STUDIES ON REPRODUCTIVE PERFORMANCE OF BEEF CATTLE IN CENTRAL QUEENSLAND

Thesis submitted by
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DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institute of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

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ABSTRACT

Reproductive rates in beef cattle observed during a five year study (1979-1983) in the Central Highlands of Queensland were shown to be comparable with rates obtained in coastal areas. Conception rates as high as 88.8% (mean 82.6%) and branding rates as high as 84.1% (mean 76.6%) were recorded. An effect of the 1982 drought was to decrease mean branding percentage for thirteen properties surveyed in 1983. The 1983 mean branding percentage was 61% which was significantly below the 72% obtained in 1981 ($p < 0.05$) and the 74% obtained in 1984 ($p < 0.01$). This is consistent with results obtained elsewhere.

Of 27 properties experiencing poor reproductive performance tested for vibriosis using the Campylobacter vaginal mucus agglutination test, twelve were found to be infected with a cow prevalence of 28.9% (201 cows tested and 58 infected). Only one property was found to be infected with trichomoniasis.

The 13 properties in the major survey were sampled before and after the wet season for a two year period (November 1980 to November 1982). Serovar hardjo was significantly ($p < 0.01$) more prevalent (13%) than serovar pomona (4.1%) in this area. When the properties were divided into two groups on the basis of water holding capacities (WHC) of their predominant soil types, serovar hardjo was significantly ($p < 0.05$) more prevalent (18%) on properties with high (> 30%) WHC soils than on properties with low (< 20%) WHC soils (7.3%). Such differences in prevalence were not detected for serovar pomona.

Graphical display of monthly rainfall and hardjo prevalence demonstrated a cyclic pattern for both. Hardjo peaks tended to follow the rainfall peaks by 2 to 3 months. Similar effects were not demonstrable for pomona. As this area frequently has a midyear rainfall peak, hardjo prevalence may often peak when the majority of the area's beef cows are in the last half of pregnancy when they are most susceptible to leptospiral infection.

Twenty-six stock horses sampled were shown to have high hardjo (30%) and pomona (40%) prevalence in this area. This could have been due to

local husbandry practices which may increase the exposure of these horses to young cattle and household dairy cattle and their urine under conditions of high stocking rates. Forty-two feral pigs sampled in this area were found to be virtually free of hardjo and pomona. Stock horses may therefore play a part in disseminating leptospirosis in this area.

In a single herd study, a group of pregnant cows (177) was tested before and after the last trimester of pregnancy for both serovars. Those cows with rising titres (34) had a significantly ($p < 0.05$) lower branding rate (64.7%) than those with steady or declining titres (83.2%). Thus leptospiral infection has been shown to correlate with branding percentage.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

1 GENERAL INTRODUCTION

Most major studies on factors influencing bovine reproduction in Queensland have been carried out either in the coastal area usually near Brisbane or Townsville. No such work has been carried out in the Central Highlands and few surveys are reported from other subcoastal or western areas in Queensland (Entwistle 1983).

The present aim was to establish the reproductive rates obtainable in Central Queensland and to examine factors which might adversely affect those rates. Two of the major factors influencing fertility in herds of beef cattle, environmental factors and disease, were studied in some detail (Murray and Entwistle 1978).

The effect of drought and rainfall on branding percentages are examined. Other authors have found that drought depresses reproductive performance (Carroll and Hoerlein 1966, Daly 1971) whilst rainfall tends to lift reproductive performance (Donaldson 1962). The climate of the Central Highlands is characterized by great variability in rainfall with frequent droughts and floods (O'Sullivan 1977).

Some observations were made to determine how commonly the two venereal diseases of cattle, vibriosis and trichomoniasis, may cause infertility in beef cattle in this area. Although trichomoniasis is thought to be uncommon in Queensland (Seddon and Albiston 1966) vibriosis is endemic in north Queensland with 15.8% of cows being positive to the vaginal mucus agglutination test in one abattoir survey (Summers, Campbell and Dennett 1974).

Particular attention was paid to leptospirosis and the possible effect of the predominant soil type of a property on its period prevalence. Whilst several authors have examined the relationship of soil type and leptospiral prevalence the present study concentrates on the effect of differences in moisture holding capacities of the major soil groups in this area may have on prevalence. Black cracking clay soils similar to those in some parts of the highlands have been considered favourable to the spread of leptospirosis (Knott and Dadswell 1970).

The effect of the seasonal rainfall pattern on prevalence was also examined as it has been shown that the prevalence of leptospiral nephritis in coastal north Queensland peaks soon after the seasonal summer (February) rainfall peak (Amatredjo, Campbell and Trueman 1976). In central Queensland a midyear rainfall peak is a feature of the climate. Should the leptospiral prevalence peak after this midyear rainfall it would coincide with the last trimester of pregnancy for most beef cows in this area.

The prevalence of the disease in the other two major species of domestic animals in this area (pigs and horses) was also determined. Finally the possible effect of leptospirosis on pregnant cows and calves was examined in a single herd study. Thus some of the factors which may influence the epidemiology of leptospirosis and determine the effect the disease has on cattle in the Central Highlands were analysed.

2 FACTORS INFLUENCING REPRODUCTIVE PERFORMANCE IN BEEF CATTLE

2.1 Measurement of reproductive performance

The most commonly used methods of measuring fertility in beef cattle are: conception rate, pregnancy rate, calving rate, branding rate and weaning rate. Conception and pregnancy rates are measured by manually pregnancy testing cows either once (if restricted mating is used) or twice (if mating is all year round) per year. This method requires trained personnel and is thus not used routinely by cattle producers. Calving rate is assessed by observing the number of calves born dead or alive but is only practical for small well-managed herds. The method most widely used by producers to measure reproductive performance in northern Australia is branding rate by comparing the number of calves branded to the number of cows mated the previous year. The main source of inaccuracy then lies in the estimation of breeder numbers in extensively reared herds. Weaning rate is similar in principle to branding rate but estimates the number of calves weaned (Murray and Entwistle 1978).

Conception rates of 90% (Osborne 1960) and branding rates of 80% in subtropical Australia (Osborne 1960) or 70% in subcoastal Queensland (Alexander 1962) are considered normal. In his recent review, Entwhistle (1983) stated that reproductive rates in cattle are lower in northern than southern areas. His summary of reproductive performance from several tropical herds showed pregnancy rates in Queensland varying from 34 to 91%. In north Queensland Holroyd, Arthur and Mayer (1979) found a mean annual conception rate of 83.1% and losses between conception and branding ranging from 5.9 to 27.7% over a three-year period in five herds under investigation.

2.2 Factors influencing herd fertility

2.2.1 General

The major factors influencing fertility in beef cattle herds can be divided into three general groups: environmental (rainfall, temperature and

consequent nutrition), physiological (body weight and body weight change, body condition, lactation status and age) and disease (for example trichomoniasis, vibriosis, brucellosis and leptospirosis) (Murray and Entwistle 1978).

2.2.2 Environmental influences on herd fertility

It has been shown that higher reproductive performance often follows years of high rainfall (Donaldson 1962) and that low reproductive performance is usual after drought years (Carroll and Hoerlein 1966; Daly 1971). Highest conception rates occur about one month after the onset of rain due to changes in dietary intake resulting from new pasture growth (Murray and Entwistle 1978). During years of severe nutritional stress reduced fertility is particularly evident in heifers and lactating cows (Entwhistle, Goddard, Hodge 1983).

Lamond (1970), has shown that undernutrition decreases herd reproductive performance and can also increase the age of puberty, prolonging post partum anoestrus and if particularly severe during late pregnancy reduce calf birthweight and therefore survival. Entwhistle (1983) indicated that there are complex interrelationships between these factors and that the physiological mechanisms involved are still poorly understood.

High temperatures can decrease bovine fertility but under grazing conditions this is compensated for by factors such as adaption and breed and strain effects (Murray and Entwhistle 1978). Entwistle (1983) suggested that high temperatures had been incriminated as a cause of early embryonic death in temperate zone cattle but this effect is less likely in tropical breeds.

2.2.3 Physiological influences on herd fertility

Heifer calves which are heavier tend to reach puberty earlier and are more likely to conceive as are heavier first calf cows. The dampening effect of obesity on fertility is not likely to be encountered under grazing

conditions. Lactating cows have a lower fertility than non-lactating cows. Aged cows (8-9 years old) lose body condition more quickly during stress than younger cows and are slower to recover their condition (Murray and Entwistle 1978).

2.2.4 Reproductive function of bulls

In Central Queensland, approximately on the tropic of Capricorn, Chenoweth and Osborne (1975) examined the effect of breed on the reproductive function of young beef bulls. In their sample they found significant differences between breed for the prevalence of testicular hypoplasia and variation in libido and mating ability when Brahman, Africander, Hereford, Brahman cross-bred, Africander cross-bred, and Shorthorn Hereford cross-bred bulls were compared. The prevalence of testicular hypoplasia in Brahman and Brahman cross-bred bulls was higher than in the other groups. Cross-bred bulls showed a higher libido and Africander and Africander cross-bred bulls had a better mating ability. The Africander crosses obtained significantly higher conception rates (79.1%) than the Brahman (54.9%) or Brahman cross-bred (64.6%) bulls.

2.2.5 Reproductive diseases

Bovine reproductive diseases can be readily sorted into two types: specific infections which occur without a predisposing cause and may be enzootic, and infections which require a predisposing cause and thus tend to affect individual cows (Arthur 1975).

Trichomoniasis is caused by the protozoon Trichomonas fetus. Three main serovars of Trichomonas fetus (T fetus var Belfast; T fetus var Brisbane and T fetus var Manley) have been observed in Australia (Wosu 1977). In north-eastern Australia Dennett et al. found serovar Brisbane to be the most common (79.6%) whilst serovar Belfast accounted for all other isolations they made in the area. Although the organism varies in shape it is generally piriform, from 5u. to 15u. long and 0.7u. wide. It is freely motile at 37°C by means of an undulating membrane and anterior and posterior flagellum (Laing 1970). Clinical signs of the infection result

from early embryonic mortality and are characterized by repeat mating and long, irregular oestrus cycles. Observable abortions occasionally occur but always prior to the fifth month. Postcoital pyometra occurs in up to 5% of infected cows. The infection in cows is self-limiting but bulls become carriers. Trichomoniasis is a true venereal disease and the only significant method of infection is via service (Laing 1970, Siegmund 1973, Arthur 1975, Hungerford 1975, Blood, Henderson and Radostits 1979).

Clark et al. (1974) found that by culling all bulls over four years of age and introducing non-infected bulls aged between 1 and 3 years the disease can be controlled in large beef herds. Christensen and Clark (1979) confirmed that older bulls are more likely to be infected than younger bulls within a herd and that although the disease can be spread passively by non-infected bulls (Clark, Dufty and Parkonson 1977) the disease can be controlled by culling bulls routinely at four years of age.

Diagnosis is made by demonstrating the organism either in vaginal washings from infected cows or preputial washings from infected bulls. It has been shown that the organisms' distribution in the genital tract of bulls is limited to the penis and prepuce and that it is localized in the prepucial secretions (Parsonson et al. 1974). Tedesco, Errico and Del Baglivi (1979) showed that scraping the preputial mucosa was preferable to aspiration for diagnosis when using direct microscopic examination but both methods were equally good when samples were cultured within two hours of collection. Alternatively the abomasum of aborted foetuses usually contain an abundance of the organisms (Seddon and Albiston 1966). Although Seddon and Albiston (1966) considered trichomoniasis to be uncommon in Queensland, Ladds, Dennett and Glazbrook (1973) found a prevalence of 30.2% in culled bulls from north Queensland and The Northern Territory. Turnbull (1977) found a 4% prevalence in bulls slaughtered in Western Australian abattoirs and Dennett et al. (1974) found a 4% prevalence in North-eastern Australia in a survey of herds. It was also found that the larger extensive herds west of the Great Dividing Range had a higher prevalence than those to the east.

Vibriosis is caused by the motile, Gram-negative, spirillar bacterium Campylobacter fetus (formerly known as Vibrio fetus) (Trinca 1979). The organism has a single polar flagellum and is 1.5 to 5.0u. long by 0.2 to

0.3u in breadth. There are two pathogenic strains of this organism, C fetus subsp venerealis and C fetus subsp intermedius which are catalase positive, reduce nitrates to nitrites and do not produce H_2S on culture. The saprophytic strains such as C bubulus and C faecalis are catalase negative, reduce nitrates to nitrites and produce large quantities of H_2S on culture (Laing 1970, Arthur 1975). Subspecies venerealis and intermedius have been identified in Australia (Clark, Monsborough and Dufty 1974). The primary effect of vibriosis is temporary infertility characterized by irregularity of the oestrus cycle. If abortions occur, they occur at the five to six month stage but these are of only secondary importance in the disease. Infected cows build up immunity and eventually recover whilst bulls usually become carriers. This is also a true venereal disease (Laing 1970, Siegmund 1973, Arthur 1975, Hungerford 1975, Blood, Henderson and Radostits 1979).

Diagnosis is made by culturing the organism from prepucial washings of infected bulls or aborted foetuses or by the vaginal mucus agglutination test on infected cows (Hungerford 1975, Clark and Dufty 1978) or demonstrating it by immunofluorescence. The use of enrichment medium in the field greatly increases the chances of recovering the organism at the laboratory (Clark and Dufty 1978). This disease is relatively common in Queensland. Up to 50% of herds may be affected in some northern districts (Hungerford 1975). Summers, Campbell and Dennett (1974) found 15.8% of cows were positive to the vaginal mucus agglutination test during an abattoir survey in north Queensland.

It has been shown that vaccination with killed bacterins protects bulls from infection with vibriosis (Clark, Dufty, Monsborourgh and Parsonson 1974). Such bulls however can become passively contaminated and although not a cause of spread under normal conditions (Clark, Dufty, Monsborough and Parsonson 1975) can result in the spread of disease under conditions of intensive sexual activity (Fivaz, Swanepoel, McKenzie and Wilson 1978). Vaccination of infected bulls is effective in eliminating infection in most cases but should not be recommended as the sole measure of control (Vasques et al. 1983). Dual vaccines active against both pathogenic subspecies have been used successfully in Australia on bulls, cows and heifers (Clark et al. 1977, Clark et al. 1979).

Brucellosis caused by Brucella abortus is now a very uncommon cause of infertility in Queensland due to the success of the National tuberculosis and brucellosis eradication scheme and by 1990 the State should be free of this once common disease.

Leptospirosis is a complex infection in cattle as it is usually not venereal, produces a variable and wide range of signs, does not always affect fertility and can be carried by non-ruminants. The organisms often spend a short time free in the environment before infecting a new host. The disease is also a zoonosis. Since particular attention has been given to leptospirosis in this study a detailed review of this disease complex is presented.

3 LITERATURE REVIEW OF BOVINE LEPTOSPIROSIS

3.1 Microbiology

3.1.1 Taxonomy

The genus Leptospira can be divided into two species, Leptospira interrogans representing the pathogenic types while Leptospira biflexa represents the saprophytic types (Kenzy and Ringen 1967). Others prefer to use only one species - Leptospira interrogans, divided into the pathogenic interrogans complex and the saprophytic biflexa complex (W.H.O. 1967).

The Leptospira interrogans complex or species is divided into sixteen (16) serogroups on the basis of antigenic structure. Into these serogroups the 180 odd serovars are assigned (W.H.O. 1967 and Robertson 1983). The serovar remains the basic taxon when discussing leptospirosis (Abdussalam, Alexander, Babudieri, Bogel, Borg-Petersen, Faine, Kmety, Lataste-Dorelle, and Turner 1972).

3.1.2 Morphology and staining characteristics

The different serovars of leptospires are indistinguishable on the basis of morphology. They are extremely thin (0.1u. - 0.2u.), elongated (8u. - 12u.) and tightly spiralled organisms. Their ends are commonly bent into a hook. Due to their extreme thinness they cannot be seen in unstained wet preparations unless dark background microscopy is used (Kenzy and Ringer 1967, and Michna 1970).

Leptospires do not stain readily with the aniline dyes. They can however, be stained to some degree with Hoyer's congo red, Giemsa, basic fuschin in formalin-fixed smears though preferably silver impregnation (Kenzy and Ringer 1967). Fluorescence microscopy and immunoperoxidase methods are being used increasingly.

Leptospires are actively motile organisms by means of a spinning action on the long axis and perhaps also through a serpentine undulating motion (Kenzy and Ringer 1967).

Ultrastructurally organisms consist of a cytoplasmic body with an axistyle inserted subterminally at each end, and a sheath or envelope that encloses both structures. Due to the similarities between the axistyle of leptospirae and the axil filaments of bacterial flagellae it is felt that the axistyle is concerned with motility. The body wall consists of the following concentric structures from the outside inwards: an envelope or enveloping sheath; a perimural layer in which the axistyle is embedded and a cell wall that seems to be closely associated with the cytoplasmic membrane. The wall is structurally and chemically analogous to that of Gram-negative bacteria and in fact leptospires are Gram-negative (Abdussalam et al. 1972).

3.1.3 Immunochemical and antigenic characteristics

Abdussalam et al. (1972) considered that the role of agglutinogens (serovar specific antigens) and other cellular factors in immunity should be reexamined. On the basis of empirical evidence it has always been thought that agglutinogens were related to immunogens. However experimental cross-protection has been demonstrated with some serovars which are antigenically unrelated.

3.1.4 Genetics

Pathogenic Leptospira interrogans can be divided into two genetic groups on the basis of the percentage of guanine plus cytosine in their DNA. The two groups can also be differentiated phenotypically. It was proposed that these two groups may be considered as distinct species (Abdussalam et al. 1972).

3.1.5 Growth requirements and metabolism

Most types of media used for growing leptospires require 5 to 10 percent serum (usually rabbit), a pH of 7.2 to 7.4 and an incubation temperature of 28.5° to 29.5°C for optimal growth. After inoculation with field samples, cultures may take 6 weeks before observed growth

occurs. However strains adapted to laboratory media may produce satisfactory growth in several days (Kenzy and Ringer 1967). The importance of lipids as a source of energy for leptospires is well known and there is evidence that chemically defined media may eventually be used to culture different strains. The differences between lipolytic activity of saprophytic (lipase-positive) and pathogenic (lipase-positive or negative) strains is also of continuing interest (Abdussalam et al. 1972).

3.1.6 Resistance

Leptospirae do not withstand conditions of dryness or heat well (Kenzy and Ringer 1967). With regard to disinfectants Chang, Buckingham and Taylor (1948) found that residual iodine (0.7 ppm for 10 minutes) or 3 to 5 ppm residual chlorine would kill serovar icterohaemorrhagiae. Ten ppm cationic detergent for 30 minutes or 2.2 percent salt also destroyed this organism.

Surface waters with low pH (5.1) do not encourage the survival of leptospires (Chang et al. 1948) but at a higher pH the organisms can survive for periods ranging from 4 to 99 days (Chang et al. 1948, Okazaki and Ringen 1957 and Smith and Turner 1961). Okazaki and Ringen (1957) found a longer survival time for serovar pomona at pH 6.2 for 7°C than at 20°C to 26°C, while at pH 8.4 the reverse effect occurred. Smith and Turner (1961) found that four serovars (icterohaemorrhagiae, tarassovi, australis A and javanica) had shorter-mean survival times (10-117 days) at pH 5.3 to 7 than at pH 7 to 8 (21-152 days).

3.2 History of leptospiral epidemiology

3.2.1 General

The first report of leptospires causing disease in cattle was in 1935 when serotype grippotyphosa was isolated from calves with haemoglobinuria in the USSR. (Michen and Azinov 1935). This finding was followed by Jungherr (1944) who described the disease in cattle in the United States. Table 1 shows the serovars isolated from cattle in various countries and the date of their first isolation.

