Studies On Some Aspects Of The Epidemiology Of Bovine Leptospirosis.

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ABSTRACT

A survey of bovine sera indicated that titres to *Leptospira interrogans* serovar *hardjo* were present in 60% of New Zealand cattle; 18% of sera were positive against serovar *pomona* and 9% against serovar *tarassovi*. Detailed studies were undertaken in a herd with a high prevalence of *hardjo* and *pomona* titres. It was determined that more than 90% of newborn calves acquired titres detectable by the microscopic agglutination test (MAT) after suckling sero-positive dams. The magnitude of the titre acquired by a calf was strongly correlated with that of its dam. These titres declined steadily, with a half-life of 15 to 17 days; most calves were sero-negative after 100 days of age and all by 190 days of age. Following natural infection cattle developed peak titres ranging from 1:768 to 1:8689 within fourteen days after sero-conversion. Initially these titres fell rapidly, declining on average by 62% in the first year and 89% in the first two years after infection. Thereafter titres declined slowly with a mean loss of 5% of residual titre per year.

Chromotographical studies on sera from cattle of various ages and at various stages of infection revealed that both IgM and IgG were involved in MAT response to leptospiral infection. Passively acquired titres in calves were largely attributable to IgG_1 antibodies. The MAT response of infected cattle was initially attributable IgM antibodies but IgG_1 antibodies were involved within the first week after sero-conversion and became the predominant immunoglobulin within 42 days. In convalescent titres of more than one year's duration IgM antibodies frequently were not involved and, in some animals apparently infected for some years, IgG_2 accounted for up to 14% of the MAT response.

Prospective cohort studies on groups of calves indicated that most became infected with *hardjo* at about 12 months of age during the winter and early spring. Analysis of climatic factors indicated that outbreaks of infection were strongly correlated with the level of moisture in the environment rather than rainfall *per se*. Susceptible calves became infected only following close contact with other leptospiruric cattle; no other wild, feral or domestic animal

reservoir of *hardjo* infection was detected. Leptospiruria was demonstrated to persist for up to 14 months in *hardjo*-infected cattle. The existence of an endemic cycle was demonstrated in which calves infected during one winter infected younger, susceptible calves in the following winter.

Evidence was presented to support an hypothesis that bovine *pomona* infection is a self-limiting sporadic disease which is attributable to direct or indirect contact with infected pigs. The survival of *pomona* for six weeks in an acidic soil (pH 5.5) under simulated Manawatu winter conditions and the potential for spread of this serovar from pigs to cattle via the environment was demonstrated.

The existence of a period of up to three months after the loss of detectable colostral MAT titres during which calves appear to be refractory to experimental inoculation with *hardjo* was demonstrated. It was also shown that convalescent *hardjo* titres are protective against experimental challenge of cattle with that serovar. On the basis of experimental studies it is believed that cattle are susceptible to infection with *balcanica* and that it is not possible to distinguish between bovine infections with this serovar and *hardjo* except by cultural techniques.

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CHAPTER ONE

BOVINE LEPTOSPIROSIS IN NEW ZEALAND: A REVIEW OF THE LITERATURE.

In contrast to the situation in many countries only four leptospiral serovars have been recovered from cattle in New Zealand. Although the syndrome of icterohaemoglobinuria in calves, now attributed to infection with *Leptospira interrogans* serovar *pomona*, had been observed in New Zealand over the previous 30 years (Salisbury, 1954; Dodd, 1955; Jamieson, Davidson and Salisbury, 1970) this organism was first isolated from infected calves and sheep in 1951 (Anon, 1951a). Subsequently *pomona* was associated with outbreaks of bovine abortion in 1953 and was recovered from tissues of aborted foetuses (Te Punga and Bishop, 1953; Anon, 1954a).

