rate of 0.05/100,000 and in the U.K. in 1978, 65 cases of human leptospirosis were reported giving a crude attack rate of 0.2/100,000. Thus the attack rate in this country is approximately 300 times that of the U.S.A. and 80 times that of the U.K. and undoubtedly reflects the relatively higher proportion of New Zealanders who are at risk working in agriculture-based industries.

In N.Z., in the 1970s, over 90% of human cases of leptospirosis were reported as occupationally associated (see Chapter Four) while Penniket (1977) reported that dairy farm workers comprised 90% of reported cases in the Hamilton Health District which annually records nearly half of the reported cases in N.Z. However, other occupations are also at risk. Historically, the first reported case of human leptospirosis in this country was due to an Icterohaemorrhagiae serogroup organism and was referred to as a case of "Weils disease" (Kirschner and Gray, 1951). However, within a year it became apparent that the most commonly identified cause of human leptospirosis was *pomona* and the condition was referred to as "swineherd's disease" (Kirschner *et al*, 1952). This name originated in Europe where the association between human *pomona* infection and the keeping of pigs was demonstrated (Gsell, 1952). However, in the 1950s and 1960s most pigs were kept on dairy farms and the majority of clinical cases occurred in

dairy farm workers. Consequently the name "dairy farm fever" was coined by Kirschner and Maguire (1957) for the disease. Since then the major emphasis has been on the association between leptospirosis and dairy farming while other occupational groups, including pig farmers, meat industry workers, stock truck drivers, livestock agents, veterinarians and butchers, have tended to be overlooked. It is only recently that some of these groups have been investigated. Blackmore *et al* (1979) and Blackmore and Schollum (1980) showed that 10.3% of meat inspectors and 6.3% of meat workers had leptospiral titres and Blackmore and Schollum (pers.comm.) have found that approximately 30% of pig farmers in the Manawatu area had leptospiral titres. Dairy farm workers, pig farm workers and meat industry workers appear to be the groups most at risk and crude estimates of the total number of seropositive persons in these occupational groups can be calculated from the samples examined by Blackmore and his co-workers. Approximately 2000 seropositive workers could be expected in the meat industry (6.3% of 30,000 freezing workers plus 10.3% of 1300 meat inspectors) and approximately 1800 seropositive pig farmers could be seropositive (30% of 6000 pig farmers) making a total of 3800 in these two groups. If the serological prevalence of 34% obtained in the survey of Manawatu dairy farm workers (see Chapter Five) is applied to the estimated 40,000 dairy farm workers in N.Z. approximately 13,600 seropositive dairy farm workers could be expected. If it is assumed that these three populations are the main occupational groups at risk and that there is a constant proportion of seropositive people in each group who had suffered from clinical leptospirosis,then dairy farm workers are likely to account for approximately 78% of occupationally associated leptospirosis. Meat industry workers should account for a further 11.5% and pig farm workers for 10.5% of cases. These estimated proportions appear to be of the same order as those reported to the

Department of Health (Christmas, 1976),although there is an indication that there is a degree of under-reporting of cases involving pig farmers. Consequently, it would appear that the control of "dairy farm fever" could directly reduce the annual incidence of human leptospirosis in this country by over 75%.

The major occupational risk factors associated with contracting leptospirosis are those which influence the degree and duration of exposure to infection. In Chapters Four and Five it was shown that over 60% of leptospiral infections in dairy farm workers were due to *hardjo* and were associated with the milking of cows. Factors that influenced the risk of contracting *hardjo* included the length of time spent in the milking shed, the size of the dairy herd, the type of milking shed, the wearing of shorts and, most important of all, the presence of *hardjo* infection in the dairy cows. *Pomona* infections in dairy farm workers were associated with the keeping of pigs, the milking of cows and the occurrence of *pomona* infection in the dairy herd. In contrast to the situation in dairy farmers, over 60% of leptospiral infections in meat industry workers were due to *pomona* and *tarassovi* and were associated with the killing, dressing and inspecting of pigs (Blackmore and Schollum, 1980). In the survey of Manawatu dairy farm workers (Chapter Five) it was shown that although the wearing of shorts increased the risk to the farmer, the wearing of aprons appeared to give no protection. It seems likely therefore that milkers are directly exposed to urine splash and infection is contracted by leptospires penetrating mucous membranes or cuts and abrasions on exposed skin. It would be impractical to recommend that milkers wear face shields, gloves and waterproof clothing which cover the entire body surface. It is also not possible to vaccinate dairy farm workers and their families as an appropriate vaccine is unavailable. The obvious alternative is to control leptopiral infections in dairy cattle

which are the most important source of leptospirosis for humans in this country.