TABLE 1

FIRST PRINCIPAL LEPTOSPIRAL SEROVAR ISOLATIONS
FROM CATTLE IN VARIOUS COUNTRIES
(adapted from Amatredjo and Campbell 1975)

SEROGROUP	SEROVAR	COUNTRY	DATE
Australis	australis A	Japan	1960
	peruviana	Peru	1967
Autumnalis	autumnalis	Japan	1960
		China	1960
Ballum	ballum	New Zealand	1973
Bataviae	bataviae	China	1960
Canicola	canicola	Israel	1955
		USA	1958
		Brazil	1961
		French Union	1961
		Germany	1962
		USSR	1966
		Columbia	1966
	azuli	Argentina	1967
	galtoni	Colombi	1969
Grippotyphosa	grippotyphosa	USSR	1935
		Israel	1948
		Tunisia	1953
		Denmark	1956
		Kenya	1958
		USA	1964
		France	1966
*Hebdomadis	hebdomadis	Japan	1955
		USSR	1956
		Israel	1964
	sejroe	USSR	1965
		Britain	1969
	saxkoebing	USSR	1964

*Recently renamed Sejroe (Ellis, Hustas, Robertson and Mayberry 1984).

	hardjo	USA	1960
		Canada	1964
		Britain	1974
		Italy	1968
		Australia	1969
		New Zealand	1973
		Japan	1953
Icterohaemorrhagiae	icterohaemorrhagiae	Britain	1949
		Ireland	1956
		USSR	1957
		Germany	1962
		Brazil	1961
		Peru	1966
		USA	1970
	copenhageni	New Zealand	1960
Pomona	pomona	USSR	1940
		Argentina	1944
		USA	1948
		Canada	1957
		Australia	1949
		Hungary	1952
		Turkey	1956
		Denmark	1958
		Yugoslavia	1959
		Bulgaria	1961
		Germany	1962
		Brazil	1957
	kennewithe	USA	1967
Semaranga	patoc	USA	1969
Tarassovi	tarassovi	USSR	1962
	barbotin	USA	1962
	illini	USSR	1973

Serological evidence of the disease is more widespread and has usually preceded isolation of the bacteria in the countries mentioned above.

3.2.2 Australia

Human leptospirosis of bovine origin in Australia was first diagnosed by isolation in 1937 when Clayton, Derrick and Cilento (1937) isolated serovar pomona from a human seven-day fever case.

Serovar pomona was the first isolated from Australian cattle. In 1949 it was shown to cause icterohaemoglobinuria in calves in Queensland (Sutherland, Simmons and Kenny 1949). By 1958 the disease was reported in all states of Australia and to be widespread in all dairying areas of Queensland (Parkinson 1958). Serovar hardjo has also been isolated from cattle in Australia (Sullivan and Stallman 1969). Leptospira australis was isolated from a subclinical case of interstitial nephritis in a steer in north Queensland by Campbell and Stallman (1975). Although there is widespread serological evidence of infection by serovar tarassovi (hyos) in Australian cattle, as yet this organism has not been isolated and it is not regarded as having pathological significance.

3.3 Clinical signs

3.3.1 General

Michna (1970) described four clinical responses in animals to natural infection with various leptospiral serovars.

(i) Sub-clinical infections occur in both wild and domestic animals, especially pigs and cattle. Animals thus affected are usually healthy carriers whose infection remains unnoticed unless investigated by serology or urine culture.

(ii) Acute or sub-acute infections whose clinical signs include fever, depression, anorexia and loss of milk production and, in severe cases, jaundice and haemoglobinuria. Neurologic manifestations

may also be observed. Mastitis can occur with serovar hardjo.

(iii) Reproductive disorders such as infertility, abortion and stillbirths are found especially in pigs, cattle, ewes and mares.

(iv) Ocular disease (periodic ophthalmia) has been reported in horses, man and pigs.

3.3.2 Bovine leptospirosis

Acute leptospirosis is seen mainly in calves (Michna 1970, Sullivan 1974). It is characterized by sudden onset of high fever, haemoglobinuria, jaundice and anorexia. Calves usually die within 3 to 5 days of illness though some may survive poorly developed and a few may recover (Michin and Azinov 1935, Parkinson 1958, Prescott 1967, Corbould 1972, Marshall 1972, Sullivan 1974, Shield 1974). The younger the calf, the more severe the disease seems to be (Parkinson 1958). Shield (1974) considered that this 'red-water' form of leptospirosis was less frequently seen in western Queensland than reproductive disorders. The condition has occasionally been described in older cattle (Jungherr 1944).

Ris, Lake and Holland (1973) isolated serovars copenhageni and balcanica from healthy calves and Durfee and Presidente (1979) failed to produce clinical signs after infecting calves with serovar balcanica.

Serovar pomona may be the most common leptospiral infection in Australian cattle (Prescott 1967, Sullivan 1974). Reproductive disorders attributed to leptospirosis include late pregnancy abortions, stillbirths, mastitis and subsequent lactation failure and orchitis (Parkinson 1958, Corbould 1972, Marshall 1972, Sullivan 1974, Little and Hathaway 1983 and Robertson 1983). Herr, Riley, Neser, Roun and De Lange (1982) found serovar pomona to be the cause of an abortion storm in South Africa.

In Britain Michna (1971) found that 62.6% of sera obtained from aborting cattle were serologically positive to a member of the Hebdomadis serogroup serovar sejroe whilst in control cattle only 28.3 to 59.3% of animals reacted. During an experimental outbreak of leptospirosis in

Queensland (serovar pomona) one of five pregnant heifers aborted (Doherty 1967b).

In North Queensland Johnson, Allan and Dennett (1974) found a correlation between abortions and serovar hardjo antibody formation in a group of 12 heifers of which 10 were serologically positive and 3 aborted. A severe abortion storm on a grazing property in Queensland in which 50% of cows aborted in two months has been described by Knott and Dadswell (1970). Of 34 cows tested 26 gave positive agglutination tests for serovars pomona and tarassovi. One cow out of six was also positive for leptospires on dark background microscopic examination of urine. Features of the outbreak were abortions, stillbirths, retained placenta and deaths in young calves. A survey of literature showed significant differences in the seropositive reactions of herds with an infertility syndrome compared with normal herds (Amatredjo and Campbell 1975).

Leptospires of the Hebdomadis serogroup were isolated from both aborted foetuses and a premature calf in Northern Ireland (Ellis and Michna 1976, Ellis, O'Brien, Neill, Hanna and Bryson 1976). Antibodies were found in the sera of 15 out of 218 aborted foetuses whilst no antibodies were detected in the sera of 196 non-aborted foetuses (Ellis et al. 1978). Leptospiral infection was diagnosed in 41.6% of randomly selected aborted bovine foetuses and 68.9% of foetuses from farms with abortion problems (Ellis et al. 1982) whilst it was found in only 4.6% of normal foetuses collected from an abattoir (Ellis, Neill, O'Brien, Cassells and Hanna 1982). It has been estimated that leptospirosis and vibriosis cause a 20% reduction in the reproduction rates of cattle in Queensland (Queensland Department of Primary Industries 1981).

After studying the effects of experimental infection of bulls with serovar pomona, Sleight, Atallah and Steinbauer (1964) postulated that a leptospiral orchitis of sufficient severity to produce sterility might be a possibility. Ladds, Dennett and Glazebrook (1973) however found no correlation between testicular lesions in bulls and leptospirosis during an abattoir survey.

Sullivan and Callan (1970) described an outbreak of bovine mastitis due to serovar hardjo in a Queensland dairy herd. This organism produced

the following clinical signs: fever, depression, inappetance, depressed milk production and a flaccid udder with a yellowish secretion containing clots obtainable from all quarters. The milk returned to normal in 4 days and full production was restored after 14 days (Sullivan 1974). A similar condition has been described in New South Wales with abortions following mastitis (Hoare and Claxton 1972). Ellis, O'Brien, Pearson and Collins (1976) in Ulster found that in an outbreak of serovar hardjo mastitis in a dairy herd there was a significant number of infected animals which did not display any signs of disease. A feature of leptospiral mastitis in a herd is the high incidence of mammary infection marked only by a fall in milk production and an increased milk leucocyte count. Thus the fall in milk production may be greater than that accounted for by the number of clinical cases (Sullivan 1974).

Cordes, Carter, Townsend, Lewis and Holland (1982) concluded after a study in New Zealand that clinical disease was much less common than subclinical infection with serovar hardjo. Hathaway and Little (1983) maintained that clinical disease may pass unnoticed where the disease was endemic but hardjo infection causes greater problems in first and second calf dairy heifers than in the rest of the herd. First calf heifers are particularly susceptible when raised in isolation (Robertson 1983).

In north Queensland nephritis has been shown to be caused by pomona (Amatredjo and Campbell 1976) and australis (Campbell and Stallman 1975) but in adult cattle this condition was subclinical. Serovar tarassovi does not appear to be pathogenic for cattle (Campbell 1979) although serological evidence of infection may be widespread.

3.3.3 Clinical signs in other animals in Australia

Pigs: Serovars pomona and tarassovi cause abortion, stillbirths, neonatal mortality and retained placenta with pomona being regarded as the most important in Australia (Seddon and Albiston 1965, Corbould 1972, and Sullivan 1974). Other serovars (for example canicola) may be found in Britain and elsewhere (Michna and Campbell 1969).

Sheep: Durfee and Presidente (1979) failed to produce any clinical signs in pregnant ewes inoculated with serovar balcanica. Sheep appear to be relatively resistant to leptospirosis but outbreaks of haemolytic disease due to pomona have been reported (Seddon and Albiston 1965, Sullivan 1974). Serovar hardjo has been suggested as a cause of lamb mortality in Victoria (McCoughan, Gordon, Rakaby, Slee and Presidente 1980) but Andreeni, Tolari and Farina (1983) considered that hardjo produced only subclinical disease in sheep. In Northern Ireland leptospiral infection with members of the Hebdomadis, Australis and Pomona serogroups has been implicated as a cause of late pregnancy abortion, stillbirth and new born lamb mortality (Ellis et al. 1983).

Dogs: Clinical leptospirosis caused by icterohaemorrhagiae producing nephritis and widespread haemorrhage is occasionally seen in Australia (Sullivan 1974) though in other countries such as Britain serovar canicola predominates.

Cats: The disease appears to be subclinical in this species (Sullivan 1974, Robertson 1983).

Horses: Clinical leptospirosis has rarely been recorded in Australia (Sullivan 1974). Kirkman et al. (1982) concluded that leptospirosis is not a common clinical disease of horses in North Queensland. They found 8.2% of horses surveyed reacted to pomona while only 1.4% reacted to hardjo. Hogg (1974) isolated pomona from a sick foal which recovered without treatment. Leptospirosis is thought to be one of the causes of periodic opthalmia. Swan, Williams and Taylor (1981) found that an ocular syndrome manifested by severe, painful keratitis or an iridocyclidis was the most consistant clinical finding of leptospirosis in the horse. These lesions may not occur until 12 to 24 months after the clinical episode. Slatter and Hawkins (1982) found 33% of normal horses had titres to leptospiral serovars. In Northern Ireland Ellis et al. (1983) found infection due to the serogroups Australis, Pomona, Hebdomadis and Icterohaemorrhagiae caused abortion in mares.

Feral animals: Durfee and Presidente (1979a) managed to infect bush-tailed possums, common wombats and water rats with serovars balcanica and hardjo. The disease caused mild to moderately severe focal interstitial nephritis. In north Queensland Glazebrook et al. found fifteen strains of leptospires being actively excreted by feral rodents. English (1982) found serological evidence of serovar pomona in wild fallow deer in New South Wales but the prevalence was low (2.7%).

3.4 Pathogenesis

After entry of leptospires into the host they migrate via the lymphatics and blood to the liver and kidneys where they multiply (Sullivan 1974). This incubation period may take from 2 to 21 days (Michna 1970, Marshall 1972 and Sullivan 1974). A leptospiraemia with a febrile response lasting up to 7 days may occur (Marshall 1972, Sullivan 1974) but is terminated at this stage by the development of specific antibody and subsequent phagocytosis (Michna 1970).

Some strains of serovar pomona produce a haemolysin which results in haemoglobinuria in some cases near the end of the leptospiraemic stage, while hardjo does not possess this enzyme (Sullivan 1970, Marshall 1972 and Amatredjo and Campbell 1975). Kasarov (1970) found haemolysins in some strains of serovars copenhageni, icterohaemorrhagiae, ndambari and pomona. During the leptospiraemic stage organisms can be demonstrated in every organ of the body but after clearance of leptospires from the blood they appear to localize in various regions. The kidneys are a prime site for the localization of leptospires which arrive from the blood. They traverse the intertubular spaces, the tubular epithelial cells or their junctions and enter the lumina of the renal convoluted tubules. Here they multiply forming small clumps and finally escape into the urine causing leptospiruria (Michna 1970).

Amatredjo, Campbell and Trueman (1976) cultured leptospires from 16% of bovine nephritis cases during an abattoir survey in north Queensland. Serovars pomona and less frequently australis were isolated (Campbell and Stallman 1975, and Amatredjo et al. 1976). Here the organisms produce

an interstitial nephritis probably of immunological origin caused either by an auto-immune mechanism (Spradbrow and Seawright 1963) or a simple host-organism reaction (Amatredjo et al. 1976). Cellular responses are dominated by lymphocytes and some plasma cells.

Faine (1962) showed that older animals or animals exposed to smaller infective doses tended to progress to leptospiral colonization of the kidney tubules whilst younger animals or animals exposed to higher infective doses were more likely to die. If a host-parasite equilibrium is established, the animal then becomes a reservoir host or carrier (Michna 1970) a condition which may last for 6 months or longer (Marshall 1972). Sleight et al. (1964) found leptospires resident in testes and epididymides of bulls and noted that the interstitial lesions were similar. Leptospires have been cultured from both the milk and blood of cows affected with hardjo mastitis (Ellis, O'Brien, Pearson, Collins 1976).

It is thought that organisms enter the foetus via either haematogenous spread (Ellis and Michna 1977), persistent genital tract infection or descending infection from the peritoneal cavity (Ellis, O'Brien et al. 1982). The organism can be isolated from the kidneys of aborted foetuses (Ellis et al. 1982).

Leptospirosis does not always lead to abortion in pregnant cattle. If cattle are infected during the second half of pregnancy when the leptospires can more easily penetrate the placenta, foetal leptospirosis can occur (Amatredjo et al. 1976).

According to Fennestard and Borg-Petersen (1960) the features of bovine genital leptospirosis are:

(i) Abortion is not a constant sequel to leptospirosis in pregnant cattle. Abortion due to leptospirosis occurs in the latter half of pregnancy. When clinical signs are observed there is a 2-3 week interval between the onset of signs and abortion.

(ii) The dam may be free of clinical signs but is serologically positive with a rising titre.

(iii) Abortion is often the only clinical sign.

(iv) Aborted foetuses usually appear to have been dead 24 hours or more before expulsion.

(v) The most consistent changes in aborted foetuses are oedema of certain tissues, e.g., subcutaneous tissues and intercotyledonary areas and haemorrhagic fluids in body cavities.

(vi) Foetal membranes are usually oedematous and have ischaemic vessels but no signs of inflammation. Uniformly affected, fawnish-yellow cotyledons are seen.

3.5 Pathology

3.5.1 Post-mortem findings

Calves which have died of acute leptospirosis reveal the following changes on autopsy: anaemia, petechial haemorrhages throughout subcutaneous and muscular tissues, icterus, enlarged, often friable livers or livers with focal necrotic areas, engorged kidneys with occasional necroses and inflammation of the abomasum with or without ulceration (Prescott 1967). In chronic cases of unthrifty calves the only finding may be swollen and scarred kidneys (Parkinson 1958). Amatredjo et al. (1976) described sub-clinical leptospiral renal lesions from an abattoir survey as dull white or fawn-coloured foci, only occasionally associated with hyperaemia and usually measuring 1 to 3mm. in diameter. Lesions occurred chiefly in the outer cortex. In some cases there was evidence of mild focal fibrosis, which when subcapsular, was associated with contracted scars and capsular adhesions. Jungherr (1944) also reported so-called 'nutmeg' lesions in the liver representing areas of diffuse central congestion.

Severe oedema of the allanto-chorion and separation and necrosis of the maternal and foetal placenta has been described in cases of experimental bovine leptospiral abortion (Murphy and Jensen 1969).

3.5.2 Histopathology

Microscopically the renal lesions in cattle consist of various degrees and forms of focal interstitial nephritis. The principal cellular reaction involves small lymphocytes distributed between tubules and frequently in perivascular situations. Lymphoid follicles are sometimes formed in the renal tissue. Marked tubular degeneration is detectable in and around the interstitial foci. Mild focal fibrosis can be found in many cases (Amatredjo et al. 1976). Intertubular lymphocytic infiltration can also be demonstrated in bull testes after pomona infection (Sleight et al. 1964).

Hadlow and Stoenner (1955), investigating histopathological changes in cows spontaneously infected with pomona, described similar kidney lesions to those described above. They also found haemosiderosis in the spleen; variable, but never marked, portal and interlobular mononuclear cell infiltration in the liver but no significant changes in the uterus or lungs. Slee, McOrist and Skilbeck (1983), found that foetuses aborted due to hardjo showed only mild interstitial nephritis and could find no specific placental lesions.

The most striking histopathological findings in aborted foetuses are widespread and severe vascular lesions in most organs. These involve mainly arterioles, venules and capillaries. The lesions are particularly severe in the liver where centrilobular degeneration is also present. Renal tubular degeneration and areas of necrosis are present in the kidneys. Autolytic changes in aborted foetuses can make accurate histopathological examination impossible (Ellis, et al. 1976), but does not interfere with the demonstration of leptospires by silver impregnation (Slee, McOrist and Skilbeck 1983).

3.6 Epidemiology

3.6.1 General

Of the four major bovine reproductive diseases in Australia (brucellosis, leptospirosis, trichomoniasis and vibriosis) the epidemiology of leptospirosis is arguably the most complex. Both vibriosis and trichomoniasis are

diseases which under natural conditions are spread by mating (Hungerford 1975). These two venereal diseases produce cyclic waves of infertility as the herd immunity ebbs and flows. Although Campylobacter foetus occurs also in sheep they are unimportant in the epidemiology of these diseases in cattle.

The epidemiology of brucellosis is complex as this disease is not venereal in nature but can be transmitted to a non-immune cow by ingestion (mainly), inhalation, direct contact, coitus (artificial insemination) or by congenital passive transfer (New Zealand Ministry of Agriculture and Fisheries 1977). Br. aboatus must therefore survive in the environment. However non-female cattle hosts are of limited importance and the infection is usually shed from the reproductive tract as a consequence of the infection at abortion.

Leptospires spend a considerable time free in the environment and the disease is mainly non-venereal in spread. The organism is usually spread in urine rather than from the reproductive tract. Non-bovine and non-female carriers may be of importance.

3.6.2 Dissemination of leptospires from the host into the environment

Urinary excretion of leptospires is the most significant factor in the epidemiology of the disease (Sullivan 1974). Hellstrom and Blackmore (1979) found calves began excreting organisms in the urine about two weeks after sero-conversion. Mean shedding time was 215 $\pm$ 26 days. Doherty (1967) found adult cattle to be excretors for 10 to 118 days with a mean of 36.4 $\pm$ 3.1 days. The highest level of excretion occurred during the first half of the leptospiruric phase; older animals tend to have a lesser degree of leptospiruria.