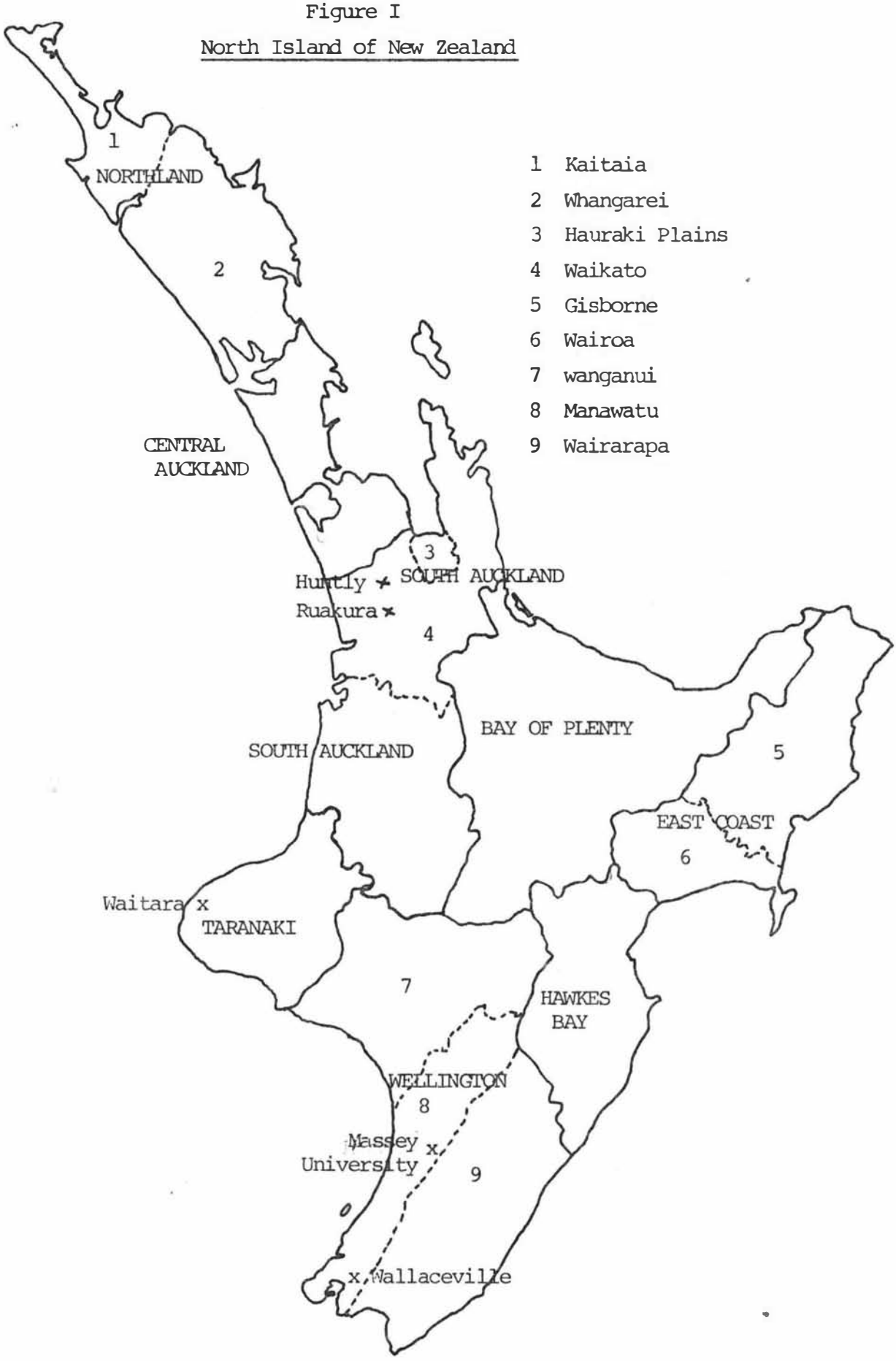
The isolation of serovar *copenhageni* from a rat was first reported in 1949 (Anon, 1950; Kirschner and Gray, 1951; Kirschner, 1960), but the first isolates typed at a W.H.O. Reference Laboratory were obtained from calves, exhibiting a syndrome of hyperacute kidney failure and sudden death, on several properties in the South Auckland district* in 1960 (Dodd and Brakenridge, 1960). More recently *copenhageni* was recovered from an apparently healthy three-month-old calf in the Hauraki Plains (Ris, Lake and Holland, 1973).

Serovar *ballum*, first isolated from two Hauraki Plains' dairy farmers in 1968 (Till, 1968; Christmas, Till and Bragger, 1974b), was subsequently recovered from two healthy three-month-old calves in the same district in 1971 (Ris, Lake and Holland, 1973). Serovar *hardjo* was first recovered from four dairy farmers, again in the Hauraki Plains, in 1968 (Till, 1968; Christmas *et al*, 1974b) and three years later from a healthy calf on a nearby farm (Lake, 1972). Two other leptospiral serovars are known to occur in New Zealand; *tarassovi*, isolated from pigs (Ryan and Marshall, 1976) and man (Till, 1977) and

* Geographical regions and locations referred to in the text are indicated in Figures I and II.