There are two basic approaches to the control of leptospirosis in dairy cattle. The first approach, suggested by Hellstrom (1978), entails the maintenance of an endemic cycle of *hardjo* infection in the young replacement cattle on a property by management practices whereby the calves aged nine to 12 months are mixed with 21 to 24 month old "yearlings", which had been infected the previous year. Hellstrom (1980) suggested that in this way "the disease" in these animals "has usually disappeared by the time they are milked", thereby preventing the milker from being exposed to leptospiruric animals. Limited evidence is presented in Chapter Five to give some support to this theory as the results in the case-control survey indicated that milkers were at a lower risk if cattle were infected with *hardjo* as yearlings than as two year olds. However, there are several problems associated with this approach. As described in Chapter Ten susceptible animals run together with those that are leptospiruric do not necessarily become infected. Management practices on factory supply farms usually necessitate that calves and yearlings are grazed separately as they have different feed requirements, and the yearlings usually join the main herd in winter when the cows have ceased lactating prior to calving in spring. Consequently radical changes in management procedures must be made to ensure that the calves become infected at nine to 12 months of age. It is possible that cattle could become infected with *hardjo* as yearlings if a small number of the previous season's infected yearlings, which failed to become pregnant, were held back and grazed with them. However, even if all the susceptible animals did become infected as yearlings they could still be leptospiruric for much of their first milking season as leptospiruria can persist for up to 21 months. In such circumstances the milker would still be exposed to

infected urine, although probably to a lesser degree than if *hardjo* infection was cycling in the two year old cows. Finally, such an approach would have no effect on the control of sporadic outbreaks of *pomona* infection in dairy cattle such as those described in Chapter Nine.

The other approach is to control both *hardjo* and *pomona* infections by the vaccination of all the cattle on the property. The vaccine trials reported in Chapter Ten and elsewhere (Marshall *et al*, 1979a, b: Flint and Liardet, 1980b) have shown that *hardjo/pomona* bacterins provide a significant degree of protection against infection with *hardjo*. The results of the investigation described in Chapter Nine indicate that a *pomona* bacterin also gives significant protection against infection and abortion caused by *pomona*. The vaccination of all the cattle on a commercial factory supply dairy farm, reported in Chapter Ten, apparently eliminated endemic *hardjo* infection which had been present in the herd prior to the trial. It is believed that a continued programme of annual revaccination of all calves, yearlings and adult cattle on this property will maintain the herd free from *hardjo* and *pomona* infection. Thus the cattle should no longer be a source of infection for the milkers. It is considered advisable to vaccinate all the cattle in a herd irrespective of whether or not a number of them have been previously infected with *hardjo*, because it is not possible to determine which cows are susceptible to infection and which are not by routine serological methods. For example in Chapter Ten it was found that over 30% of all the adult cows were seronegative to *hardjo* but it was not possible to determine if they had never been infected or if protective titres due to past infection had decayed to less than 1:24 and were undetectable. Vaccination of all the animals eliminated this problem.

The vaccination of cattle against infections with *hardjo* and *pomona* would have a number of benefits. In addition to the social and economic benefits of preventing leptospirosis in dairy farmers, it would prevent the economic losses resulting from *pomona* epidemics in calves or pregnant cows, such as those described in Chapters Six and Nine. There is also some evidence (see Chapters One and Ten) that *hardjo* infections in cattle can result in agalactia and sporadic abortions, and vaccination would prevent these.