Leptospires have been isolated from aborted foetuses and it is thought that these could be a source of human infection. As large numbers were observed in the peritoneal fluid of an aborted foetus, it is possible that aborted foetuses could also be a source of infection to carnivores (Ellis et al. 1976). Both semen (Sleight et al. 1964) and milk (Ellis et al. 1976) have had leptospires isolated from them and as the

organisms are found in the blood during the leptospiraemic phase of the disease, blood must also be considered as a potential source of infection.

3.6.3 Survival of leptospires in the environment

Okazaki and Ringen (1957) considered that the survival time of serovar pomona outside the animal body is related to an interaction of several factors, including temperature, pH, moisture, the various constituents of soil and water and the presence of naturally occuring microorganisms. It seems reasonable to assume that this principle is universal for all serovars.

Outbreaks of leptospirosis are often linked with cattle in low lying, swampy conditions or poorly drained and muddy yards (Hoare and Claxton 1972, Shields 1974). Knott and Dadswell (1970) describe an abortion outbreak due to leptospirosis where they found higher abortion rates in low lying, highly stocked and black soil paddocks. The outbreak also followed higher than normal rainfall.

Leptospirosis due to grippotyphosa had a higher incidence in pastured animals than in animals kept in tie-up cowsheds (Raetz and Herr 1973), whilst that due to icterohaemorrhagiae may be seen in permanently housed animals in an environment contaminated by carrier rats. This may however be due to different geographical distributions of the two serovars (Twigg, Cuerden, Hughes and Medhurst 1969) as well as differences in the microenvironments.

Amatredjo et al. (1976) found an increase in leptospiral nephritis in north Queensland following the wet summer period. Also in Queensland Elder and Ward (1978) found by studying the mean annual rainfall in a district that serovar pomona had a higher prevalence in low rainfall areas whilst hardjo prevalence was uniformly distributed.

It is probable that net environmental water levels and not net rainfall is critical in the survival and spread of leptospires (Hellstrom and Blackmore 1979). Leptospirosis is more easily transmitted within a herd during static wet environmental conditions than during dry periods. This

is particularly so when the level of environmental contamination of leptospires is low (Doherty 1967a). Hellstrom and Blackmore (1979) however claimed that the size of the infective challenge to a group of susceptibles is not a major factor in establishing a propagating epidemic. It has been suggested that leptospiral transmission would be less likely in hot, arid environments (Songer, Chilelli, Marshall, Noon and Meyer 1983). Glazebrook et al. (1977) found rodent leptospirosis appeared to be most prevalent in areas of high rainfall.

Leptospires can survive for long periods in water. Smith and Turner (1961) showed survival in buffered distilled water for up to 152 days but this was dependent on both the serovar and the pH of the water. They found that for all four serovars examined (icterohaemorrhagiae, tarassovi, australis A and javanica) survival was longer in alkaline water up to pH 8 than in acid water. Although fast waters are detrimental to leptospiral survival slow-moving waters promote the spread of leptospires over great distances (Okazaki and Ringen 1957).

Urine, being strongly acid, does not allow the growth of leptospires unless it is neutralized or alkaline. Survival of leptospires in undiluted faeces is limited to less than 24 hours (Noguchi 1978). The presence of bacterial contamination also decreases survival time in water (Chang et al. 1948, Okazaki and Ringen 1957).

Serovar pomona survives best within the temperature range 7°C - 36°C and pH range 6 - 8.4. Survival in the lower pH range is longer at lower temperatures and in the higher pH range is longer at higher temperatures (Okazaki and Ringen 1957).

The degree of soil moisture is an important influence in the survival time of leptospires in soil. The wetter the soil the longer the organisms survive (Twigg et al.1968; 1969). Okazaki and Ringen (1957) found pomona could be cultured after 2.5 hours in dry soil, 5 days in damp soil and 183 days in supersaturated soil.

The role of soil pH in leptospiral survival is a complex one. Smith and Turner in Malaysia (1961) and Twigg et al. (1969) in the United Kingdom found leptospires in acid soils but Martin, Hanson and Schnurrenberger

in the USA (1967) considered that alkaline soils aided leptospiral survival. Twigg et al. (1969) stated that leptospires shed in urine on acid soil should survive for shorter periods than those shed on chalk. In the Roma district of Queensland cattle on black soil paddocks within a property where the pH was 6.1 to 6.5 had higher abortion rates during a leptospiral outbreak (Knott and Dadswell 1970). Kingscote (1970) in Canada who found a higher prevalence of leptospirosis where the bedrock was composed of limestone and dolomite concluded that bedrock type was more important than surface soil characteristics.

Whilst studying the unexpected low prevalence of leptospirosis in ricefield workers in Malaysia, Smith and Turner (1961) postulated that the montmorillonite clays of the ricefields might absorb leptospires and thus decrease the numbers free in the water. They found kaolin did not absorb leptospires but bentonite in 0.25% suspension reduced the number of live leptospires in the water by half. Bentonite is however similar to montmorillonite and the authors recommended that more work needed to be carried out on this subject. Ricefield rodents had a high prevalence of leptospirosis whilst the human population had a low prevalence. Kingcote (1970) in Canada found that clay is common in leptospiral habitats. Bacteria and other soil microorganisms are highly colloidal. Due to its 2 to 1 type crystal lattice montmorillonite clays have a very high cation-absorption capacity and can thus bind organic matter within its matrix. This property also means that a distinct favourable microenvironment around the clay particles is produced (Buckman and Beady 1960).

3.6.4 Non-ruminant reservoirs

In most foci of infection, one or more species of domestic or wild animal act as maintenance hosts. However during epizootics other animals living in the same biocenosis are also involved in the circulation of leptospires in the focus (W.H.O. 1967).

Domestic animals probably act as their own reservoir of infection although wildlife can play an important role (Marshall 1972). Twigg et al. (1968) in Britain suggested that the reservoir of leptospires in wildlife is

probably of considerable importance. Serovar hardjo in Ulster however appeared to be solely maintained by cattle (Ellis, O'Brien and Cassells 1981).

Wild pigs may be a source of infection for cattle when the cattle drink at sites used as wallows (Shield 1974). Spradbrow (1964) found positive titres to five serovars (icterohaemorrhagiae, robinsoni, esposito, pomona and tarassovi) in the sera of domestic pigs in the Brisbane area. Elder and Ward (1978) found a particularly high prevalence of pomona antibodies in the sera of feral pigs in Queensland. Corbould (1971) reported that hardjo had not been detected in pigs in Tasmania and this serovar has so far not been isolated from rodents (Ellis et al. 1981).

Whilst Spradbrow (1964) found serological evidence of leptospirosis in goats in Brisbane, Schollum and Blackmore (1981) considered that goats are not a natural maintenance host for leptospires.

Animals from the orders Rodentia, Lagomorpha, Insectivora, Carnivora and Artiodactyla have been found to be infected with leptospirosis (Twigg et al. 1969). Michna and Campbell (1970) in Scotland detected feral leptospirosis on farms where both domestic animals and humans were affected. Leptospires have also been detected in the American opossum (Martin et al. 1967). Songer et al. (1983), isolated serovar ballum from mice in a dairy.

In South Australia wombats (Corbould 1972), bush-tailed opossums (Durfee and Presidente 1977) and wallabies, koalas and deer (Milner, Spratt, Presidente 1981) have been shown to carry leptospirosis. Emmanuel, Mackerras and Smith (1964) working in north Queensland sampled 5 monotremes, 643 marsupials, 2355 rodents, 67 bats, 30 birds, 28 reptiles and 21 toads for leptospirosis. They found evidence of infection in 223 marsupials, 309 rodents and 6 fruitbats. In the same area Glazebrook, Campbell and Hutchinson (1977) found 6.4% of rodents with evidence of leptospirosis. They detected serotypes australis and celledoni in four species (Hydromys chrysogaster, Uromys caudimaculatus, Rattus fuscipes and Rattus norvegicus). Only one species is non-indigenous (R norvegicus).

It has been found in New Zealand that predator-chain transmission does not appear to be an important natural route for leptospirosis as free-living carnivores appear to be poor maintenance hosts (Hathaway and Blackmore 1981). In the United Kingdom however Hathaway, Little, Headlam and Stevens (1983) isolated serovar australis from free living carnivores.

Dogs in the Sydney area have shown positive serological reactions to seven serovars including pomona and hardjo (Watson, Wannan, Porges and Testoni 1976). The full significance of these findings remains to be determined.

The possible role of ticks in transmitting and maintaining leptospires has been studied. Krephogorskaia and Rementsova (1957) isolated grippotyphosa from the tick Dermacentor marginatus S. Ornithoctoros turicata has been shown to be able to infect guinea pigs with pomona and to maintain the organisms for up to 518 days (Burgdorfer 1956). The Ixodid ticks Dermacentor andersoni and Amblyomma maculatum have also been shown to transmit pomona to guinea pigs (Burgdorfer 1959).

Noguchi (1918) did not find that the larva or adults of the Culex mosquito, the larva of the house fly or bluebottle, wood ticks (Dermacentor andersoni), or leeches could play the part of an intermediate host for icterohaemorrhagiae.

Whilst no evidence of leptospirosis can be found in amphibia or fish, poultry in Italy showed evidence of infection (Babudieri 1958).

Great care must be taken when using serological investigations to study possible reservoirs among wild and domestic animals. They do not supply firm data for definitive identification of infecting serovars nor do they make allowance for non-specific low titre reactions that occur in certain species (W.H.O. 1967).

3.6.5 Transmission to the host

Indirect contact, that is, contact with an environment contaminated with infected urine is probably the most common source of infection of domestic animals (Marshall 1972). Infection may be cutaneous (mucus membranes or abraded skin), oral, by inhalation or conjunctival routes less commonly. Direct contact infection occurs by the venereal, transplacental and mammary pathways (Amatredjo and Campbell 1975).

As mentioned previously ticks may sometimes play a role in transmitting leptospirosis.

3.6.6 Prevalence patterns within Australia

Spradbrow (1964) sampled 464 cattle from the Sub-tropics of south-east Queensland and found titres of 1:100 or greater to the following serovars: icterohaemorrhagiae (4), canicola (1), zanoni (2), robinsoni (6), australis (3), esposito (8), pomona (44), tarassovi (53), celledoni (1) and the Hebdomadis serogroup (16). In north Queensland Lucas (1966) found the serovars pomona (3.37%), tarassovi (6.3%), australis (0.9%), canicola (0.38%), icterohaemorrhagiae (0.38%), zanoni (0.15%) and the Hebdomadis serogroup (1.67%). No reactions to robinsoni or grippotyphosa were found. Until the seventies the widespread nature of serovar hardjo was not recognized. Elder and Ward (1978) later found a higher prevalence for hardjo than for pomona in a Queensland-wide survey. They admitted that theirs was a biased sample and likely to overestimate prevalence but they found 41.1% of herds and 16.7% of animals positive for pomona and 62.0% of herds and 34.6% of animals positive for hardjo. In Victoria Milner, Winks and Calvert (1980) found hardjo to be the most common serovar by far.

3.7 Diagnosis

3.7.1 Diagnosis of the disease in cattle

Due to the broad diversity of clinical signs and their non-specific nature the diagnosis of leptospirosis can only be confirmed by laboratory

investigations. In cattle leptospirosis can be diagnosed by serology or the isolation of leptospires from blood, milk or urine. Histopathological diagnosis using silver impregnation, fluorescent antibody techniques or immunoperoxidase staining (Ellis, Robertson, Hustas and Kirby 1983) may be applied.

The microscopic agglutination (M.A.T.) test is the standard reference procedure for the serological diagnosis and classification of leptospires (W.H.O. 1967). The World Health Organization recommends that the test be carried out with live antigens 4-14 days old with an antigen density of approximately 100 million organisms per millilitre and to an end point of 50% agglutination.

Winks (1962) considered that a 50% reaction at 1:100 or more could be regarded as a positive result for this test and reactions at 1:30 could be classified as suspect. Ellis et al. (1978) however regarded reactions of 50% lysis at dilutions of 1:30 or more to be positive. Whilst others considered that any detectable antibody was indicative of past or present infection (Songer et al. 1983).

Whilst paired sera from individual animals showing a rising titre is required for positive diagnosis this is often impractical when doing herd tests for infertility under extensive conditions. In these circumstances a significant proportion of the group should have titres of 1:3000 or greater to vindicate a diagnosis of active leptospirosis (Sullivan 1974).

Agglutination titres rise for 1 to 3 weeks and then drop to a low level in a month in a few animals, persist for 2 years in most animals, and may last as long as 8 or 10 years in an occasional animal (Hanson 1977).

Durfee and Allen (1980) found that during the early stages of an outbreak due to serovar hardjo titres were more prevalent in younger cows than older cows. They further found a bimodal configuration of titres in the herd peaking at 6 and 33 weeks after the initial outbreak. Ellis and Michna (1977) found that nine weeks after infection with hardjo, in 8 out of 20 heifers the titre had dropped to 1:100 or less and with sejroe infection in 15 out of the 20 heifers the titre had dropped to 1:100 or less.

A major problem with the microscopic agglutination test is cross-agglutination, where agglutinins elicited by leptospires of a particular serovar often agglutinate leptospires of related serovars (W.H.O. 1967). Definitive identification of the infecting strain can only be established by recovery and specific typing of the organism (W.H.O. 1972).

The microscopic agglutination test has been carried out using monoclonal antibodies and found to be useful for the classification of leptospires as well as for antigenic analysis of the organisms (Yoshida 1983).

The complement-fixation test has been found to be sensitive enough for diagnostic purposes but is unable to differentiate between current or past-infection (Gordon 1979). Both the indirect haemagglutination and complement-fixation tests were shown to be highly cross-reaction when used to test rabbit hyperimmune serum but showed no or negligible cross-reaction when used to test buffalo calf hyperimmune serum (Palit and Sharma 1971). Ellis et al. (1982) considered that the complement-fixation and plate agglutination tests were of less value than the M.A. test.

Adler, Faine and Gordon (1981) considered that the enzyme-linked immunosorbent assay (E.L.I.S.A.) would be a useful screening test for serovar hardjo in sheep. Thiermann (1983) found that the E.L.I.S.A. test was a more sensitive test than the M.A. test for detecting pomona and hardjo antibodies in cattle. The fluorescent antibody test has been used by Hodges and Ekdahl (1973) to differentiate different leptospiral serovars from cultures and urine.

Hoare and Claxton (1972) observed spirochaetes by dark field examination of urine and isolated leptospires from that urine in liquid medium from cows showing mastitis and abortions. Whilst comparing laboratory techniques for the detection of leptospiraemia and leptospiruria, Hathaway (1981) found inoculation of hamsters (not available in Australia) to be more sensitive than culture for the detection of leptospiraemia. He also found dark field microscopy to be far less sensitive than either hamster inoculation or culture for detecting leptospiruria. Herr et al. (1982), found E.M.J.H. media with 5-fluorouracil as a selective agent to be very effective for isolation.

3.7.2 Diagnosis of environmental contamination

Baker and Baker (1970) developed a screening method for soil and water samples for the presence of pathogenic leptospires using hamsters. They relied upon the sharply circumscribed time of death from leptospirosis following inoculation.

3.8 Treatment and control

3.8.1 Treatment of infected animals

Sutherland, Simmons and Kenny (1949) reported that sulphamerazine given orally at the rate of 1g/15lb to calves appeared beneficial when given early in the course of the disease. Prescott (1967) however reported better results by using streptomycin at the rate of 0.5g twice a day for 4 days in sick calves and for treating in-contacts with 15mg/kg of oral oxytetracycline hydrochloride for 4 days.

Streptomycin is now considered the drug of choice for the treatment of leptospirosis (Sullivan 1974). A single intramuscular injection of streptomycin at 1g for calves or 4g for adult cattle is sufficient (Parkinson 1958). Given at a dose rate of 25mg/kg streptomycin will eliminate the renal carrier state of leptospirosis (Marshall 1972). The same dose rate appears useful in the treatment of hardjo mastitis (Hoare and Claxton 1972). Udder infusions are of no use in treating serovar hardjo mastitis (Corbould 1972).

3.8.2 Vaccination

Vaccination of cattle for leptospirosis usually results in immune response sufficient to prevent clinical illness, abortions and production losses for 6 months to 1 year. However the vaccine serovar must be homologous with the challenge strain to be effective (Hanson 1977). Revaccination of cattle every 12 months in closed herds with good management and every 6 months in herds where new cattle are being frequently introduced was recommended by Hanson, Tripathy and Killinger (1972).

Until recently the bacterins used in vaccines produced a low magnitude IgM response and did not greatly interfere with the microscopic agglutination test. However they do pass colostral immunity on to the calf for a few months. Thus calf vaccination should be delayed until the calf is 3 to 5 months old (Hanson 1977). Recent vaccines are however agglutinogenic.

Cargill and Davos (1981) found that in pigs vaccination did not completely prevent renal colonization by leptospires. Stalheim (1965) also reported that vaccination did not prevent the development of a carrier state for leptospirosis. However when a living attenuated vaccine was used in cattle instead of the normal killed bacterins, Stalheim (1968) found that the live vaccine did prevent renal leptospirosis. Hanson (1973) originally found killed bacterins did not prevent the urinary shedding of serovar pomona but a later study (Hanson 1976) showed that such vaccination did prevent the urinary shedding of serovar hardjo. A recently released killed vaccine also reduces the likelihood of urinary shedding of hardjo (Robertson 1983). A Victorian dairy trial (Hancock, Wilks, Kotiw and Allen 1984) demonstrated that calfhood vaccination with a commercially prepared vaccine significantly reduced the development of hardjo leptospiruria for at least 55 weeks but failed to stop urinary shedding of hardjo in already leptospiruric adults. It is therefore necessary to vaccinate dairy replacement heifers as calves to prevent urinary shedding of serovar hardjo. Vaccination with hardjo bacterins may also lower the incidence of lactation failures in beef heifers (Holroyd and Smith 1976). It has been recommended that all breeders in problem herds receive two doses of vaccine, one at mating and another 1 month before calving (Shield 1974). Holroyd and Smith (1976) however achieved a significant reduction in wastage between pregnancy diagnosis and branding using a single dose of serovar hardjo vaccine. In vaccinated animals the wastage was 11.9% whilst in unvaccinated animals it was 19.4%. Holroyd (1980) found that two doses of hardjo vaccine given midterm significantly reduced prenatal loss but not perinatal or postnatal losses. Nor was there any difference between the growth rate of progeny from vaccinated and non vaccinated dams.

Tripathy, Manson and Mansfield (1978) working in the U.S.A. demonstrated that long term annual vaccination freed a herd from active

infection from a mixed pomona, hardjo and grippotyphosa infection even though leptospires were consistently present in surrounding wildlife. During a five year vaccination trial in Scotland, Little and Hathaway (1982) found a gradual decrease in leptospiral titres which was most dramatic in the lower age ranges as vaccinated heifers entered the herd and remained relatively free of infection.

Adler and Bragger (1979) showed that cyclophosphamide treated (immunosuppressed) hamsters make a useful model for hardjo protection studies.

3.9 Zoonotic implications

Any leptospiral serovar can infect man, but the clinical signs of the disease vary considerably from sub-clinical to acute fatal infections. The disease begins suddenly and the initial stages resemble influenza with a high fever, very severe headache, perspiration, chills and pain in the muscles and joints, lasting for 5 to 7 days. Sore throat, headache, gastro-intestinal disorder, anorexia, nausea and vomiting may also be evident. Hyperaemia of the conjunctivae is characteristic. Complications such as icterus, haemoglobinuria, haemorrhages, nephritis and meningitis may be caused by some serovars e.g. icterohaemorrhagiae (Michna 1970, Robertson 1983).

One hundred and thirty (130) cases of human leptospirosis were reported in Queensland during 1982, mainly in farm and abattoir workers.