Figure I

North Island of New Zealand



balcanica isolated from the possum, *Trichosurus vulpecula* (Marshall *et al*, 1976). Neither have been recovered from cattle but there is serological evidence of bovine infection by the former (Kirschner, 1954; Ryan and Marshall, 1976).

Since 1951 there have been many reports in this country on the occurrence of leptospiral titres to various serovars in cattle. Although the serovars most frequently employed in testing have been *pomona* and *hardjo*, in addition *andaman, australis, autumnalis, ballum, bataviae, biflexa, bratislava, canicola, copenhageni, grippotyphosa, medanensis, pyrogenes, tarassovi* and other unspecified serovars have been used (Tables 1.1, 1.2, 1.3 and 1.4). Unfortunately there has been no nation-wide survey to estimate the prevalence of titres based on a substantial random sample of bovine sera. Some early reports on the prevalence of *pomona* titres were based on random samples of dairy cattle (Anon, 1952; Kirschner, 1954; Salisbury and McDonald, 1955) and beef cattle sera (Blunt, 1959; Young, 1965) from selected districts. However most of the reports in Tables 1.1 to 1.3 relate to sera submitted from cases of bovine abortion. Those sera submitted for the export testing of cattle would appear to provide a more valid sample for the estimation of the national prevalence of leptospirosis but, curiously, there was a higher prevalence of titres to both *hardjo* and *pomona* in that sample than in sera submitted concurrently from cases of bovine abortion (Roach, 1973). Two factors may explain this higher prevalence. Export tests are read more rigorously than routine diagnostic tests and sera from export tests generally come from cattle in pedigree herds and thus represent a small biased sample of the New Zealand cattle population.

More relevant results may be those obtained from two large samples of bovine sera submitted for a variety of reasons including suspected leptospiral abortion to the Ruakura Animal Health Laboratory. These submissions came from an area containing approximately half of New Zealand's dairy cattle (Anon,1974a; 1974b). The small random sample of 100 sera from 20 Taranaki dairy herds taken in 1974 (Brockie, 1976) and Ryan and Marshall's (1976) sample of 300 sera, from a nation-wide serum bank, appear

Table 1.1

Prevalence of Pomona titres in New Zealand cattle 1951-1975.

Year[α]	Serovar	Titre	Sample[β]	+ve[ψ]	% +ve[φ]	Comment	Reference
1951-52	pomona	?[ε]	134	34	25.4%	R.S.[λ] Waitara Freezing Works	Anon (1952)
1951	pomona	≥1:200	379	68	17.9%	Abattoir survey	Salisbury and McDonald (1955)
1953	pomona	≥1:150	100	20	20.0%	Dunedin Abattoir survey	Kirschner (1954)
1953	pomona	≥1:20	4 941	780	15.8%	Wallaceville A.H.L.[κ] sera	Salisbury (1954)
1953	pomona	≥1:200	4 941	504	10.2%	" " "	Salisbury (1954)
1953	pomona	≥1:20	108	73	67.6%	Cattle in contact to abortion outbreak	Te Punga and Bishop (1953)
1953	pomona	≥1:200	108	66	61.1%	" " " " " "	Te Punga and Bishop (1953)
1954	pomona	≥1:20	13 031	1 909	14.7%	Wallaceville A.H.L. sera	Salisbury and McDonald (1955)
1954	pomona	≥1:200	13 031	1 285	9.9%	" " "	Salisbury and McDonald (1955)
1952-55	pomona	?	40 000	?	10.0%	Wallaceville A.H.L. sera all reasons[ν]	McDonald and Rudge (1957)
1958	pomona	≥1:200	1 101	95	8.6%	Gisborne district beef cows	Blunt (1959)
1967	pomona	?	?	?	0.6%	Gisborne district wet-dry heifers	Young (1967)
1967	pomona	?	?	0	0.0%	R.S. 20% of pregnant heifers	Young (1967)
1968	pomona	≥1:200	728	116	16.0%	Wallaceville A.H.L. abortion sera	Anon (1969c)
1967-69	pomona	≥1:200	403	71	17.5%	Massey University clinical sera	Marshall (1978)
1969-71	pomona	≥1:200	1 207	69	5.7%	Lincoln A.H.L. sera all reasons	Smith (1973)
1971	pomona	≥1:200	890	45	5.1%	Hauraki Plains survey	Lake (1973a)
1971	pomona	≥1:200	1 015	142	14.0%	Ruakura A.H.L. abortion sera	Gardner (1973)
1970-72	pomona	≥1:20	1 133	0	0.0%	R.S. Southland brucella test sera	Porter (1973)
1972	pomona	≥1:20	2 416	741	30.6%	Wallaceville A.H.L. export sera	Roach (1973)
1972	pomona	≥1:200	6 083	987	16.2%	" " "	Roach (1973)
1972	pomona	≥1:200	1 516	234	15.5%	" " abortion sera	Roach (1973)
1972	pomona	≥1:200	1 573	170	10.8%	" " non-export sera	Roach (1973)
1972	pomona	≥1:200	?	?	14.3%	Lincoln A.H.L. abortion sera	Smith (1973)
1973	pomona	≥1:200	12 888	557	4.3%	Ruakura A.H.L. sera all reasons	Anon (1974a)
1973	pomona	≥1:2000	12 888	245	1.9%	" " " " "	Anon (1974a)
1973	pomona	≥1:100	102	10	9.8%	Taranaki herd with human *hardjo* case	Brockie (1976)
1974	pomona	≥1:200	6 607	406	6.1%	Ruakura A.H.L. sera all reasons	Anon (1974b)
1974	pomona	≥1:2000	6 607	139	2.1%	" " " " "	Anon (1974b)
1974	pomona	≥1:100	112	5	4.5%	Taranaki herd with human *hardjo* case	Brockie (1976)
1974	pomona	≥1:100	100	4	4.0%	R.S. 5 sera from 20 Taranaki herds	Brockie (1976)
1975	pomona	≥1:2000	1 305	111	8.5%	Whangarei A.H.L. abortion sera	Anon (1975d)