Other potential sources of leptospiral infection on dairy farms include pigs, dogs, sheep, horses, possums and other wildlife. Ryan (1978) demonstrated a high prevalence of titres to *pomona* and a low prevalence of titres to *tarassovi* in pigs and showed that pigs are the maintenance hosts for these serovars in this country. The results of the survey of Manawatu dairy farm workers (see Chapter Five) showed that titres to *pomona* were associated with the keeping of pigs as well as the milking of cows. Thus, for the complete control of *pomona* on a dairy farm it is necessary to eliminate infection from pigs as well as cattle on the property. Studies of wildlife (Brockie, 1976; Hathaway, 1978; Carter and Cordes, 1980) have failed to show any evidence of reservoirs of *pomona* infection in wildlife.

The role of the dog in the epidemiology of leptospirosis on dairy farms was investigated (see Chapter Six) and, although dogs are apparently susceptible to infection with all the serovars present in N.Z. and kidney infections with *tarassovi* and *pomona* can persist for up to a year, it appears that they are probably "dead end" hosts and are unlikely to act as maintenance hosts for these serovars. Therefore, they are probably of limited importance as carriers of leptospiral infection on dairy farms. If *hardjo*, *pomona* and *tarassovi* infections were eliminated from herds of

cattle and pigs then it is believed that dogs would not become infected with these serovars.

There is little published information on the role of sheep in the epidemiology of leptospirosis in N.Z. However, studies by Webster and Reynolds (1955), Durfee (1978), Bahaman (1981) and Blackmore (pers.comm.) suggest that sheep are not maintenance hosts for *hardjo* or *pomona*, although they are susceptible to infection with both serovars and may act as short term carriers. It is believed that they are of little significance as sources of infection for either cattle or humans. Similarly horses are not recognised as significant sources of leptospirosis in this country (Doe,1979).

Possums have been shown to be the maintenance hosts for *balcanica* (Hathaway, 1978) and they are widely distributed throughout N.Z. As shown in Chapters Seven and Eight of this study, cattle are susceptible to infection with *balcanica*, although this appears to result in only sporadic outbreaks and not endemic infection and such infections probably occur only in herds where endemic *hardjo* is not present. Cattle infected with *balcanica* may be leptospiruric for a period and are therefore a potential risk to milkers. However, this risk is probably minimal due to the apparent low intensity and limited duration of leptospiruria compared to the risk associated with long term leptospiruria demonstrated in cattle infected with *hardjo*. The results of a passive hamster protection test described in Chapter Ten indicate that the *hardjo/pomona* bacterin used in the vaccine trials is likely to provide some degree of cross-protection in cattle against infection with *balcanica*. Thus the vaccination of cattle to prevent *hardjo* infection should also prevent infection with *balcanica*. Although dairy farm workers are unlikely to be exposed directly to infected possum urine, they should be made aware of the potential risks associated with handling possums.

The only other significant sources of leptospirosis infection on dairy farms in N.Z. are rodents. Brockie (1976), Hathaway (1978) and Carter and Cordes (1980) have shown a high prevalence of *ballum* infection in rodents throughout the North Island and *copenhageni* infection in rodents in the South Auckland and Waikato districts. Human infections with these serovars have been shown to occur by others (Kirschner and Gray, 1951; Josland *et al*, 1957; Anon, 1967) and serological evidence of *ballum* infection was found in Manawatu dairy farm workers (see Chapter Five). These infections probably resulted from indirect contact with infected rodent urine via the environment. Sporadic *ballum* and *copenhageni* infections and leptospiruria have also been demonstrated in dairy cattle (Dodd and Brakenridge, 1960; Ris *et al*, 1973) which could be potential sources of infection for dairy farm workers although the serological prevalence of such infections in cattle in this country is low (Hellstrom, 1978). Compared with the incidence of human infections due to *hardjo* and *pomona*, those caused by *ballum* and *copenhageni* have been of little significance and comprised less than 1% of diagnosed cases in the early 1970s (Brockie, 1976). Nevertheless it would be prudent to control the numbers of rodents on dairy farms to reduce the possibility of infection with these serovars.

In conclusion, it is believed that the incidence of leptospirosis in dairy farm workers could be significantly reduced by the elimination of *hardjo* and *pomona* infections in the cattle and pigs on dairy farms using a programme of routine vaccination. Similarly a vaccination programme to eliminate *pomona* and *tarassovi* infection in pigs should prevent pig farm workers from contracting leptospirosis due to these serovars. If a sufficient number of dairy and pig farmers eliminated leptospirosis from their animals in this way the risk of contracting leptospirosis would be

reduced for all the occupational groups associated with agriculture-based industry, including meat industry workers, stock truck drivers, veterinarians, butchers and even housewives preparing kidneys in the kitchen. Human leptospirosis is a preventable disease, and it is the responsibility of the veterinary and medical professions to educate the farming community and to provide them with the means to eliminate leptospirosis in the animals most responsible for transmitting infection to man in this country.