Whilst rodents are a significant source of leptospirosis in man when working in wet environments, domestic animals now constitute the major factor. The serovars most commonly involved are pomona, hardjo and to a lesser extent tarassovi when cattle are the source of infection (Sullivan 1974). White, Sulzer and Engle (1982) working in Florida cultured leptospires from 36% of normal kidneys during an abattoir survey. Serovar pomona was first isolated in Australia from a dairy farmer (Clayton et al. 1937). Hirschner (1954) also diagnosed a pomona infection in two dairy farmers in New Zealand. Both Gordon (1977) and Milner, Winks, Morgan and Rosen (1980) have reported hardjo infection in dairy workers in Australia.

Michna and Campbell (1970) found that farm workers working on farms during an active leptospiral outbreak were in danger of becoming infected and a high correlation between human and animal infection has been found on Tasmanian dairy farms by Corbould (1972) and in New Zealand by Ryan, Sceats and Penniket (1982).

Campbell and Stallman (1975) considered that as serovar australis had been cultured from a bovine kidney during an abattoir survey and the serovar had already been isolated from meatworkers the possibility of transmission from cattle to humans requires investigation. Serovars australis and celledoni were found to be actively excreted by rats adjacent to the Paluma dam system during a survey by Glazebrook et al. (1978).

Although they found a high serological and cultural prevalence of leptospirosis in domestic and feral animals on an Illinois farm Schnurrenberger et al. (1970) found no reactive sera amongst the human population. Andrew and Marrocco (1977) indicated that there was a potential for waterborne epidemics in humans when farm ponds are used for recreational purposes.

It has been suggested that herd vaccination with hardjo - pomona bacterin reduces the incidence of leptospirosis amongst dairy workers (Ryan, Sceats and Penniket 1982). Therefore it is not only the occupational hazards of the veterinary profession, nor work in sewers, abattoirs, mining, meat handling or fish trading, but also farming which must be considered as significant in the epidemiology of human leptospirosis (Michna 1970).

4 THE ENVIRONMENT AND BEEF CATTLE INDUSTRY OF THE CENTRAL HIGHLANDS OF QUEENSLAND

4.1 Geographical features

The Central Highlands area of Queensland is situated 300km west of Rockhampton and comprises the shires of Belyando, Peak Downs, Emerald and Bauhinia as well as the far western portion of Duringa Shire and the south-western portion of Broadsound Shire (see Figures 1 and 2). It has an area of 74,500 square kilometres. The main towns, Clermont, Emerald and Springsure, only have elevations of 265, 177 and 287m above sea level and therefore the term 'highlands' is misleading (O'Sullivan 1977).

4.2 Climate

The climate of the area is characterized by great variability in rainfall, temperature and evaporation with droughts, heatwaves, frosts and floods all recurrent features of the environment. Rainfall is summer dominant mainly from November to April with November and December usually being the two months of greatest evaporation. A minor peak of rainfall may occur in winter, during June and July. December and January are the months of greatest heat risk whilst July and August are the months of greatest frost (O'Sullivan 1977, D.P.I. 1979).

Table 2 shows the average monthly and total rainfall figures for the principal "Highland" towns, whilst Figure 3 shows the long term rainfall weekly totals at Emerald (D.P.I. 1979). The Central Highlands lie within the 500mm to 700mm isohyets (O'Sullivan 1977) which are the intermediate levels of rainfall for Queensland.

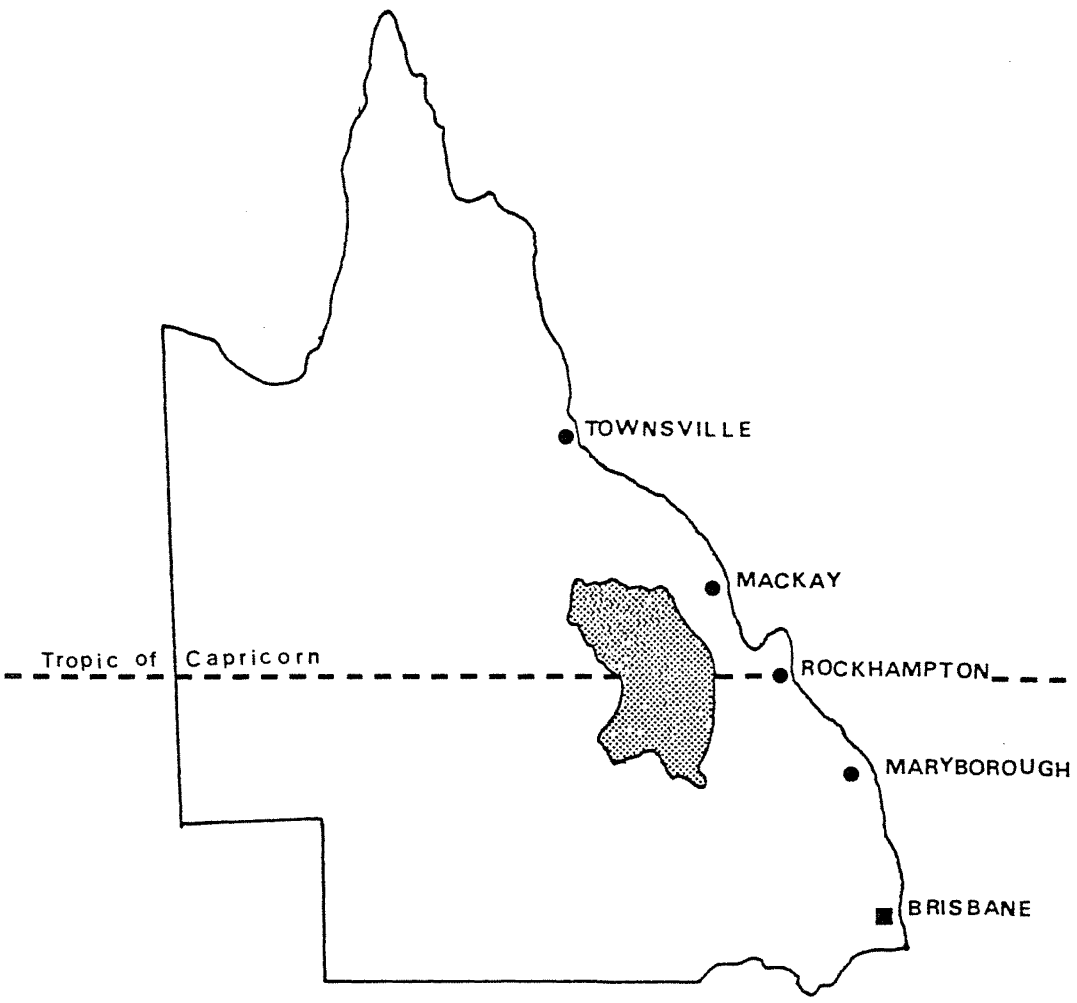
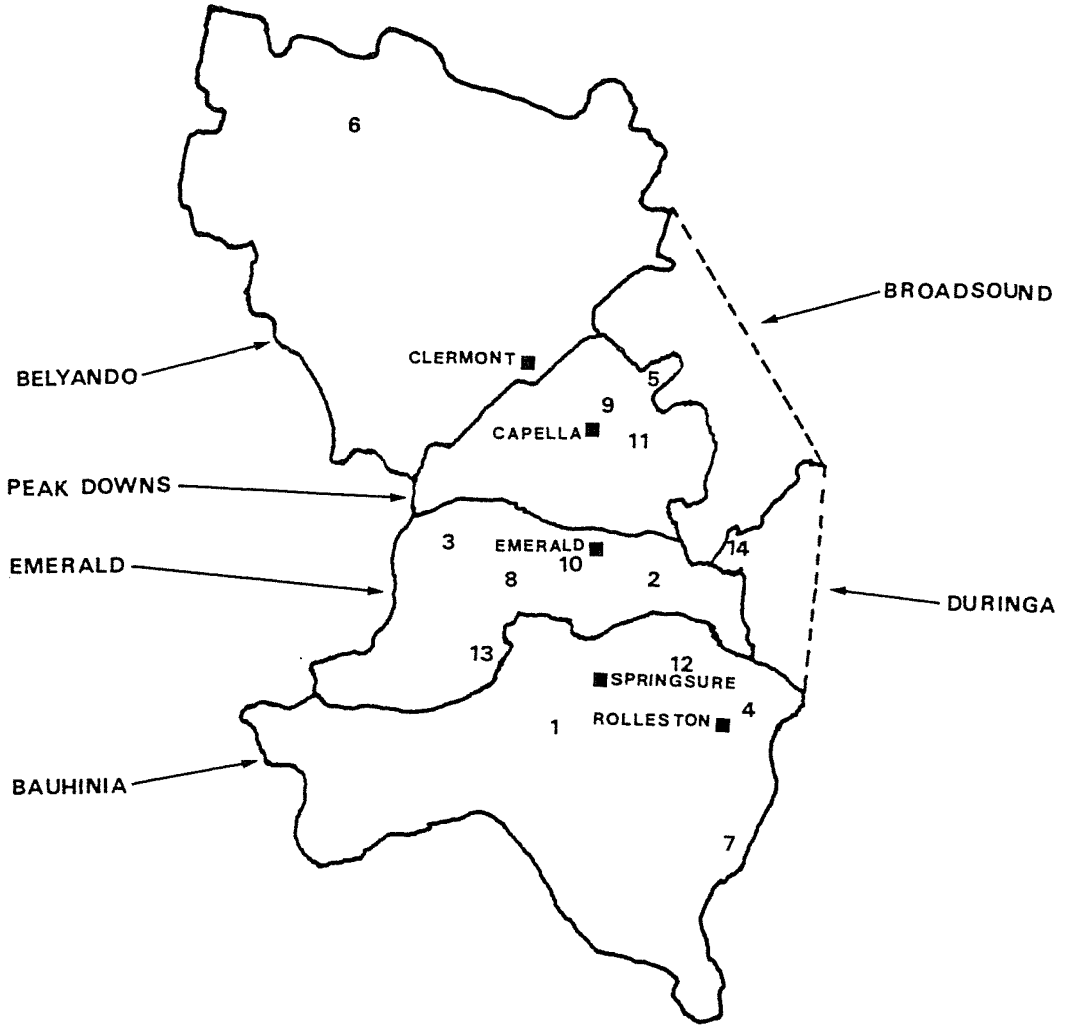


Figure1. Map of Queensland showing the Central Highlands



BAUHINIA ⟶ shire name

⟵ shire boundry

■ EMERALD town

1	Hillview	8	Seventeen Mile
2	Galgartha	9	Janibee
3	Ryhope	10	Saint Helens
4	The Lake	11	Glencoe
5	Anncourye	12	Comet Downs
6	Moray Downs	13	Jo-Jo Station
7	Vesta	14	Berrigurra

Figure 2 Map of the Central Highlands showing shire boundries, towns and survey properties

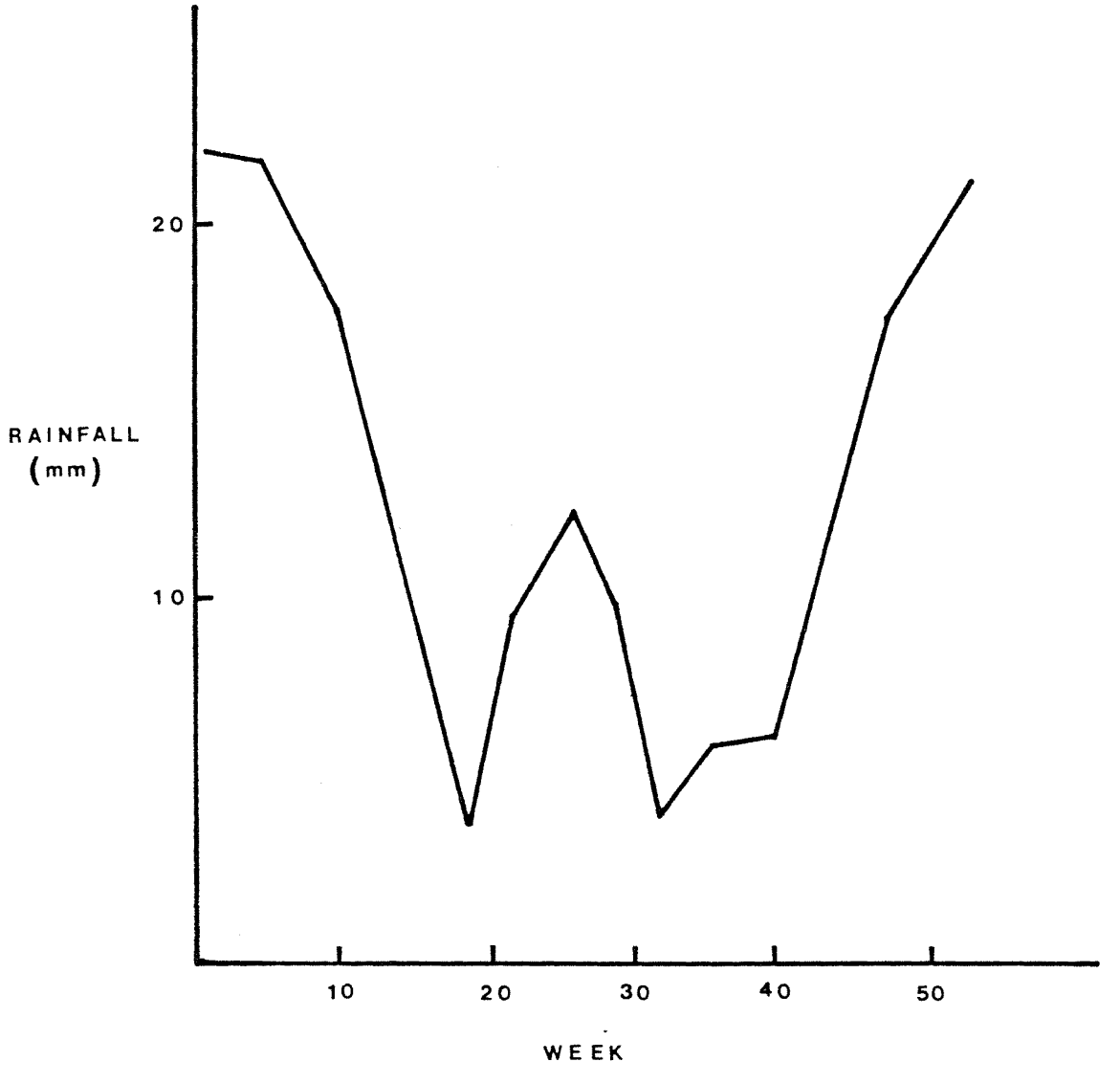


Figure 3 Mean Annual Rainfall in Central Queensland

TABLE 2

MEAN MONTHLY AND ANNUAL RAINFALL
FOR CENTRAL HIGHLANDS (mm.)
(1968-1978)

MONTH	CLERMONT	CAPELLA	EMERALD	SPRINGSURE
January	126.2	102.4	106.4	105.9
February	115.3	91.9	100.3	107.2
March	80.5	69.6	74.2	74.4
April	38.9	29.5	35.6	41.1
May	32.8	24.4	28.2	32.0
June	39.9	36.6	39.9	43.4
July	28.4	25.7	29.2	31.0
August	17.5	14.5	21.1	24.9
September	23.1	20.3	26.2	29.5
October	35.6	37.3	39.1	45.7
November	53.1	54.9	56.6	63.2
December	89.7	80.0	81.3	82.6
Annual	681.0	587.1	638.1	680.9

4.3 Soil Types

Through the centre of the Central Highlands runs a strip of dark cracking clay soil. On either side of this are zones of other soil types that include sands, sandy loams, loams and texture contract soils (O'Sullivan 1977, D.P.I. 1979). These are shown in Figure 4.

There are several ways of measuring the moisture holding capacity of a soil. Water capacity is the number of cm. of water contained in a 10cm. depth of soil expressed as a percentage after the soil is thoroughly wetted and the rate of drainage is negligible. This is also called the field capacity (Baver, Gardner and Gardner 1940, Cassidy 1975). Moisture equivalent is a laboratory approximation obtained by subjecting wet soils to a one-third atmosphere pressure (Buckman and Brady 1960).

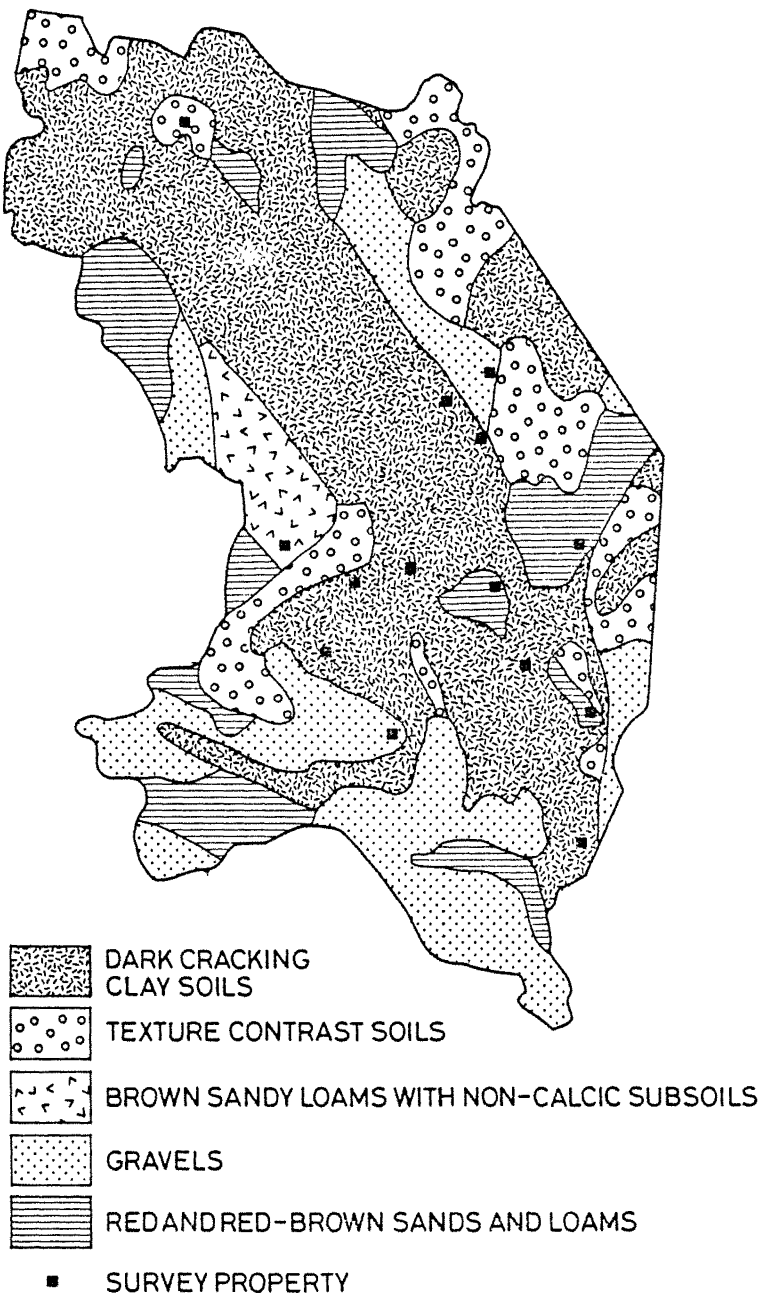


Figure 4 Soil structure in Central Queensland

Field capacity has also been defined as the limit to the amount of water which a permeable well-drained soil horizon can hold one to two days after rain, expressed as a percentage (Leeper 1957). Field capacities for various soils are shown in Table 3.