α Year in which survey conducted
β Number of animals tested
ψ +ve = positive
φ % +ve = % positive

ε ? = not specified
λ R.S. = Random sample
κ A.H.L.= Animal Health Laboratory
ν submissions for all reasons including abortion

Table 1.2

Prevalence of *Hebdomadis, Icterohaemorrhagiae* and *Ballum* titres in New Zealand cattle 1953 - 1974.

Year[α]	Serovar	Titre	Sample[β]	+ve[ψ]	% +ve[φ]	Comment	Reference
1967-69	*hardjo*	1:200	403	12	3.0%	Massey University clinical sera	Marshall (1978)
1970-72	*medanensis*	1:20	1 133	67	6.0%	R.S.[λ] Southland brucella test sera	Porter (1973)
1971	*medanensis*	1:100	890	161	18.0%	Hauraki Plains survey	Lake (1973a)
1972	*hardjo*	1:20	948	530	56.0%	Wallaceville A.H.L.[κ] export test sera	Roach (1973)
1972	*hardjo*	1:200	948	456	48.0%	" " " " "	Roach (1973)
1972	*hardjo*	1:200	1 491	592	39.7%	" " abortion sera	Roach (1973)
1972	*medanensis*	1:200	583	86	14.8%	" " non-export sera	Roach (1973)
1973	*hardjo*	1:200	12 888	1 610	12.5%	Ruakura sera all reasons [ν]	Anon (1974a)
1973	*hardjo*	1:2000	12 888	180	1.4%	" " " "	Anon (1974a)
1973	*hardjo*	1:100	102	59	57.8%	Taranaki herd with human *hardjo* case	Brockie (1976)
1974	*hardjo*	1:100	100	49	49.0%	R.S. 5 sera from 20 Taranaki herds	Brockie (1976)
1974	*hardjo*	1:200	6 385	1 739	27.2%	Ruakura A.H.L. sera all reasons	Anon (1974b)
1974	*hardjo*	1:2000	6 385	281	4.4%	" " " " "	Anon (1974b)
1974	*hardjo*	1:100	112	58	51.8%	Taranaki herd with human *hardjo* case	Brockie (1976)
1975	*hardjo*	1:200	77	39	50.8%	Wanganui herd in contact with possums	de Lisle *et al* (1975)
1975	*hardjo*	1:2000	1 305	28	2.2%	Whangarei A.H.L. abortion sera	Anon (1975d)
1953	*copenhageni* [ρ]	1:150	100	8	8.0%	Dunedin abattoir survey	Kirschner (1954)
1971	*copenhageni*	1:100	890	4	0.4%	Hauraki Plains survey	Lake (1973a)
1972	*copenhageni*	1:20	2 413	26	1.0%	Wallaceville A.H.L. export sera	Roach (1973)
1972	*copenhageni*	1:200	6 080	44	0.7%	" " " "	Roach (1973)
1972	*copenhageni*	1:200	983	8	0.8%	" " non-export sera	Roach (1973)
1973	*copenhageni*	1:200	12 888	11	0.1%	Ruakura sera all reasons	Anon (1974a)
1973	*copenhageni*	1:2000	12 888	2	0.0%	" " " "	Anon (1974a)
1974	*copenhageni*	1:100	100	0	0.0%	R.S. 5 sera from 20 Taranaki herds	Brockie (1976)
1974	*copenhageni*	1:200	6 422	24	0.4%	Ruakura A.H.L. sera all reasons	Anon (1974b)
1974	*copenhageni*	1:2000	6 422	4	0.1%	" " " " "	Anon (1974b)
1967-69	*ballum*	1:200	403	1	0.2%	Massey University clinical sera	Marshall (1978)
1971	*ballum*	?[ε]	890	0	0.0%	Hauraki Plains survey	Lake (1973b)
1973	*ballum*	1:200	10 680	3	0.0%	Ruakura A.H.L. sera all reasons	Anon (1974a)
1973	*ballum*	1:2000	10 680	1	0.0%	" " " "	Anon (1974a)
1974	*ballum*	1:200	6 409	3	0.1%	" " " " "	Anon (1974b)
1974	*ballum*	1:2000	6 409	0	0.0%	" " " " "	Anon (1974b)
1974	*ballum*	1:100	100	0	0.0%	R.S. 5 sera from 20 Taranaki herds	(Brockie (1976)