APPENDIX I

PREPARATION OF JS MEDIUM ACCORDING TO THE METHOD OF JOHNSON AND SEITER (1977)

Analytical grade reagents and double-distilled deionised water were used in the preparation of this medium. Glassware was thoroughly washed in an automatic laboratory washing machine and rinsed with distilled water before being autoclaved at 121°C for 20 minutes.

STOCK SOLUTIONS : Fresh stock solutions of chemicals were prepared for each batch of medium as follows :

		grams per 100 ml deionised water
NH_4Cl	(B.D.H.)	25.0
$ZnSO_4.7H_2O$	(M & B)	0.4
$MgCl_2.6H_2O$	(B.D.H.)	1.5
$CaCl_2.2H_2O$	(B.D.H.)	1.5
$FeSO_4.7H_2$)	(B.D.H.)	0.5
$CuSO_4.5H_2O$	(B.D.H.)	0.3
Sodium pyruvate	(B.D.H.)	10.0
Glycerol	(B.D.H.)	10.0
Tween 80	(Sigma)	10.0
Thiamine.HCl	(Sigma)	0.5
Cyanocobalamin	(Sigma)	0.02

ALBUMIN SUPPLEMENT : The albumin supplement was prepared by dissolving 20g bovine albumin fraction V powder (Pentex-Miles) in 100 ml deionised distilled water. While this was stirred the following stock solutions were slowly added :

$MgCl_2$	2.0 ml
$CaCl_2$	2.0 ml
$ZnSO_4$	2.0 ml
$CuSO_4$	0.2 ml
$FeSO_4$	20.0 ml
Cyanocobalamin	2.0 ml
Tween 80	25.0 ml

When the powder was completely dissolved the pH was adjusted to 7.4 and the solution brought to a final volume of 200 ml by the addition of deionised distilled water. The solution was then sterilised by filtration using a 0.22μm filter (Millipore) and stored in 30 ml batches in sterile glass bottles. The sterility of the solution was checked by the addition of 1ml albumin supplement to 10 ml nutrient broth and incubation at 37°C for 24 hours, after which time it was examined for bacterial contamination.

BASAL MEDIUM : To 996 ml of deionised distilled water the following were added :

Na_2HPO_4 (anhydrous)	B.D.H.	1.0 g
KH_2PO_4 (anhydrous)	B.D.H.	0.3 g
NaCl	B.D.H.	1.0 g

plus the following stock solutions :

NH_4Cl	1 ml
thiamine	1 ml
sodium pyruvate	1 ml
glycerol	1 ml

The pH of the resulting solution was adjusted to 7.4, and the solution was decanted into 270 ml screw capped bottles which were autoclaved at 121°C for 20 minutes and stored until used.

LIQUID MEDIUM : Liquid medium was prepared by adding 30 ml of albumin supplement to 270 ml of basal medium.

SEMISOLID MEDIUM : Semisolid medium was prepared by adding 0.5 g of agar (Difco, Bacto-Agar) to 270 ml of basal medium. This was autoclaved at 121°C for 20 minutes, then cooled to 56°C before the addition of 30 ml of albumin supplement.

Medium was dispensed in 5 ml aliquots into screw-capped McCartney bottles.

All batches of medium were checked for bacterial contamination by incubation at 37°C for three days and 27°C for three days then examined.

All new batches of basal medium and albumin supplement were tested to see that they supported the growth of a recent isolate of *hardjo*.