TABLE 3

FIELD CAPACITIES OF SOME WELL KNOWN SOIL TYPES (AFTER LEEPER 1957 AND CASSIDY 1975)

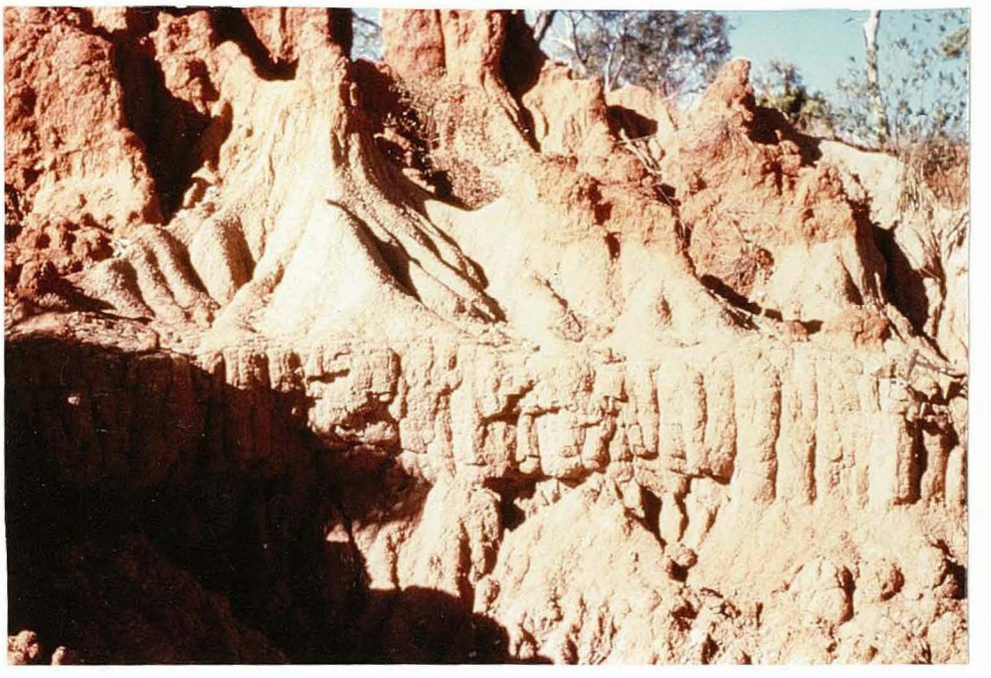
SOIL	FIELD CAPACITY %
Sand	6.2
Sandy loam	17.6
Heavy grey soils (thin)	32.4
Clay	39.4
Krasnozem	45.4
Clay loams	46.0
Heavy grey soils (thick)	46.6

Heavy soils hold more water than sands, etc. and therefore after rains the top layers of heavy soils remain very moist (Leeper 1957). The dark cracking clays of the Central Highlands are heavy grey soils (thin and thick), clays and clay loams with water holding capacities of greater than 30%, whilst the other soil types in this area have water holding capacities of less than 20%. Examples of these soil types are illustrated in Photographs 1-4.

4.4 Vegetation

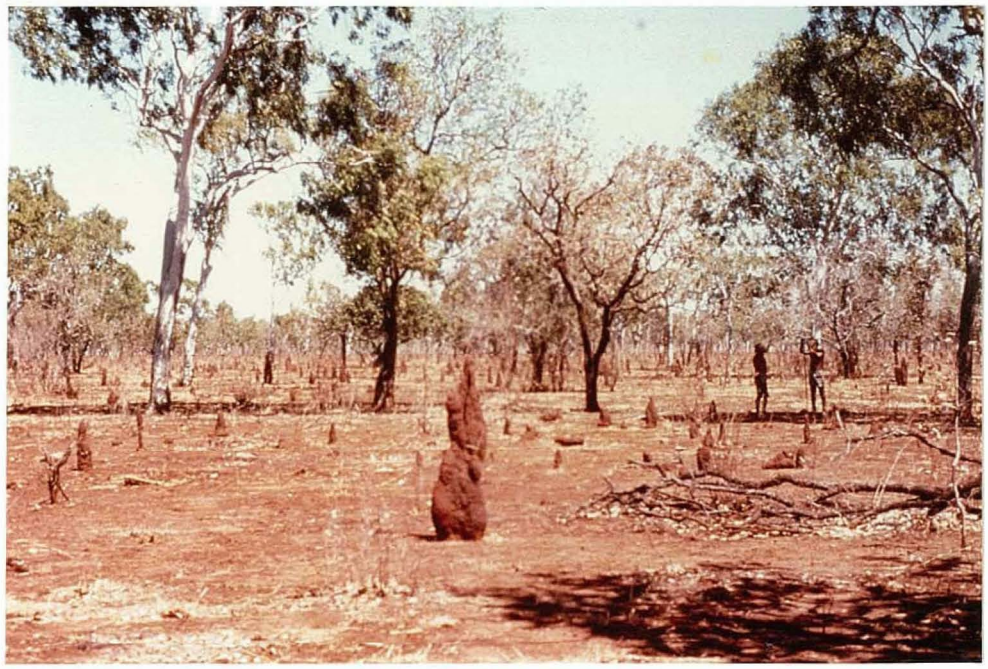
Through the centre of the Highlands on the strip of dark cracking clay soils is open downs country with Queensland blue grass (Dichanthium sericeum), black top spear grass (Heteropogon contortus) and buffel grass (Cenchrus ciliario) predominating. Much of this land is cleared brigalow country. On the poorer quality country on either side of this zone are brigalow (Acacia harpophylla) and softwood scrubs; poplar box (Eucalyptus populnea) and silver leaf ironbark (Eucalyptus melanophloia) are encountered (O'Sullivan 1977, D.P.I. 1979, Queensland Premiers Department 1980). Cleared brigalow country is shown in Photograph 5.

1. Soil profile of black cracking clay showing assistant standing on basalt bedrock

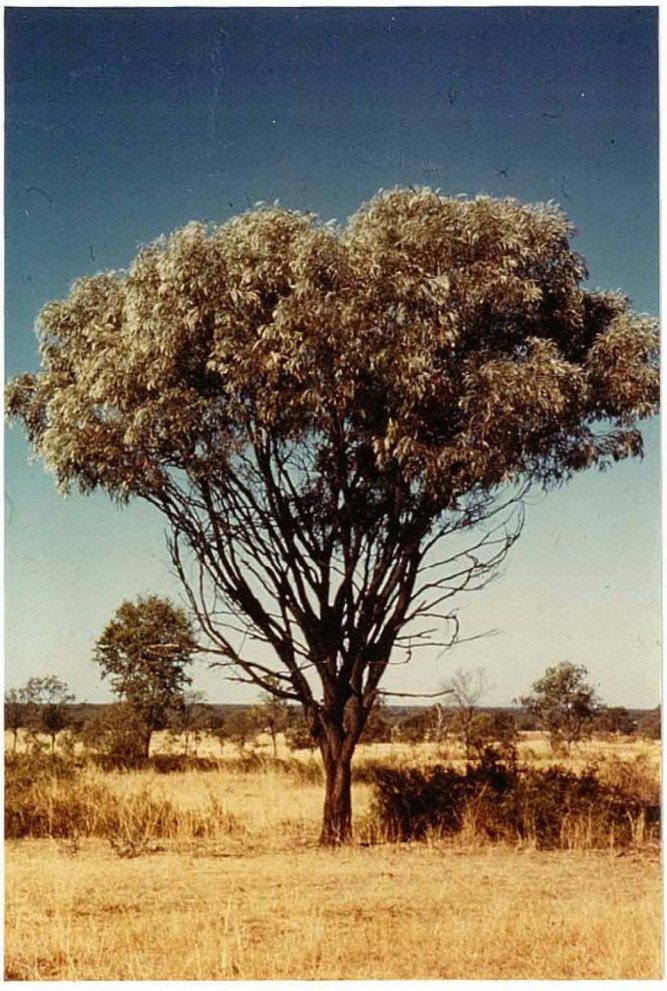


2. Soil profile of duplex soil showing clear demarcation between textures

3. Sandy soil showing erosion following over-grazing



4. Red earth showing termite mounds



5. Cleared brigalow country with a large brigalow in foreground

4.5 Cattle Production

There are approximately 800 beef cattle holdings in the Central Highlands and 800,000 cattle, 260,000 of which are breeding cows. 80% of animals in this region are Bos taurus X Bos indicus crosses (D.P.I. 1981, 1982).

Properties are predominantly breeding and fattening concerns with only a small number engaged solely in a fattening operation. The majority are owned privately or by families (D.P.I. 1982). The major disease problems so far identified are Yellowwood poisoning on the better soils; botulism, saw fly poisoning and phosphate deficiency on the poorer country; tick-fevers (Babesia bovis, Anaplasma marginale and Babesia bigemina), bovine ephemeral fever and poor reproductive performance (D.P.I. 1982). Reproductive diseases diagnosed in the Highlands include leptospirosis, vibriosis and trichomoniasis, with brucellosis all but eradicated.

Branding percentages of calves which in the Central Highlands average 66% are shown in Table 4 according to shire (Australian Bureau of Statistics 1977-78, 1978-79, 1979-80, 1980-81).

Supplementary feeding of cattle with commercially produced concentrate blocks is widely carried out in late winter (September - November) as a survival strategy, especially for breeding stock.

TABLE 4

COWS MATED AND CALVES BRANDED IN
THE CENTRAL HIGHLANDS
1977-78 to 1980-81

YEAR	BELYANDO	PEAK DOWNS	EMERALD	BAUHINIA	TOTAL
1977-78					
Cows	92,548	29,351	45,906	99,531	267,336
Calves	59,066	19,907	29,373	66,120	174,466
Percent	63.8	67.8	64.0	66.4	65.3
1978-79					
Cows	89,564	29,788	41,998	89,043	250,393
Calves	57,748	19,531	28,701	64,999	170,979
Percent	64.5	65.6	68.3	73.0	68.3
1979-80					
Cows	75,471	22,491	37,165	79,819	214,946
Calves	51,653	14,777	24,054	55,534	146,018
Percent	68.4	65.7	64.7	69.6	67.9
1980-81					
Cows	80,625	25,583	39,198	78,734	224,140
Calves	48,493	16,242	24,518	52,297	141,550
Percent	60.1	63.5	62.5	66.4	63.2
Total					
Cows	338,208	107,213	164,267	346,947	956,635
Calves	216,960	70,457	106,646	238,950	633,013
Percent	64.1	65.7	64.9	68.9	66.2

(Australian Bureau of Statistics 1977-78, 78-79, 79-80, 80-81)

5 STATISTICAL CONSIDERATIONS

5.1 Measurement of Incidence and Prevalence

5.1.1 Incidence

Incidence is the measure of the number of new cases of a disease which occur in a population during a specified period of time. It is usually expressed as a rate with the number of new cases being the numerator and the population at risk being the denominator. When using incidence to measure disease occurrence it is most important that only new cases of a disease are measured (Durfee 1978, Blackmore and Harris 1979).

5.1.2 Prevalence

Prevalence is the measure of the number of cases of a disease (both new and old) which occur in a population. There are two types of prevalence: point prevalence and period prevalence. Point prevalence measures the number of cases of a disease which are detected in a population at a designated period of time. Period prevalence measures the total number of cases of a disease which are known to have occurred during a given period. It is therefore the sum of point prevalences plus incidence during the survey period (Durfee 1978, Blackmore and Harris 1979). Serological surveys nearly always measure period prevalence as there is often no way of knowing when the serologically positive animals become infected (Durfee 1978).

5.2 Biometrical Analysis

5.2.1 General

The following biometrical analyses were performed on the data collected: t test, paired t test, simple linear correlation coefficient and chi square test for homogeneity. The t, r or chi value obtained was compared to standard tables (Fisher and Yates 1963, Balaam 1972).

5.2.2 t test

The formula used for the t test is:

$$t = \frac{\overline{x_1} - \overline{x_2}}{SE\ (\ \overline{x}_1 - \overline{x}_2\ )} \qquad \text{with Degrees of Freedom (D.F.)} = n_1 + n_2 - 2$$

$\overline{x}_1$ = mean of sample one

$\overline{x}_2$ = mean of sample two

SE = standard error calculated using the pooled variances
n_1 = number of cases in sample one
n_2 = number of cases in sample two

5.2.3 Paired t test

The formula used for the paired t test is:

$$t = \frac{\overline{d}}{SE_{\overline{d}}} \qquad \text{with D.F.} = n - 1$$

$\overline{d}$ = mean of the differences between the paired observations

$SE_{\overline{d}}$ = standard deviation of the differences between the paired observations

n = number of differences between the paired observations

5.2.4 Simple linear correlation coefficient

The formula used to calculate r (the simple linear correlation coefficient) is:

$$r = \frac{\Sigma (Y_i - \bar{y})(X_i - \bar{x})}{[\Sigma (Y_i - \bar{y})^2 \quad (X_i - \bar{x})^2]^{1/2}} \qquad \text{with D.F.} = n - 2$$

Y_i = value of case i for variable Y
X_i = value of case i for variable X
$\bar{y}$ = mean of variable Y
$\bar{x}$ = mean of variable X
n = number of cases

5.2.5 Chi square test of homogeneity

The formula used for the chi square test of homogeneity is:

$$X^2 = \Sigma [(| O - E | - 0.5)^2 / E] \qquad \text{with D.F.} = I$$

O = observed frequency
E = expected frequency
0.5 = continuity correction factor

6 THE PREVALENCE OF VIBRIOSIS AND TRICHOMONIASIS IN BEEF HERDS EXPERIENCING POOR REPRODUCTIVE PERFORMANCE IN CENTRAL QUEENSLAND

6.1 Introduction

Vibriosis and leptospirosis may lower the branding percentage in cattle by as much as 20% (Queensland Department of Primary Industries 1981). Vibriosis alone is considered to be a major problem (Seddon and Albiston 1965). Trichomoniasis does occur in Queensland but is considered by some authors to be a less important cause of infertility (Seddon and Albiston 1966) and it has been suggested that its prevalence in Australia appears to be decreasing (Hungerford 1975). However among older bulls in a northern Queensland abattoir survey Ladds et al. (1973) found a 30.2% prevalence whilst Dennett et al. (1974) detected a 4% prevalence in bulls in a herd survey in the same region. Summers et al. (1974) also found 15.8% of cows positive to the vaginal mucus agglutination test in an abattoir survey. Care must be taken to differentiate epideminological changes in beef and dairy cattle.

This investigation was designed to assess the prevalence of these two diseases in herds where the owners observed branding rates lower than expected.

6.2 Materials and Methods

During the period January 1980 to October 1984 the owners of all properties who consulted the Department of Primary Industries office at Emerald about poor reproductive performance in their herds were assisted by investigations for vibriosis. Where there was evidence of vaginal discharge the herd was sampled for trichomoniasis. Three of the 27 properties included in this analysis were part of the leptospirosis investigations reported later.

Samples for vibriosis studies were collected by inserting tampons in the vaginas of 5 to 15 cows on each property. The tampons were removed after 20 minutes and placed in sterile bottles containing 15ml of Campylobacter agglutination test diluent supplied by the Animal Research

Institute, Brisbane. Specimens were then forwarded to the Institute where they were tested using the Campylobacter vaginal mucus agglutination test (Clark, Dufty and Monsborough 1970).

Bulls aged between three and five years were tested for trichomoniasis by means of a 'Bull Testing Kit' supplied by the Animal Research Institute. Using a sterile pipette, samples of preputial discharge were obtained and mixed with sterile saline then poured into bottles containing Trichomonas fetus medium (Appendix 14.1). Samples were then forwarded to the Institute where they were microscopically inspected for the presence of the organism.

6.3 Results

Twelve properties out of twenty-seven had serological evidence of vibriosis infection and one proved positive by culture for trichomoniasis. None of the herds involved in later trials were positive for either disease. No herd was positive for both diseases (Table 5).

Table 5 RESULTS OF VIBRIOSIS AND TRICHOMONIASIS SURVEY

	ANIMALS			HERDS		
	Tested	Positive	(%)	Tested	Positive	(%)
Vibriosis (VMA)	201	58	(28.9%)	27	12	(44.4%)
Trichomoniasis (culture)	39	1	(2.6%)	7	1	(14.3%)
Trichomoniasis (clinical and culture)	-	-		27	1	(3.7%)

6.4 Discussion

The vaginal mucus agglutination test is useful only as a herd test and should not be relied on as an individual animal test (Anon. 1982). Due to the comparatively small number of animals tested (39) the prevalence

data of trichomoniasis in individual animals is less useful than the herd prevalence.

The sero-prevalence of vibriosis indicates that this disease is endemic in the area as it was diagnosed in 44.4% of the specific infertility investigations carried out by the Queensland Department of Primary Industries in the Central Highlands from January, 1980 to October 1984.

As with the work of other authors little evidence of trichomoniasis was obtained in the area even in herds which were experiencing poor reproductive performance (Seddon and Albiston 1966, Hungerford 1975). On clinical and cultural grounds only one herd of the twenty-seven investigated was diagnosed as positive (3.7%). On this property 50% of cows had a copious muco-purulent vaginal discharge.

7 STUDIES ON THE REPRODUCTIVE PERFORMANCE OF BEEF CATTLE IN CENTRAL QUEENSLAND WITH SPECIAL REFERENCE TO RAINFALL PATTERN

7.1 Introduction

It has been shown in the United States and Australia that years of high rainfall tend to be followed by higher reproductive performance in cattle (Donaldson 1962) while droughts are usually followed by lower reproductive rates (Carroll and Hoerlein 1966, Daly 1971). Droughts usually result in severe undernutrition which decreases reproductive efficiency by increasing the age of heifer puberty, prolonging post-partum anoestrus and, when particularly severe, by reducing calf birthweight and therefore survival (Lamond 1970, Entwistle 1983). Entwistle et al. (1983) also noted that decreased fertility during times of severe nutritional stress was particularly evident in heifers and lactating cows. In the Central Highlands of Queensland within the mean 500mm to 700mm isohyets (O'Sullivan 1977), annual rainfall well below 500mm or well above 700mm are considered respectively as drought conditions or above normal rainfall.

The present investigation was designed to study calf branding percentage in this area in relation to patterns of rainfall.

7.2 Materials and methods

Calf branding percentages were obtained from the 13 properties involved in the study of leptospirosis reported later in this thesis (Chapters 8 and 9). The total number of cows mated was compared with the total number of calves branded for the years 1981, 1982, 1983 and 1984. Annual rainfall for the regional towns of Emerald, Springsure, Clermont, Rolleston and Capella was obtained for the years 1980, 1981, 1982 and 1983 and from these figures the mean Highland rainfall for each year was calculated.

7.3 Results

Table 6 shows the annual rainfall for each Highland town and the mean rainfall for each year while Table 7 shows the calf branding results for each property and the mean branding percentage for each year. The mean branding percentage and the mean annual rainfall figures are shown graphically in Figure 5.