For symbols α, β, ψ, φ, ε, λ, κ, ν, see Table 1.1. ρ = *icterhaemorrhagiae AB*

Table 1.3

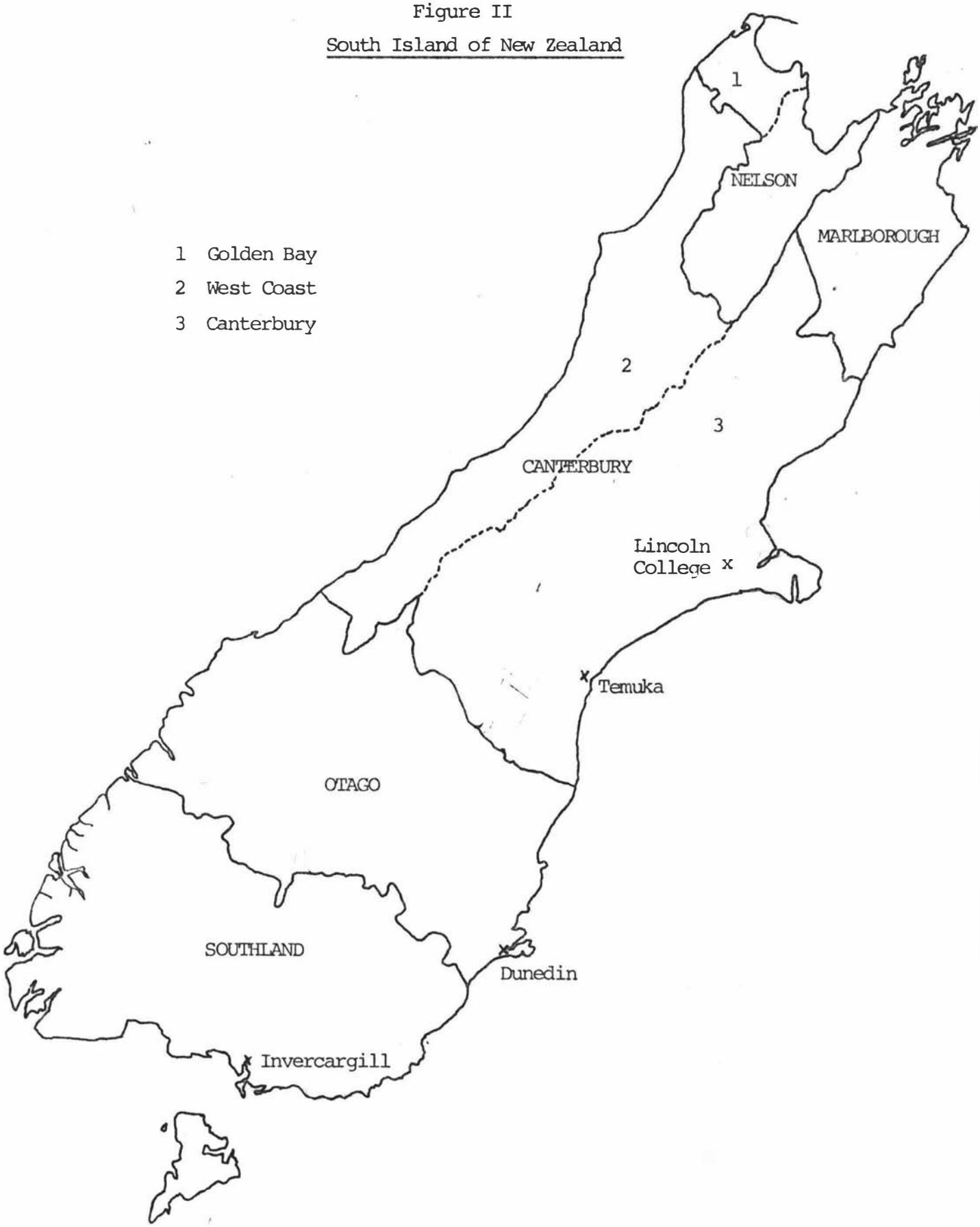
Prevalence of titres in New Zealand cattle sera to various serovars not isolated from cattle in that country.