SELECTIVE MEDIUM : Selective media were prepared by the addition of 200 or 400 µg 5FU/ml to liquid or semisolid medium. A stock solution of 5FU (Sigma) was prepared by the addition of 1.0 g of 5FU to 50 ml of distilled water. This was placed in a 56°C water bath to dissolve the 5FU, and the pH was then adjusted to 7.4 - 7.6 by the addition of 1M HCl. The solution was then made up to 100 ml by the addition of deionised distilled water and sterilised by filtration through a 0.22 µm filter (Millipore). Twenty ml aliquots of 5FU solution were held at 4°C until required, when they were dissolved by placing in a 56°C water bath prior to their addition to prepared medium. To prepare medium with 200 µg 5FU/ml 6.0 ml of 5FU stock solution were added to 300 ml of medium; and 12.0 ml 5FU were added to 300 ml medium to produce a final concentration of 400 µg/ml.

B.D.H. - British Drug House Chemicals Ltd., Poole, England.

Difco - Difco Laboratories, Detroit, Michigan, U.S.A.

M & B - May and Baker Ltd., Dagenham, England.

Miles - Miles Laboratories Inc., Research Products, Elkart,Indiana,46514,USA.

Millipore - Millipore Corp., Bedford, Mass., 01739, USA.

Sigma - Sigma Chemical Co., P O Box 14508, St. Louis, Missouri, 63178, USA.

APPENDIX II

MANAWATU FARM SURVEY QUESTIONNAIRE

PART A : GENERAL SECTION

Survey No. _____

1. Supply No. _____ 2. Farm Brucella Ref. No. _____

3. Occupier's Name(s) _____

_____

4. Address of Farm _____

_____

5. Name and Address of Occupier's Veterinarian

_____

_____

Herd Information:

6. Average number of milking cows _____

6A. Do you dock your cows?

Yes, all	( )
Yes, some	( )
No.	( )

7. Town supply ( )
Factory supply ( )

8. Are pigs normally present?

Yes	( )
No	( )

If YES, are they for home consumption only ( )
or produced for sale ( )

Milking Techniques:

9. Do you wash the udders before milking? Yes ( )
No ( )

If YES,

Method	Product Used	Concentration
( ) Automatic Spray ( ) Hand Spray ( ) Hose ( ) Bucket and Cloth		

10. Stimulation. None ( )
Hand ( )
Brush ( )

11. Have you used a teat salve or a product for teat dipping or spraying after milking in the last twelve months?

Yes ( )
No ( )

If YES,

Month	Morning	Evening	Teat Salve	Teat Spray	Teat Dip	Product Used
July						
August						
September						
October						
November						
December						
January						
February						
March						
April						
May						
June						

12. Do you dip your cups into any sanitiser after the milking of any cow?

Yes ( )
No ( )

If YES,

Some cows ( )
All cows ( )
Part of the season ( )
All of the season ( )

13. Strength of the concentrate used:

_____

14. Strength of the dilution used:

_____

15. How is it mixed?

By hand ( )
Automatically ( )

Plant Hygiene:

16. Do you use iodophors as a sanitiser?

Yes ( )
No ( )

IF NO, PROCEED TO QUESTION 23.
IF YES, ANSWER QUESTIONS 17 to 22.

17. Is it heated before use?

Yes ( )
No ( )

If YES, Temperature _____

18. What product do you use?

_____

_____

_____

19. How is it mixed?

By hand ( )
Automatically ( )

19A. Do you use this product to clean out the vat?

Yes ( )
No ( )

20. Concentration of diluted iodophor finally used:

_____

_____

_____

21. Do you use iodophors in the

Morning ( )
Evening ( )

22. After you have used the iodophor, does any of the waste solution get discharged into or flow into the area of the shed where the cows are milked?

Yes ( )
No ( )

If YES, is this

Before milking ( )
After milking ( )

IF NO TO QUESTION 16, ANSWER THE FOLLOWING QUESTIONS.

23. Do you use any other type of sanitiser other than iodophors?

Yes ( )
No ( )

IF NO, PROCEED TO QUESTION 30.
IF YES, ANSWER QUESTIONS 24 to 29.

24. What product do you use?

_____

_____

_____

25. Does this product need to be heated before use?

Yes ( )
No ( )

If YES, Temperature _____

26. How is this product mixed?

By hand ( )
Automatically ( )

26A. Do you use this product to clean out the vat?

Yes ( )
No ( )

27. Concentration of diluted sanitiser finally used:

_____

_____

_____

28. Do you use this sanitiser in the

Morning ( )
Evening ( )

29. After you have used this product, does any of the waste solution get discharged into or flow into the area of the shed where the cows are milked?