Table 6 MEAN ANNUAL RAINFALL IN THE CENTRAL HIGHLANDS OF QUEENSLAND, 1980 TO 1983 (mm)

	1980	1981	1982	1983	Mean(±SD)
Emerald	542	777	359	985	666(±273)
Springsure	540	731	316	1042	657(±308)
Clermont	497	697	273	1072	635(±339)
Rolleston	561	852	323	962	675(±289)
Capella	536	614	341	1092	646(±319)
Mean (± SD)	535(±23)	734(±89)	322(±32)	1031(±56)	656(±302)

Table 7 REPRODUCTIVE PERFORMANCE OF BEEF HERDS IN CENTRAL QUEENSLAND

CALF BRANDING RESULTS 1981 TO 1984

Property	Cohort	1981	1982	1983	1984	Mean (±SD)
Hillview	cows	350	406	256	304	329 (± 64)
	calves	254	256	128	195	208 (± 61)
	percent	73	63	50	64	63 (± 9)
Galgartha	cows	351	418	400	412	395 (± 30)
	calves	268	372	330	379	337 (± 51)
	percent	76	89	82	92	85 (± 7)
Ryhope	cows	237	254	296	271	265 (± 25)
	calves	177	185	226	196	196 (± 21)
	percent	75	72	76	72	74 (± 2)
The Lake	cows	223	242	212	200	219 (± 18)
	calves	151	211	153	148	166 (± 30)
	percent	68	87	72	74	75 (± 8)
Anncourye	cows	402	345	309	321	344 (± 41)
	calves	308	159	136	241	211 (± 79)
	percent	76	45	44	75	60 (± 18)
Moray Downs	cows	4661	4236	4088	4184	4292 (± 253)
	calves	2386	2640	2167	2301	2374 (± 199)
	percent	51	62	53	55	55 (± 5)
Vesta	cows	510	573	528	550	540 (± 27)
	calves	308	220	243	358	282 (± 63)
	percent	60	38	46	65	52 (± 12)
Seventeen Mile	cows	230	228	203	230	223 (± 13)
	calves	186	156	124	175	160 (± 27)
	percent	81	68	61	76	72 (± 9)
Janibee	cows	154	218	172	236	195 (± 38)
	calves	93	146	112	217	142 (± 55)
	percent	60	66	65	92	71 (± 14)
Saint Helens	cows	264	410	398	408	370 (± 71)
	calves	240	306	275	335	289 (± 41)
	percent	91	75	69	82	79 (± 9)
Glencoe	cows	309	321	282	310	306 (± 17)
	calves	211	202	175	208	199 (± 16)
	percent	68	63	62	67	65 (± 3)
Comet Downs	cows	751	636	640	687	679 (± 54)
	calves	572	414	448	536	493 (± 74)
	percent	76	65	70	78	72 (± 6)
Jo-Jo Station	cows	1021	1303	908	1005	1059 (± 170)
	calves	816	980	436	704	734 (± 229)
	percent	80	75	48	70	68 (± 14)
Mean percent		72 (± 11)	67 (± 14)	61 (± 12)	74 (± 11)	69 (± 6)

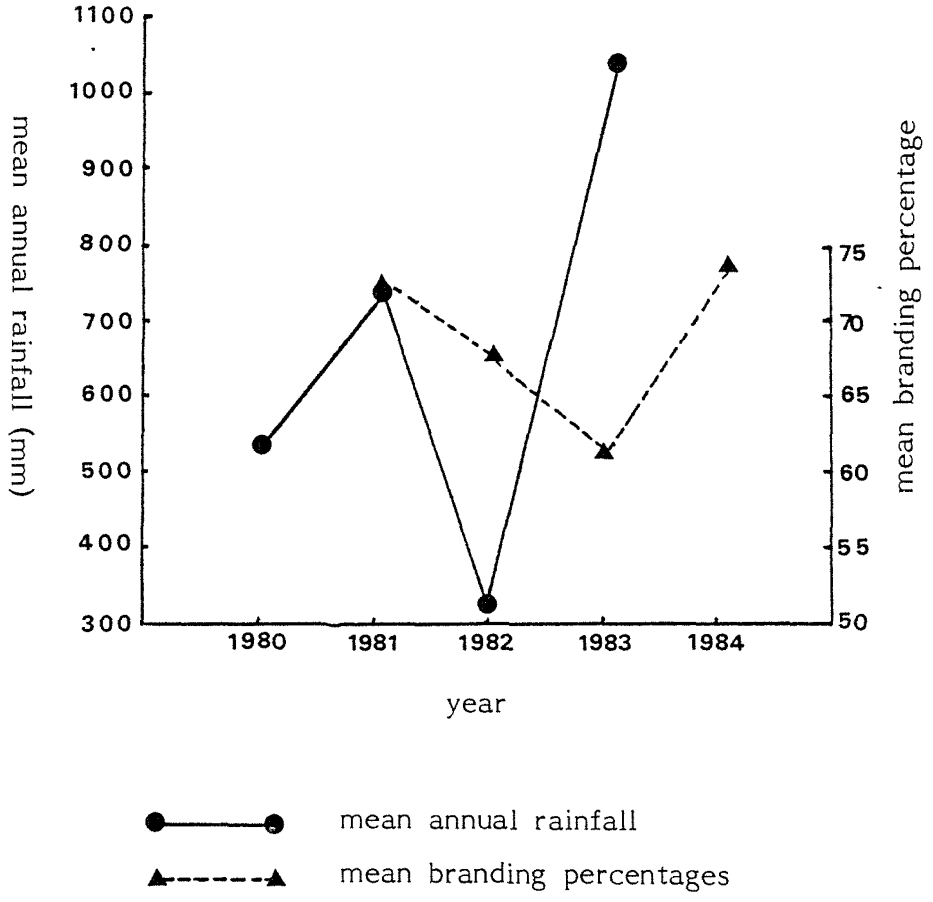


Figure 5 Mean annual rainfall and mean branding percentages in the Central Highlands 1980-1984

7.4 Discussion

The graphic representation (Figure 5) indicates the depressant effect of the 1982 drought on the 1983 branding percentage. All four shires were declared drought stricken by the Queensland Department of Primary Industries. Paired 't' tests were carried out (Appendix 14.2) showing that the branding percentages for 1983 were significantly lower than those for 1981 ($p < 0.05$) and 1984 ($p < 0.01$). The above normal but late rainfall of 1983 failed to raise branding percentages in 1984 above those of 1981 or 1982 as much of it fell after May and following the normal peak conception time. Due to the cooler weather when the bulk of the rain fell in April and May the pasture response was slower than after rainfall in summer between December and March.

This demonstrates the pronounced effect environmental factors such as rainfall and time of rainfall have on bovine reproductive performance in beef cattle herds under extensive management making between-year interpretation of infertility incidence difficult.

8 A STUDY OF SOIL TYPE OF PROPERTIES AND LEPTOSPIRAL PREVALENCE IN SELECTED HERDS

8.1 Introduction

Leptospiral survival in the external environment depends on an interaction of several factors including temperature, soil pH and moisture (Okazaki and Ringen 1957). Moisture, which appears to be one of the most crucial factors, is dependent on both rainfall and the water-holding capacity of the soil. Annual rainfall is fairly constant over the Highlands but there is a wide variation in soil types which results in significant differences in water-holding capacities (Table 3). In Queensland as elsewhere leptospirosis seems to be more easily transmitted within a herd when environmental moisture is high (Doherty 1967a). Twigg et al. (1968, 1969) in England found that the survival time of leptospires in soil increased as soil moisture increased.

This investigation was designed to compare the soil type which is predominant on a property and the leptospirosis prevalence in the herds. The soils of the Highlands can be divided broadly into the black earths with high water-holding capacities and other soils of low water-holding capacities.

8.2 Materials and methods

8.2.1 Properties

Thirteen properties were selected for the study on leptospirosis. As about 55% of properties in the Highlands contain predominantly black earths, a stratified sample of 7 black earth (high water-holding capacity) properties and 6 properties with soils of low water-holding capacity were chosen. Their locations are shown in Figures 2 and 4.

Detailed property descriptions were then constructed using data from the Australian National Animal Disease Information System, the Queensland Valuer General's Property Data Listings and the CSIRO Land Research Series 18 and 19 on Lands of the Nogoa-Belyando Area, Queensland (Gunn,

Galloway, Pedley and Fitzpatrick 1967) and Lands of the Isaac-Comet Area, Queensland (Storey, Galloway, Gunn and Fitzpatrick 1967) respectively.

Information obtained for each property included name, area, normal number of breeding cattle, stocking rate (number of cattle/ha), soil description and soil water-holding capacity classification (see Table 8).

8.2.2 Serology

Cattle on each property were sampled between 4 to 6 times over a two-year period from October, 1980 to October 1982. With the exception of the first sample blood was collected after the wet summer (December-March) and dry winter (May-August) seasons. Each sampling consisted of 40 sera collected at random from the herd. A total of 2,376 samples were obtained from the 10,300 breeding cows on the 13 properties.

Samples were collected by transecting the coccygeal artery and vein using a scalpel blade and collecting the blood in sterile 25ml plastic vials. The blood was allowed to clot and the serum poured off and frozen at -8°C before testing.

All leptospiral serology was carried out using the microscopic agglutination test (WHO 1967). Sera were tested at the School of Tropical Veterinary Science, James Cook University or at the Oonoonba Animal Health Laboratory or the Animal Research Institute of the Queensland Department of Primary Industries. All reactions of 1:30 or greater were regarded as positive (Ellis *et al.* 1976).

Each serum sample was tested for serovars *hardjo* and *pomona*. A random sample of 530 sera representing all 13 herds was also tested for the Hebdomadis serogroup and *australis* serovar whilst another 410 sera from the group of 530 sera were tested for serovar *celledoni*, which is known to occur in north Queensland.

The total number of positive reactions divided by the total number of cows tested for each property over the two years expressed as a percentage was used to represent the period prevalence of leptospirosis (Durfee 1978).

Table 8 PROPERTY INFORMATION

PROPERTY	AREA(Ha)	BREEDERS/ BREED	STOCKING RATE (Cattle/Ha)	SOIL DESCRIPTION	WATER-HOLDING CAPACITY*
Group 1					
Hillview	17,400	400 Braford =	0.03	Sands to sandy loams	++
Galgartha	17,200	420 Braford =	0.03	Very shallow sandy soils	+
Ryhope	6,300	250 Brahman	0.04	Sandy to sandy loam soils	++
The Lake	4,450	240 Santa Gertrudis	0.05	Texture contrast soils with thick sandy surface	++
Anncourye	18,000	590 Brahman-Shorthorn cross	0.03	A mixture of sandy to sandy loam soils and very shallow sands	+
Moray Downs	117,200	4240 Brahman	0.04	Yellow or grey texture contrast soils	++
Group 2					
Vesta	8,500	570 Hereford	0.06	Deep; dark grey to brown cracking clay soils with gilgais	++++
Seventeen Mile	4,520	220 Brahman	0.05	Dark brown to dark grey; deep cracking clay soils	++++
Janibee	2,120	200 Brahman	0.10	Moderately shallow dark cracking clay soils	++++
Saint Helens	10,300	410 Brahman	0.04	Moderately shallow to deep; dark to brown cracking clay soils	++++
Glencoe	4,470	310 Brahman	0.07	Moderately shallow to deep; dark cracking clay soils	++++
Comet Downs	26,300	960 Santa Gertrudis	0.04	Dark grey and brown cracking clay soils	++++
Jo-Jo Station	37,750	1500 Shorthorn-Brahman cross	0.04	Dark medium to shallow cracking clay soils	++++

*Soil Water-Holding Capacity
+ = less than 10% (very low WHC)
++ = 10% to 20% (low WHC)
+++ = 20% to 30% (medium WHC)
++++ = greater than 30% (high WHC)

= Brahman-Hereford cross

8.3 Results

The results of individual property tests for serovars hardjo and pomona are shown in Table 9.

Table 9 LEPTOSPIRAL SEROLOGY OF PROPERTIES IN CENTRAL QUEENSLAND

PROPERTY	SAMPLING DATES	TOTAL ANIMALS	POSITIVE HARDJO (%)	POSITIVE POMONA (%)
Group 1				
Hillview	80.11	60	0 (0)	0 (0)
	81.8	16	0 (0)	0 (0)
	82.2	20	3 (15)	0 (0)
	82.9&10	30	4 (13)	1 (3)
Galgartha	80.11	40	0 (0)	0 (0)
	81.6	19	4 (21)	1 (5)
	82.1	20	2 (10)	4 (20)
	82.7	30	7 (23)	0 (0)
Ryhope	80.12	60	0 (0)	0 (0)
	81.7	14	2 (14)	0 (0)
	81.9	49	9 (18)	1 (2)
	82.2	80	5 (6)	0 (0)
	82.4&5	56	16 (29)	2 (4)
The Lake	81.1	40	0 (0)	0 (0)
	81.5	40	0 (0)	0 (0)
	82.4	40	3 (8)	5 (13)
	82.9	37	0 (0)	10 (27)
Anncourye	80.10	60	0 (0)	0 (0)
	81.8&9	49	5 (10)	4 (8)
	82.3	25	6 (24)	2 (8)
	82.9	40	3 (8)	0 (0)
Moray Downs	81.1	40	0 (0)	0 (0)
	81.5	40	0 (0)	0 (0)
	81.9	60	0 (0)	1 (2)
	82.4	40	5 (13)	4 (10)
	82.5	40	5 (13)	2 (5)
	82.7	40	2 (5)	0 (0)
Group 2				
Vesta	80.10	60	0 (0)	0 (0)
	81.9	80	26 (33)	0 (0)
	82.1	80	4 (5)	9 (11)
	82.2	40	39 (98)	0 (0)
	82.8	40	16 (40)	2 (5)
	82.9	40	28 (70)	0 (0)
Seventeen Mile	81.1	40	1 (3)	0 (0)
	81.8	25	3 (12)	0 (0)
	82.1	27	0 (0)	0 (0)
	82.5	25	7 (28)	2 (8)
Janibee	80.12	60	11 (18)	4 (7)
	81.8&10	27	3 (11)	0 (0)
	82.4	16	8 (50)	1 (6)
	82.9	20	10 (50)	0 (0)
Saint Helens	80.11	68	0 (0)	0 (0)
	81.9	20	0 (0)	0 (0)
	82.2	16	0 (0)	1 (0)
	82.5&6	49	17 (35)	17 (14)
Glencoe	80.10	60	0 (0)	0 (0)
	81.8	20	3 (15)	1 (5)
	82.3	35	9 (26)	1 (3)
	82.9	21	4 (19)	2 (10)
Comet Downs	80.11	60	0 (0)	0 (0)
	81.7	40	0 (0)	0 (0)
	81.9	32	4 (13)	9 (28)
	82.3	13	5 (38)	3 (23)
	82.8	40	6 (15)	5 (13)
	82.9	40	9 (23)	2 (5)
Jo-Jo Station	81.1	40	0 (0)	0 (0)
	81.5	40	0 (0)	0 (0)
	81.9	60	0 (0)	1 (2)
	82.4	40	5 (13)	4 (10)
	82.5	40	5 (13)	2 (5)
	82.7	40	2 (5)	0 (0)

Table 10 summarizes the individual period prevalence of leptospirosis by property from the serological test. Analysis of these figures (Appendix 14.3) reveals that serovar hardjo is more prevalent on properties with high water-holding capacity soils (18%) than on properties with low and very low water-holding capacity soils (7%). There are no significant differences between the two property groups for serovar pomona. Table 11 summarizes these findings.

Table 10 PERIOD PREVALENCE OF LEPTOSPIROSIS PER PROPERTY

PROPERTY	ANIMALS SAMPLED	POSITIVE SEROVAR HARDJO		POSITIVE SEROVAR POMONA	
		NUMBER	%	NUMBER	%
Group 1					
Hillview	126	7	4.8	1	0.8
Galgartha	109	13	11.9	5	4.6
Ryhope	259	32	12.4	3	1.2
The Lake	157	3	1.9	15	9.6
Anncourye	174	14	8.0	6	3.4
Moray Downs	260	12	4.6	6	2.3
Group 2					
Vesta	340	113	33.2	11	3.2
Seventeen Mile	117	11	9.4	2	1.7
Janibee	123	32	26.0	5	4.1
Saint Helens	153	17	11.1	8	5.2
Glencoe	136	16	11.8	4	2.9
Comet Downs	235	24	10.2	19	8.1
Jo-Jo Station	187	45	24.1	12	6.4
Total	2376	339	14.3	97	4.1
High WHC Soils	1291	258	18.0	61	4.5
Low WHC Soils	1085	81	7.3	36	3.7

Table 11 SUMMARY OF ANALYSIS: SOIL TYPE AND SEROVAR

SOIL TYPE	SEROVAR	PREVALENCE	DIFFERENCE	SIGNIFICANCE LEVEL
High WHC	hardjo	18.0	13.5	$p < 0.01$
	pomona	4.5		
Low WHC	hardjo	7.3	3.6	N.S.
	pomona	3.7		
High WHC	hardjo	18.0	10.7	$p < 0.05$
Low WHC		7.3		
High WHC	pomona	4.5	0.8	N.S.
Low WHC		3.7		

N.S. = not significant

There was no significant correlation between hardjo and pomona period prevalence or hardjo/pomona period prevalence and stocking rate (Appendix 14.4).

Only one serum was positive for serovar australis (1:100) which gave a period prevalence of 0.2%. No cows tested were positive for serovar celledoni.

Forty-six cows reacted when tested for the Hebdomadis serogroup of which hardjo is a member. There was a significant correlation ($p < 0.01$) between the seroprevalence to the Hebdomadis group and the serovar hardjo on individual properties ($r = 0.79$) (Appendix 14.4) as shown in Table 12, indicating that hardjo was the member of the Hebdomadis group responsible for the reactions.

Table 12 SERO-PREVALENCE TO HEBDOMADIS SEROGROUP AND SEROVAR *HARDJO*

PROPERTY	HEBDOMADIS GROUP PREVALENCE	*HARDJO* SEROVAR PREVALENCE
Group 1		
Hillview	0.0	4.8
Galgartha	5.0	11.9
Ryhope	10.0	12.4
The Lake	0.0	1.9
Anncourye	5.0	8.0
Moray Downs	3.3	4.6
Group 2		
Vesta	38.0	33.2
Seventeen Mile	10.0	9.4
Janibee	50.0	26.0
Saint Helens	0.0	11.1
Glencoe	6.7	11.8
Comet Downs	6.7	10.2
Jo-Jo Station	5.0	24.1

8.4 Discussion

As in other surveys in central and north Queensland (Elder and Ward 1978, Winks and Calvert 1980) serological reactions for serovar *hardjo* were more prevalent than those to *pomona*. This contrasts with some earlier surveys (Spradbrow 1964, Lucas 1966) which were carried out either in coastal areas or South-east Queensland where rainfall and stocking rates are higher and temperatures are lower than in the Central Highlands. The earlier work was also carried out before serovar *hardjo* was identified as a specific infection.

Serovar *hardjo* was significantly more prevalent on properties where the soils had a high water holding capacity. Once wet by rain, etc., these soils remain moist for a considerable time and the pH of the black earths tends towards mild alkalinity which may further favour leptospiral survival (Chang *et al.* 1948, Okazaki and Ringen 1957, Smith and Turner

1961, Twigg et al. 1969). Sands and Sandy loams tend towards mild acidity and dry rapidly so would not favour leptospiral survival.

Serovar pomona was not however more prevalent on the properties which had predominantly black earths, being uncommon (4.5 and 3.7%) on both soil types. From the lack of correlation (r = 0.043) it seems possible that factors which favour hardjo survival and spread do not favour pomona in a similar way. However the general prevalence of pomona in this area may have been too low to allow other factors to influence it significantly.

It was found that both serovars australis and celledoni are rare in this area, a finding which confirms other Queensland surveys. Most of the *Hebdomadis serogroup reactions appear to have been due to serovar hardjo.

Correlation of leprospiral prevalence, particularly L. hardjo, with soil type in Central Queensland indicates that when environmental and climatic conditions are favourable to leptospiral survival the prevalence of infection will be enhanced on properties with black earths.

*Now called serogroup Sejroe (Ellis et al. 1984).

9 BIOMETEOROLOGICAL STUDIES OF LEPTOSPIRAL INFECTIONS IN BEEF CATTLE IN CENTRAL QUEENSLAND

9.1 Introduction

Clinical outbreaks of leptospirosis have been reported as following higher than normal rainfall (Knott and Dodswell 1970) and Amatredjo et al. (1976) found the incidence of subclinical leptospiral nephritis to increase after the summer wet season in north Queensland. It is probable that net environmental water level and not net rainfall is important in the survival and spread of leptospires (Hellstrom and Blackmore 1979); the disease is therefore more easily spread during and after the wet periods than in the dry season.

The following investigation was designed to examine leptospiral activity, as measured by the prevalence of serologically positive animals at different periods of the year with special reference to dry season and post-wet season conditions.

9.2 Materials and methods

9.2.1 Serum samples

The sera collected for the previous study were used. From the total number of samples collected for each month and the numbers positive for each serovar the percentage prevalence for each month was calculated.

9.2.2 Rainfall data

Bureau of Meteorology rainfall data was obtained from the principal towns in the survey area, namely Emerald, Springsure, Clermont, Rolleston and Capella. The mean monthly rainfall figures (in millimetres) were obtained by adding monthly rainfall of each town and dividing by the number of towns.

9.3 Results

Monthly rainfall figures for each of the Bureau of Meteorology centres for the Central Highlands are shown in Table 13 and Figure 6 whilst Table 14 and Figure 7 show the mean monthly rainfall and monthly leptospirosis sero-prevalence.

Table 13 RAINFALL DATA FOR THE CENTRAL HIGHLANDS 1980-1982 (mm)

YEAR	MONTH	EMERALD	SPRINGSURE	CLERMONT	ROLLESTON	CAPELLA	MEAN
1980	June	0.4	0.6	4.2	0.0	0.0	1.0
	July	58.0	58.8	20.4	38.0	29.9	41.0
	August	6.3	8.0	11.8	7.0	9.2	9.0
	September	0.0	0.0	0.0	0.0	0.0	0.0
	October	6.8	40.2	17.2	29.0	37.4	26.0
	November	29.6	28.6	7.0	16.8	31.0	23.0
	December	73.9	77.4	80.8	138.8	89.0	92.0
1981	January	148.4	81.8	158.2	86.6	85.6	112.0
	February	96.8	129.4	148.2	161.8	132.8	134.0
	March	47.1	18.0	9.8	40.2	74.2	39.0
	April	55.3	32.6	36.6	43.6	17.8	37.0
	May	93.7	106.6	99.4	109.3	103.2	102.0
	June	37.0	68.6	22.8	52.6	24.4	41.0
	July	39.0	37.6	36.8	36.6	46.6	39.0
	August	9.0	12.8	20.6	13.4	8.4	13.0
	September	0.0	0.0	0.0	0.0	0.0	0.0
	October	31.4	60.2	9.0	65.8	11.0	35.0
	November	82.8	91.0	105.4	129.2	59.8	94.0
	December	136.8	92.6	50.6	112.8	50.2	89.0
1982	January	86.2	52.0	81.7	72.8	130.2	85.0
	February	40.6	33.4	24.2	45.4	43.2	37.0
	March	57.2	101.6	98.4	111.0	83.8	96.0
	April	39.7	1.0	4.2	5.2	7.8	12.0
	May	46.4	26.0	3.2	16.0	1.2	19.0
	June	0.0	0.0	0.0	0.0	0.0	0.0
	July	10.2	6.2	8.2	8.8	6.8	8.0
	August	0.6	5.5	2.4	6.2	0.0	3.0
	September	2.8	4.4	3.0	2.8	7.2	4.0
	October	1.4	7.2	8.2	17.4	4.0	8.0

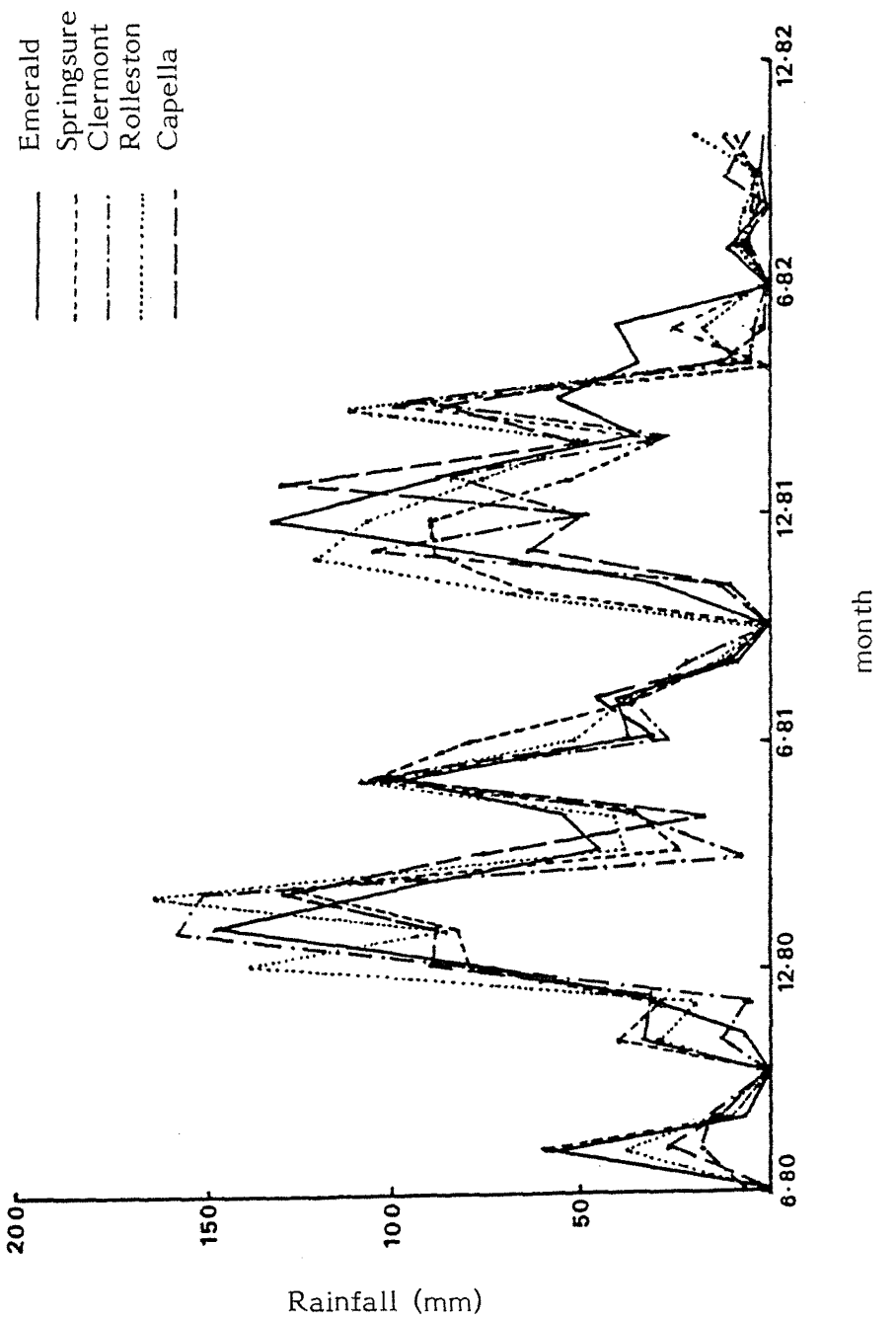


Figure 6 Monthly rainfall in millimetres for each highlands town during the trial period

Table 14 MONTHLY RAINFALL FOR CENTRAL QUEENSLAND AND LEPTOSPIROSIS PREVALENCE IN SURVEY HERDS

YEAR	MONTH	MEAN RAINFALL (mm)	TOTAL SERA	HARDJO NUMBER	HARDJO POSITIVE PERCENT	POMONA NUMBER	POMONA POSITIVE PERCENT
1980	June	1	-	-	-	-	-
	July	41	-	-	-	-	-
	August	9	-	-	-	-	-
	September	0	-	-	-	-	-
	October	26	180	0	0	0	0
	November	23	228	0	0	0	0
	December	92	120	11	9	4	3
1981	January	112	160	1	1	0	0
	February	134	-	-	-	-	-
	March	39	-	-	-	-	-
	April	37	-	-	-	-	-
	May	102	80	0	0	0	0
	June	41	19	4	21	1	5
	July	39	63	7	11	4	6
	August	13	81	6	7	2	2
	September	0	281	39	14	11	4
	October	35	47	3	6	1	2
	November	94	-	-	-	-	-
	December	89	-	-	-	-	-
1982	January	85	127	6	5	13	10
	February	37	103	56	54	9	5
	March	96	73	20	27	6	8
	April	12	136	30	23	10	7
	May	19	104	25	24	12	11
	June	0	26	6	23	1	4
	July	8	120	32	27	3	2
	August	3	117	22	19	7	9
	September	4	151	60	24	6	7
	October	8	20	3	15	0	0
	Mean	41 (± 40)	112 (± 67)	17 (± 19)	16 (± 13)	5 (± 4)	4 (± 4)

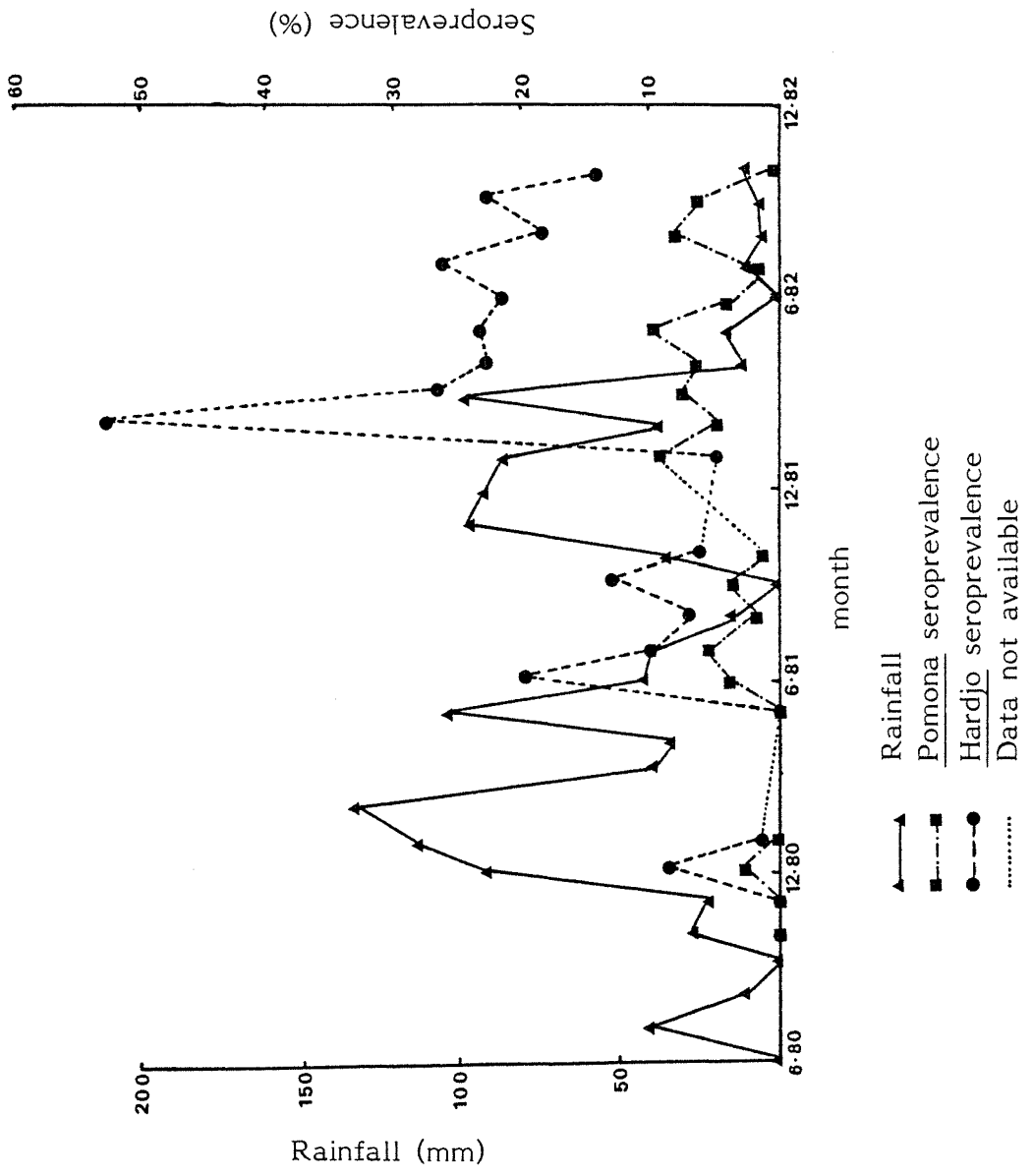


Figure 7 Monthly rainfall, pomona prevalence and hardjo prevalence in the Central Highlands

9.4 Discussion

It can be seen from Figure 6 that during the period June 1980 to October 1982 there were two distinct wet seasons - December 1980 to May 1981 and November 1981 to March 1982 with smaller rainfall peaks around July. This is typical for the area except that the 1980/81 wet season extended further into winter than normal. The winter rainfall pattern is less marked in north Queensland.

Two distinct peaks of hardjo activity occurred, one in June 1981 and the other stretching from February 1982 to September 1982. Unfortunately no hardjo prevalence data is available for early 1981. However these results demonstrate cyclic fluctuations in both hardjo activity and rainfall and that peaks of hardjo activity follow the periods of high rainfall. During 1981 there was a midyear rainfall peak which was followed by a hardjo prevalence peak later that year. This is important when considering the epidemiology of bovine leptospirosis in central Queensland as most breeders are in the last half of pregnancy during the latter stages of the year. Figure 3 shows the long term rainfall figures which demonstrates that this midyear peak is a feature of the rainfall pattern in this area. In this respect it differs from much of tropical north Queensland.

Serovar pomona activity does not clearly show the same seasonal fluctuations. The low mean prevalence of 4% has a random scatter of prevalence both above and below the mean and there is no discernible pattern. For any single month the highest pomona prevalence was only 11% compared to 54% for hardjo so that environmental conditions may not favour pomona spread or survival to such an extent that clear seasonal fluctuations were visible. Alternatively there may simply be a low prevalence of pomona in this area which does not respond significantly to periods of rainfall.

10 SEROLOGICAL STUDIES OF THE LEPTOSPIRAL STATUS OF OTHER DOMESTIC ANIMALS IN CENTRAL QUEENSLAND

10.1 Introduction

Although some authors (Twigg et al. 1968) in Britain considered that wildlife may constitute a leptospiral reservoir of considerable importance, cattle probably act as the major reservoir host of infection of certain serovars. However during epizootics other animals living in the area may be involved in the circulation of leptospires in the focus (WHO 1967). Serovar pomona has been detected in both pigs (Spradbrow 1964, Elder and Ward 1978) and horses (Hogg 1974); hardjo has not been reported in pigs (Corbould 1971) but there is evidence that both horses and sheep may be susceptible.

As both feral pigs and stock horses commonly mix with cattle in central Queensland a serological survey was carried out to determine the levels of infection in the two species.

10.2 Materials and methods

10.2.1 Porcine sera

Feral pigs captured by local graziers and farmers were bled soon after capture. Forty-two serum samples were obtained by transecting one of the ear veins and collecting the blood in 25ml sterile plastic vials. After the samples had clotted the serum was poured off and frozen at -8°C before testing.

10.2.2 Equine sera

Twenty-four stock horses chosen at random were bled via the jugular vein into sterile bottles. The sera was poured off and frozen while awaiting test.

10.2.3 Serology

Serology was carried out as for the previous experiments using the microscopic agglutination test (MAT).

10.3 Results

Only one pig gave a low positive reaction to serovar pomona (1:50) while no reactions were found to serovar hardjo.

Serological responses in the horse are presented in Table 15. One animal from property 10 showed anorexia, lethargy, lameness in the hind-legs, enlarged lymph nodes and a temperature of 38.5°C. It also displayed a mild neutrophilia. After treatment with streptomycin at a dose-rate of 25mg/kg it improved and made a complete clinical recovery after 5 days.

10.4 Discussion

The low leptospiral activity in feral porcine sera is interesting. In Queensland, commercial pigs appear to be commonly susceptible to serovar pomona. The low prevalence of pomona in cattle is reflected in the feral pig population. The equine results are most interesting in that 30% of the horses were positive for hardjo and 40% for pomona. This contrasts with a survey in north Queensland (Kirkman et al. 1982) where serological prevalence was much lower (8.2% for hardjo and 1.4% for pomona). The level of the titres in north Queensland horses were also lower (mainly in the 30 to 100 range) compared with some of those in the Highlands where, in one clinical case, titres greater than 30,000 were found. Horses in this area tend to be confined to small paddocks often along with the house milking cows and spend a considerable time in the stock yards. They are also often in close association with calves (at branding in yards) and weaners (at weaning time). These circumstances may expose stock horses to higher levels of leptospiral contamination of the environment.

Table 15 LEPTOSPIRAL SEROLOGY OF EQUINE SERA

PROPERTY	DATE	SHIRE	NUMBER TESTED	POSITIVE HARDJO (TITRE)	POSITIVE POMONA (TITRE)
1	81.9	Bauhinia	1	0	0
2	81.9	Bauhinia	2	0	0
3	81.9	Emerald	1	0	0
4	81.9	Bauhinia	1	0	1 (100)
5	81.9	Bauhinia	2	0	1 (30)
6	81.9	Emerald	1	0	0
7	81.3	Emerald	1	1 (100)	1 (1,000)
8	81.10 82.2	Emerald Emerald	2 5	0 1 (30)	0 3 (30) (100) (300)
9	81.11	Emerald	1	0	0
10	81.5 81.7 81.9	Emerald	1	1 (1,000,000) 1 (30,000) 1 (300)	1 (30,000) 1 (30,000) 1 (3,000)
11	82.2	Belyando	2	2 (30) (1,000)	2 (300) (3,000)
12	82.7	Emerald	1	0	1 (400)
13	83.10	Bauhinia	3	2 (100) (200	0
Total			24	7	10

The comparatively high prevalence among horses suggests that they could play a part in disseminating infection in the area.

One horse with a very high titre to L. hardjo (1:1,000,000) had spent a considerable time with a group of young weaners in a small cultivation paddock. The high titre and rapid recovery after streptomycin injection indicates that clinical signs were due to active leptospirosis.

11 A FIVE YEAR HERD STUDY OF REPRODUCTIVE PERFORMANCE IN THE CENTRAL HIGHLANDS OF QUEENSLAND

11.1 Introduction

In his review Entwistle (1983) reported conception rates in Queensland ranging from 34 to 91% but did not include performance in cattle in subcoastal central Queensland. Holroyd et al. (1979) found a mean annual conception rate of 83.1% in north Queensland. Conception rates of 90% and branding rates of 80 and 70% were considered normal in subtropical and subcoastal Queensland by Osborne (1960) and Alexander (1962).

The aim of this study was to determine the conception and branding rates obtainable on a semi-intensive beef cattle property in central Queensland under good management.

11.2 Materials and methods

'Berrigurra' is a property sixty-five kilometres east of Emerald administered by the Emerald Pastoral and Agricultural College. It runs 700 breeders on 7,100 hectares with 2.5 to 3% herd bulls. Controlled mating is carried out during November, December and January and the cows are pregnancy tested during May and June. Branding is carried out in February. The property was tested and found to be uninfected by vibriosis and trichomoniasis as part of the study described in Chapter 6. The predominant breed is Belmont Red (50% Africander, 25% Hereford and 25% Shorthorn).

Information obtained from records kept by the property manager included: number of cows mated, number of cows pregnant and number of calves branded. From this data the conception rate (cows pregnant/cows mated X 100) and branding rate (calves/cows mated X 100) were calculated.

11.3 Results

Results of an analysis of reproductive performance in the herd are shown in Table 16.

The mean conception rate for the five years from 1979 to 1983 was 82.6% while the mean branding rate was 76.6%.