Yes ( )
No ( )

If YES, is this

Before milking ( )
After milking ( )

Shed Design:

30. (i) Walk-through ( )
(ii) Herring-bone ( )
(iii) Rotary ( )

REMARKS _____

_____

_____

_____

31. Is the shed open to the prevailing wind?

Yes ( )
No ( )

REMARKS _____

_____

_____

_____

32. Number of sets of cups in shed _____

Effluent Disposal from Shed:

33. How do you dispose of your effluent from the shed?

(i)	Pond	( )
(ii)	Stream discharge	( )
(iii)	Pasture discharge	( )
(iv)	Pasture spray	( )
(v)	Other _____ (Please specify)	( )

Animal Leptospirosis:

34. Has your herd been affected with clinical leptospirosis?

Yes ( )
No ( )

If YES, give brief details relating to date, signs and animals affected.

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

Environmental Results:

Strength of concentrate

Strength of dilution

Air concentrations

APPENDIX III

MANAWATU FARM SURVEY QUESTIONNAIRE

PART B PERSONAL SECTION

Survey No. _____

Occupier's Name(s) _____

_____

Personal Information

35. Name _____

36. Age Group :

Under 20	( )
20 - 29	( )
30 - 39	( )
40 - 49	( )
50 - 59	( )
60 and over	( )

37. Sex

Male	( )
Female	( )

38. Own Doctor's Name and Address

_____

_____

_____

Occupational History

39. How many times a week do you milk cows?

Never	( )
Rarely	( )
1 - 4	( )
5 - 9	( )
10 or more	( )

40. When did you first begin to work in a dairy shed?

More than 39 years ago	( )
30 - 39 years ago	( )
20 - 29 years ago	( )
10 - 19 years ago	( )
5 - 9 years ago	( )
3 or 4 years ago	( )
1 or 2 years ago	( )
This year	( )

41. How long does it take you to milk your cows at the height of the season?

Less than 1 hour	( )
1 but under 2 hours	( )
2 and under 3 hours	( )
3 hours and more	( )

42. What occupation did you follow in the last five years prior to working on a dairy farm?

OCCUPATION	DURATION

3.

Personal Health

43. Have you ever had confirmed Brucellosis?

Yes ( )
No ( )

44. Have you ever had confirmed Leptospirosis?

Yes ( )
No ( )

If YES (to either Question 43 or Question 44),

	Brucellosis	Leptospirosis
Date		
Duration of Illness		
Doctor		
Blood Positive		
Symptoms		
Geographical location when infected		

45. In the past five years, have you experienced any of the following health problems:

		YES	NO
(i)	HAYFEVER	( )	( )
(ii)	RUNNY NOSE FOR MORE THAN 2 WEEKS	( )	( )

If YES, is this associated with a sore throat ( ), sneezing ( ), itchy eyes ( ), watery eyes ( ), cough ( ), was this cough productive ( ).

Symptoms	Total No. of occasions	May to Sept.	Oct. to April

(iii) SORE THROAT ( ) ( )

If YES,

Total No. of occasions	May to Sept.	Oct. to April

		YES	NO
(iv)	SORE EYES	( )	( )

If YES,

Total No. of occasions	May to Sept.	Oct. to April

		YES	NO
(v)	RASHES OR SKIN TROUBLE (Includes Erythema, Acne, Urticaria and Stomatitis)	( )	( )

If YES,

Symptoms	Total No. of occasions	May to Sept.	Oct. to April

		YES	NO
(vi)	GLANDULAR INFLAMMATION	( )	( )

Have you ever had enlargement of the glands at the side of your face (Interviewer to indicate parotid glands).

If YES,

Total No. of occasions	May to Sept.	Oct. to April

6.

		YES	NO
(vii)	LACK OF APPETITE FOR FOR A WEEK OR MORE	( )	( )

If YES,

Total No. of occasions	May to Sept.	Oct. to April

		YES	NO
(viii)	UNPLANNED LOSS OF WEIGHT	( )	( )

If YES,

Total No. of occasions	May to Sept.	Oct. to April

		YES	NO
(ix)	SLEEPLESSNESS	( )	( )

If YES,

Pattern	Total No. of occasions	May to Sept.	Oct. to April

7.

		YES	NO
(x)	NERVOUSNESS	( )	( )

If YES,

Nature of Trouble	Total No. of occasions	May to Sept.	Oct. to April

(xi) THYROTOXICOSIS - thyroid trouble ( ) ( )

If YES,

Date _____

(xii) OTHER MAJOR COMPLAINTS IN WHICH A DOCTOR WAS CONSULTED ( ) ( )

Please Specify

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

46. Do you consider any of these complaints to be associated with your work?

Yes ( )
No ( )

If YES, which complaint(s)

_____

_____

_____

_____

_____

Clothing

47. Do you wear any of the following protective clothing?

	No	Yes	Part of the year.
(i) Footwear			
None	( )	( )	( )
Jandals	( )	( )	( )
Sand Shoes	( )	( )	( )
Shoes	( )	( )	( )
Gumboots	( )	( )	( )
(ii) Shorts	( )	( )	( )
(iii) Long Trousers	( )	( )	( )
(iv) Waterproof Leggings	( )	( )	( )
(v) Overalls	( )	( )	( )
(vi) Apron	( )	( )	( )
(vii) Gloves	( )	( )	( )
(viii) Others (Please specify)	( )	( )	( )

_____

_____

_____

_____

9.

Medical Results

Leptospirosis Antibodies

Titre	Hardjo	Pomona	Tarassovi	Copenhagan	Ballum
$\frac{1}{24}$					
$\frac{1}{48}$					
$\frac{1}{96}$					
$\frac{1}{192}$					
$\frac{1}{384}$					
$\frac{1}{768}$					
$\frac{1}{1536}$					

Blood Sample / Urine specimen results

Interviewer [ ] Survey No. [ ]

MANAWATU LEPTOSPIROSIS SURVEY FOLLOW-UP QUESTIONNAIRE

1. Supply No. ..... 2. Farm Brucella ref.no. .....

3. Occupier's Name(s) .....

4. Address of Farm .....

.....

FARM INFORMATION

5. Type of farmland (a) Farm Flat [ ] Rolling [ ] Hill [ ] (b) Run off

6. Drainage of land : well drained [ ]
poor drained [ ]

7. Do neighbouring properties drain onto or through this farm especially surface water after heavy rain. YES [ ] NO [ ]

8. Approximate number of livestock at 1st March, 1979.

Dairy Cattle	Milking Cows	[ ]
	In-calf Heifers	[ ]
	Calves < 1 yr.	[ ]
Beef Cattle (including dairy beef)	No.	[ ]
Pigs	Boars	[ ]
	Breeding Sows	[ ]
	Fattening Stock	[ ]
Are they kept for:	Own consumption	[ ]
	For sale	[ ]

(N.B. (Enquire as to whether (they have kept pigs in (the last 5 years and if (so, how many and for (what purpose.

Sheep — No. Breeding Ewes ☐

No. Fattening Stock ☐

Goats — No. ☐

Dogs — No. ☐

Cats — No. ☐

Horses — No. ☐

9. Are rats or possums seen on the farm?

	Rat	Possum
Never	☐	☐
Infrequently	☐	☐
Frequently	☐	☐

10. Stock Movement and Replacement.

Buy in stock? :	Annually	Occasionally	Never
Calves	☐	☐	☐
Heifers	☐	☐	☐
Cows	☐	☐	☐
Bulls	☐	☐	☐
Weaner Pigs	☐	☐	☐
Breeding Sows	☐	☐	☐
Sheep	☐	☐	☐

(enquire about stock movements/replacements over the last 5 years)

Dairy Cattle Breeding

AI only ☐

AI + bull ☐

Bull only ☐

11. Vaccination of stock with leptospiral vaccine

YES ☐

NO ☐

Comments Type of vaccine, class of stock, frequency of vacc.

12. Hunting

Possums ☐

Deer ☐

Pigs ☐

Goats ☐

Do you feed game to dogs?