Table 16 CONCEPTION AND BRANDING RATES AT 'BERRIGURRA' FOR COWS MATED DURING THE YEARS 1979 TO 1983

YEAR MATED	1979	1980	1981	1982	1983	MEAN (± SD)
Number of cows	638	664	668	745	696	682 (± 40.7)
Number of cows pregnant	501	581	593	661	482	563 (± 72.9)
Conception rate (%)	78.5	87.5	88.8	88.7	69.3	82.6 (± 8.6)
Number of calves branded	450	534	562	611	458	523 (± 68.8)
Branding rate (%)	70.5	80.4	84.1	82.0	65.8	76.6 (± 8.0)
Calf loss conception to branding (%)	10.2	8.1	5.2	7.6	5.0	7.2 (± 2.2)

11.4 Discussion

The mean conception and branding rates obtained in this herd over the five years investigated compare favourably with the data quoted by Entwistle (1983). It should be noted that the branding percentage in this herd, free from vibriosis and trichomoniasis, is significantly higher than the average of 66.2% quoted earlier from the Australian Bureau of Statistics (1977-78, 78-79, 79-80, 80-81) for the Central Highlands. The possible effect of leptospirosis on this herd will be examined later (Chapter 12).

12 A HERD STUDY OF LEPTOSPIRAL INFECTION IN COWS AND CALVES

12.1 Introduction

Many authors have assumed that leptospirosis causes abortions and stillbirths in cows (Parkinson 1958, Knott and Dadswell 1970, Corbould 1972, Marshall 1972, Sullivan 1974, Little and Hathaway 1983, Robinson 1983) Michna (1971) found higher serological leptospiral activity in aborting cattle than in a control group of cattle. Ellis et al. (1978) in Ireland found 6.9% of aborted fetuses had leptospiral titres (6.4% Hebdomadis serogroup and 0.5% Icterohaemorrhagiae serogroup), whilst no titres were detected in a control group of fetuses collected at an abattoir. In a later study 4.6% of fetuses from apparently normal uteri collected at an Irish abattoir contained leptospires (similar to serovar hardjo), 41.6% of randomly selected aborted fetuses and 68.9% of aborted fetuses from farms with abortion problems in the same area showed leptospires of the Hebdomadis, Canicola and Icterohaemorrhagiae serogroups (Ellis et al. 1982). Fennestarad and Borg-Petersen considered that leptospirosis would cause abortion only in the last half of pregnancy and this is probably due to greater placental permeability at that time allowing leptospires to cross over into the foetus (Amatredjo et al. 1976). Both serovars hardjo and pomona have been incriminated in Australian cattle (Doherty 1967b, Johnson et al. 1974).

As yet no study has compared differences in branding percentages between pregnant cows infected with leptospirosis and those not infected with leptospirosis within a single herd, although between-herd and between-year comparisons have been made (Amatredjo and Campbell 1975). The present work was designed to investigate possible differences in infected cattle and to follow the leptospiral serology of beef calves in a single herd in Central Queensland.

12.2 Materials and methods

One hundred and seventy-seven (177) ear-tagged, pregnancy-tested in-calf heifers and second-calf cows from the Emerald Pastoral College property "Berrigurra" were selected for the trial. Serum was collected from

each cow by the method described in Chapter 8. The cows were sampled before the last trimester of pregnancy (July 1983) and at branding time (March 1984). At the time of branding, when the progeny were approximately three months of age, it was noted whether or not each cow had a calf. Forty randomly selected heifer calves were also ear-tagged and sampled at branding (March 1984), weaning (June 1984) and two months after weaning (August 1984).

Serology was carried out at the Oonoonba Veterinary Laboratory of the Queensland Department of Primary Industries, Townsville, using the microscopic agglutination test. Only antibodies for serovars hardjo and pomona were studied. Reactions of 1:100 or greater were regarded as positive. Individual cows were classified as 'infected' during the last trimester of pregnancy if their titres to either serovar increased between the two samplings. Cows without titres or with stationary or decreasing titres were considered as not being significantly infected during the last trimester of pregnancy.

12.3 Results

The serological results from the cows are shown in Table 17. Thirty-four (34) cows were classified as 'infected' by one or both serovars (20 with hardjo, 10 with pomona and 4 with both). One hundred and forty-three (143) cows were classified as 'non-infected'.

In the 'infected' group 22 cows had calves and 12 had lost their calves which gave a branding percentage of 64.7%. The 'non-infected' group had a branding percentage of 83.2% with 119 calves from 143 cows. Results show that there is a significant decrease ($p < 0.05$) in branding percentage in cattle 'infected' with leptospirosis in the last trimester of pregnancy. The Chi-square tests for these results are shown in Appendix 14.5.

The serological results in calves are shown in Table 18. Three of the forty calves had hardjo titres at branding (March 1984), no calves had hardjo titres at weaning (June 1984) and three had titres two months after weaning (August 1984). Titres to serovar pomona were not detected.

Table 17 SEROLOGICAL RESULTS FROM PREGNANT COWS

NO IN GROUP COWS	CALVES	L HARDJO 7.83	TITRE 3.84	L POMONA 7.83	TITRE 3.84
91	76	0	0	0	0
2	2	0	100*	0	0
2	2	0	200*	0	0
4	3	0	400*	0	0
2	1	0	0	0	200*
2	0	0	200*	0	200*
1	1	0	200*	0	400*
12	10	100	0	0	0
3	3	100	100	0	0
3	3	100	200*	0	0
2	1	100	400*	0	0
1	1	100	0	0	200*
1	0	100	200*	0	200*
12	10	200	0	0	0
1	0	200	100	0	0
6	6	200	200	0	0
5	3	200	400*	0	0
1	1	200	800*	0	0
1	1	200	200	0	100*
1	0	200	200	0	200*
1	0	200	0	0	400*
3	3	400	0	0	0
1	1	400	100	0	0
1	1	400	200	0	0
4	2	400	400	0	0
1	0	400	800*	0	0
1	1	400	0	0	200*
2	1	800	0	0	0
2	2	800	200	0	0
1	1	1600	200	0	0
1	0	1600	400	0	0
1	1	0	0	100	0
1	1	0	0	100	200*
1	1	200	100	100	0
1	1	200	0	100	100
1	1	0	0	200	400*
1	0	100	0	200	400*
Total		70	47	6	15
Percentage		39.5	26.5	3.4	8.5

* = Rising titres

Table 18 CALF SEROLOGY AT BRANDING, WEANING AND TWO MONTHS AFTER WEANING

Number of calves	March 1984		June 1984		August 1984	
	hardjo (titres)	pomona (titres)	hardjo (titres)	pomona (titres)	hardjo (titres)	pomona (titres)
35	0	0	0	0	0	0
2	100	0	0	0	0	0
1	800	0	0	0	200	0
1	0	0	0	0	200	0
1	0	0	0	0	6400	0

12.4 Discussion

The results indicate that cows which have rising titres to leptospiral serovars pomona and hardjo during the last trimester of pregnancy are less likely to have a calf at branding than those which have constant or decreasing titres. Decrease in branding percentage could be due to abortion, stillbirth or mortality in young calves.

While three heifer calves had hardjo titres at branding which could have been due to maternal antibody all calves were serologically negative at weaning. Two heifers however had seroconverted two months after weaning while one heifer, which had a titre at branding, had regained a lower titre two months after weaning. One calf developed a titre of 6400 L. hardjo in August 1984 after a period of good rain.

13 GENERAL DISCUSSION

Two of the major influences on reproductive performance in beef herds in central Queensland have been investigated, namely environmental factors and disease. Specifically conception and branding rates obtainable on well managed properties in this area were determined, the possible relationship of annual rainfall variations and branding percentages studied, and the contribution of trichomoniasis and vibriosis to bovine infertility in the Central Highlands carried out. A detailed study of the epidemiology of leptospirosis in terms of serological response and depressed calving rates was made.

In a single herd study the conception (69.3% to 88.8%) and branding (65.8% to 84.1%) rates obtained at "Berrigurra" during a five year analysis suggest that fertility levels in this area are potentially as high as those found in north coastal Queensland (Entwistle 1983). The low (66.2%) average branding percentage calculated from Australian Bureau of Statistics figures for the four highlands shires in 1977-1981 suggests that factors decreasing reproductive efficiency in this area are not being dealt with effectively, probably due to lack of understanding.

Droughts and floods are features of the meteological pattern in the highlands (O'Sullivan 1977) and appears to affect the reproductive performance of beef cows. The severe 1982 drought was followed by lowered branding percentages in 1983 to levels well below those obtained in 1981 and 1984. The effect the above normal rainfall of 1983 had on the 1984 branding percentages suggested that it is not only net annual rainfall but also time of rain that is important. To be most beneficial rain must fall during the peak breeding season (summer) to provide good pasture and initiate oestrus cycles.

The study of the contribution of trichomoniasis and vibriosis to bovine infertility in this area was limited in scope but suggested that vibriosis is endemic. All infected properties investigated were intensive and well run. Further surveys need to be conducted to accurately determine the prevalence of vibriosis, and the prevalence of both diseases should be investigated in extensive herds.

Some of the factors influencing the epidemiology of bovine leptospirosis in this subcoastal region were studied to determine their impact.

Results tend to confirm those of Knott and Dadswell (1970) who found leptospiral abortion to be a greater problem on black cracking clay soils. They tested however for pomona and not hardjo. In the present work the prevalence of pomona in this area was low (4.1%) and there were no significant differences between properties with regard to soil type. The prevalence of hardjo however was higher (14.3%) on properties with high water holding capacity soils (greater than 30%) having a significantly ($p < 0.05$) higher prevalence (18.0%) than that (7.3%) on properties with low water holding capacity soils (less than 20%). This is consistent with other studies in Australia, the UK and elsewhere (Okazaki and Ringen 1957, Doherty 1967a, Twigg et al. 1968, 1969) which found that leptospires survived longer in wetter soils.

The seroprevalence of hardjo type leptospirosis appears to fluctuate in relation to the rainfall pattern. This is consistent with the findings of Amatredjo et al. (1976) for leptospiral induced nephritis in north Queensland. The long term average rainfall figures for this area show that June is often a month of high rainfall and results show that when this happens it can be followed one to two months later by a peak in hardjo prevalence. This peak would coincide with the period during which most of the areas beef cows are in the last half of pregnancy. The results of a single herd trial indicate that this situation could result in severe calf loss.

Feral pigs and stock horses often mix with cattle in this area and must both be considered in any study of the epidemiology of leptospirosis in the highlands. Whilst Kirkman et al. (1982) found little serological evidence of leptospirosis in coastal north Queensland horses the present survey found that stock horses in this area have a high seroprevalence to both hardjo (30%) and pomona (40%). This may have been due to husbandry methods used in this area which result in horses often being confined with the property dairy cows and having high levels of exposure to young cattle. Stock horses may therefore play an important role in disemminating leptospires in this region. Pigs appear to be poor hosts for hardjo, and pomona prevalence is low in this area.

The single herd study agrees with work carried out by other authors in the UK (Michna 1971, Ellis et al. 1978, Ellis et al. 1982) who found higher reactor rates among aborting cows than non-aborting cows. Most of these were intensive dairy herds. This study differs from others in that differences in branding rates between cows which had increasing titres and cows without increasing titres were used as an index of infertility whilst other authors looked for differences in prevalence between animals showing clinical signs of leptospirosis (eg abortion) and those not showing signs. Although between herd and between year comparisons have been made in beef cattle in Queensland (Amatredjo and Campbell 1975) this is the first study to establish that within a single herd pregnant cows which have rising titres in the last trimester of pregnancy have a lower chance of having a calf (64.7%) than those without rising titres (83.2%). Therefore leptospiral infection appears to reduce the chance of having a live calf at branding.

Young beef calves may have maternal derived leptospiral titres at branding in this area but those titres were not detected at weaning.

The low pomona prevalence relative to hardjo prevalence in this area is of interest. It would appear that hardjo will tolerate higher temperatures than pomona (Elder personal communications). It may be that the high summer temperatures in this tropical area are sufficient to suppress serovar pomona, but do not similarly affect serovar hardjo.

These studies have investigated several factors influencing bovine fertility in the area. Due to the complex nature of bovine infertility long-term and multidisciplinary studies are necessary. The following work should be carried out to further define factors which decrease bovine reproductive efficiency in the Central Highlands of Queensland.

a) A wider survey of the prevalence of vibriosis and trichomoniasis should be carried out to assess their full impact.

b) The effect of annual rainfall on reproductive performance in the thirteen survey herds trial should be extended to investigate the possible correlation between the two factors.

c) The five year reproductive performance study at Berrigurra should be continued and duplicated in other herds with different environmental and management conditions to assist in developing methods of increasing cattle fertility.

d) The effects of aspects of soil chemistry and physics on leptospiral prevalence in the environment should be examined.

e) Longer term studies on seasonal fluctuations in leptospiral prevalence should be carried out to examine rainfall and temperature effects and other possible interactions.

f) Vaccination trials of at least three year duration should be established to gauge their effectiveness against leptospirosis in this area.

g) The 40 heifers at Berrigurra should continue to be tested for leptospirosis to examine the serological profile during their productive life.

14 APPENDICES

APPENDIX 14.1.

TRICHOMONAS FETUS MEDIUM

BASAL MEDIUM

Liver Infusion	2,000ml
Tryptose	20g
Agar	6g

Method:

1. 1,000g of minced liver are added to 2,000ml of distilled water and stood overnight in the refrigerator.
2. Following morning skim off the fat, heat the remainder to 45°C and hold between 45°C - 50°C for 1 hour.
3. Boil for ½hr without stirring. Strain out the coagulum and filter the fluid through glass wool.
4. Add distilled water to make up to 2,000ml.
5. Add 20g Tryptose and dissolve.
6. Adjust to pH 7.4.
7. Heat in steam for 15 mins.
8. Cool to below 20°C, and vacuum filter through a 'DO' filter pad (or coarse filter paper).
9. Add 6g agar and dissolve.
10. Dispense in 250ml amounts in 500ml bottles.
11. Autoclave at 15 lbs for 15 mins.

SERUM

Inactivate Sterile bovine serum at 64°C for 30 mins.

P + S SOLUTION

Penicillin	200,000 units (2ml of 100,000 unit/ml Solution)
Streptomycin	10,000 units (0.1ml of 100,000 unit/ml Solution)
Sterile Distilled Water	100ml

To prepare 100,000 u/ml Penicillin:	Add 5ml Sterile distilled water to a 500,000 unit (300mg) vial.
To prepare 100,000 u/ml Streptomycin:	Add 5ml SDW to a 1g (1,000,000 unit) vial. Add 1ml of this solution to 1ml of SDW to produce a 100,000 u/ml solution.

MEDIUM PREPARATION

Aseptically add together the following after melting basal medium in steamer and cooling to 56°C:-

Basal Medium	250ml
Serum (Inactivated)	250ml
P + S Solution	31.25ml

Dispense in 8ml quantities in 1oz McCatney bottles and store frozen until ready to use.

APPENDIX 14.2

PAIRED T TESTS ON HERD BRANDING RATES (1981-1984)

YEARS COMPARED		$\bar{d}^+$	SE $\bar{d}$	t	SIGNIFICANCE*
1981	1982	5.15	4.00	1.288	NS
	1983	10.54	3.89	2.710	$p < 0.05$
	1984	-2.08	3.20	- 0.650	NS
1982	1983	5.38	2.61	2.061	NS
	1984	-7.23	3.73	- 1.938	NS
1983	1984	-12.62	2.85	- 4.428	$p < 0.01$

NS = not significant

+ $\bar{d}$ = average difference between farm branding rates over all farms for any two years

* = all tests have 12 degrees of freedom

Thus the 1983 branding rates are lower than the 1981 and 1984 branding rates.

APPENDIX 14.3

T TESTS AND PAIRED T TESTS FOR SEROVAR AND SOIL TYPE RELATIONSHIPS IN SURVEY PROPERTIES

Table 19 DATA FOR APPENDICIES 14.3 AND 14.4

PROPERTY	POMONA %	HARDJO %	HEBDOMADIS %	STOCKING RATE (ANIMAL/HA)	SOIL TYPE (WHC)*
1	0.8	4.8	0.0	0.03	low
2	4.6	11.9	5.0	0.03	low
3	1.2	12.4	10.0	0.04	low
4	9.6	1.9	0.0	0.06	low
5	3.4	8.0	5.0	0.03	low
6	2.3	4.6	3.3	0.04	low
7	3.2	33.2	38.0	0.06	high
8	1.7	9.4	10.0	0.05	high
9	4.1	26.0	50.0	0.10	high
10	5.2	11.1	0.0	0.04	high
11	2.9	11.8	6.7	0.07	high
12	8.1	10.2	6.7	0.04	high
13	6.4	24.1	5.0	0.04	high
mean	4.12	13.03	14.21	0.048	
SD	2.65	9.19	18.64	0.020	

*WHC = water holding capacity

TESTS OF MEAN HARDJO VERSUS MEAN POMONA PREVALENCE ON SOIL TYPES

SOIL TYPE (WHC)	$\bar{d}$	SE $\bar{d}$	t	DF	SIGNIFICANCE
all	8.92	2.67	3.34	12	$p < 0.01$
low	3.62	2.60	1.39	5	NS
high	13.46	3.78	3.56	6	$p < 0.05$

Thus hardjo is more prevalent than pomona overall and this difference is mainly brought about by the difference between prevalences on the high water holding capacity soils.

TESTS OF SEROVAR PREVALENCE ACROSS SOIL TYPES

SEROVAR	$\bar{x}_H$	$\bar{x}_L$	S_p ++	t	SIGNIFICANCE*
hardjo	17.97	7.27	4.25+	2.52	$p < 0.05$
pomona	4.51	3.65	1.51	0.57	NS

+ It shoud be noted that when testing for the difference between the mean hardjo prevalences on high and low water holding capacity soils there is a significant difference between the variance of prevalences. While this affects the test used, the t value obtained (2.516) is outside the 0.05 critical points ($\pm$ 2.201) and thus the result still considered significant

++S_p = pooled estimate of standard error

* Degrees of freedom are N1 + N2-2 = 11

Therefore serovar hardjo is more prevalent on soils with high water holding capacities.

APPENDIX 14.4

CORRELATION ANALYSIS OF SEROVARS HARDJO AND POMONA, PERIOD PREVALENCE, STOCKING RATE; AND SEROGROUP HEBDOMADIS PERIOD PREVALENCE ON INDIVIDUAL PROPERTIES

VARIABLES	r	SIGNIFICANCE[+]
hardjo / pomona	0.034	NS
hardjo / Stocking Rate	0.468	NS
pomona / Stocking Rate	0.103	NS
Hebdomadis / hardjo	0.794	$p < 0.01$

[+] All tests have 11 degrees of freedom

Therefore there are no correlations between hardjo, pomona and stocking rates but there is correlation between the individual property period prevalences of serovar hardjo and serogroup Hebdomadis. This suggests that the serogroup Hebdomadis reactions were due to serovar hardjo.

APPENDIX 14.5

TEST OF INDEPENDENCE OF LEPTOSPIROSIS AND CALF AT BRANDING

Observed frequencies are:

	RISING TITRE	NON-RISING TITRE	TOTAL
without calf	12	24	36
with calf	22	119	141
total	34	143	177

Expected frequencies assuming independence:

	RISING TITRE	NON-RISING TITRE	TOTAL
without calf	6.92	29.08	36
with calf	27.08	113.92	141
total	34.0	143.0	177

Chi-square = 4.723

Degrees of freedom = 1

Result is significant $p < 0.05$

Thus cows with rising leptospiral (hardjo and/or pomona) titres during the last half of pregnancy have a lower probability of having a calf at branding.

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