(e.g. possums, rabbits) ☐

13. Do you kill and dress animals on your farm?

	YES	NO
Sheep	☐	☐
Pigs	☐	☐
Cattle	☐	☐

PERSONAL

	YES	NO
14. Smoker	☐	☐
- during milking	☐	☐

15. Drinks raw milk (cold) ☐ frequently ☐ occasionally ☐ never

16. If lepto +ve :

- can he recall lepto-like symptoms in last 5 years -
- if so has he any idea how he caught it, i.e. did any of the following events occur one month before developing lepto?

a. Arrival of new stock on property ☐

b. Abrasions on hand/arm ☐

c. cleaning sludge pump, drain, effluent disposal system ☐

d. removing cattle afterbirth ☐

e. a number of abortions in herd ☐

f. assist cow calving ☐

(cont) ...

g. Assist sheep lambing ☐

h. Assist sow farrowing ☐

i. P.M. or cutting up dead animal ☐

j. Clinical leptospirosis diagnosed in stock by vet. ☐

k. Redwater in calves ☐

l. Flabby "mastitis" - agalactia in herd (not normal mastitis problem) ☐

APPENDIX V

B.C.G. VACCINATION OF MICE.

INTRODUCTION : Vaccination with the Bacillus Calmette-Guérin (B.C.G.) strain of *Mycobacterium bovis* has been reported (Thompson, 1976) to increase nonspecifically the immunity of an animal by stimulating the cell mediated immune system. It was therefore considered possible that the vaccination of cattle with a *Mycobacterium johnei* vaccination might nonspecifically increase their resistance to infection with *hardjo*, or reduce the length of leptospiruria in infected cattle. To test this hypothesis, B.C.G. vaccinated mice infected with *ballum* were used as an experimental model. Mice are the maintenance host for *ballum* and were therefore considered an appropriate model for *hardjo* infection of cattle. B.C.G. vaccine* was used in this experiment due to the unavailability of Johnes vaccine.

MATERIALS AND METHODS : Thirty 4-week-old 32 g (range 26.1-36.4g) mice from an SPF colony free from leptospiral infection were used. They were divided into six groups of five mice (Groups A to F). All the mice in all six groups received a challenge inoculum of 0.5 ml of a 7 day culture of a recent *ballum* isolate containing 5×10^8 leptospires/ml. The mice in Groups A, B, C, D and E received a single intraperitoneal injection of 0.5ml B.C.G. vaccine 14 days before, 7 days before, on the same day, 7 days after and 14 days after challenge respectively, while Group F acted as unvaccinated controls. Urine samples were expressed from the live mice onto glass slides and examined by dfm at the time of challenge, one week after challenge and then at approximately monthly intervals. After eight months the mice were

* Glaxo Laboratories, Greenford, England

killed and their kidneys cultured.

RESULTS : Leptospiruria was first observed in 85% of the mice one week after challenge and was present in all mice on all subsequent occasions throughout the trial. There were no significant differences in the degree of leptospiruria between any of the groups of mice. When the kidneys were cultured eight months after challenge, *ballum* was isolated from all the mice except for one in the control group. All the cultures from this mouse's kidneys were contaminated with other bacteria preventing detection of leptospires.

CONCLUSION : B.C.G. vaccination did not appear to affect the susceptibility of mice to infection or kidney colonisation by *ballum* or the degree or duration of leptospiruria. Therefore, it is considered unlikely that Johnes vaccine would have had any effect on the susceptibility of cattle to infection with *hardjo*.

APPENDIX VI

HARDJO/POMONA BACTERIN

The vaccine was prepared in the Wellcome Research Laboratories, Dagenham, England, from killed cultures of *hardjo* and *pomona*. The original cultures of *hardjo* and *pomona* had been isolated from cattle and pigs in N.Z. (Marshall, pers. comm.). The cultures were "fermenter-grown" in a protein-free synthetic medium developed by Broughton (pers.comm.) and cell densities of 10^{10} organisms/ml were achieved. Separate batches of *hardjo* and *pomona* were blended so that the vaccine contained 2×10^9 *hardjo* organisms and 10^9 *pomona* per 2 ml dose, with aluminium potassium sulphate as an adjuvant at a final concentration of 1%(w/v). Each component satisfied the potency standard recommended by the U.S. Department of Agriculture and the vaccine met the requirements of the European Pharmacoepiae as regards safety, sterility, freedom from abnormal toxicity and freedom from living leptospires (Marshall *et al*,1979b).

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LIST OF APPENDED SCIENTIFIC PUBLICATIONS

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