Year[α]	Serovar	Titre	Sample[ε]	+ve[ψ]	% +ve[φ]	Comment	Reference
1953	*tarassovi*	1:150	100	3	3.0%	Dunedin abattoir survey	Kirschner (1954)
1967-69	*tarassovi*	1:200	403	1	0.2%	Massey University clinical sera	Marshall (1978)
1971	*tarassovi*	?[ε]	890	0	0.0%	Hauraki Plains survey	Lake (1973b)
1973	*tarassovi*	1:200	5	1	20.0%	Ruakura A.H.L.[κ] sera all reasons[ν]	Anon (1974a)
1973	*tarassovi*	1:2000	5	0	0.0%	" " " " "	Anon (1974a)
1974	*tarassovi*	1:100	100	0	0.0%	R.S.[λ] 5 sera from 20 Taranaki herds	Brockie (1976)
1976	*tarassovi*	1:50	300	18	6.0%	R.S. from N.Z. serum bank	Ryan and Marshall (1976)
1954	*grippotyphosa*[η]	1:20	83	0	0.0%	Wallaceville A.H.L. sera	Salisbury and McDonald (1955)
1971	*grippotyphosa*	?	890	0	0.0%	Hauraki Plains survey	Lake (1973b)
1971	*canicola*	?	890	0	0.0%	Hauraki Plains survey	Lake (1973b)
1973	*canicola*	1:200	13	1	7.5%	Ruakura A.H.L. sera all reasons	Anon (1974a)
1973	*canicola*	1:2000	13	0	0.0%	" " " " "	Anon (1974a)
1967-69	*autumnalis*	1:200	403	1	0.2%	Massey University clinical sera	Marshall (1978)
1971	*autumnalis*	1:100	890	8	0.9%	Hauraki Plains survey	Lake (1973a)
1973	*autumnalis*	1:200	14	2	14.3%	Ruakura A.H.L. sera all reasons	Anon (1974a)
1973	*autumnalis*	1:2000	14	1	7.1%	" " " " "	Anon (1974a)
1971	*andaman*	1:100	890	8	0.9%	Hauraki Plains survey	Lake (1973a)
1951-54	*"various"*	?	"several thousands"	0	0.0%	Wallaceville A.H.L. sera	Salisbury (1954)
1966	*"lepto"*	1:5000	172	10	5.8%	Huntly dairy cows with long returns	Moller (1967)
1966	*"lepto"*	1:5000	266	32	12.0%	Abortion sera Huntly district	Moller (1967)
1974	*bratislava*	1:100	100	1	1.0%	R.S. 5 sera from 20 Taranaki herds	Brockie (1976)
1967-69	*biflexa*	1:200	403	9	2.2%	Massey University clinical sera	Marshall (1978)
1967-69	*bataviae*	1:200	403	1	0.2%	" " " "	Marshall (1978)
1967-69	*australis*	1:200	403	2	0.5%	" " " "	Marshall (1978)
1967-69	*pyrogenes*	1:200	403	1	0.2%	" " " "	Marshall (1978)

For symbols α, ε, ψ, φ, ε, λ, κ, ν, see Table 1.1. η = *L. bovis*

Figure II

South Island of New Zealand



to be the only attempts in recent years to determine the true prevalence of agglutinating titres against some serovars. The data from the 1971 Hauraki Plains' survey (Lake, 1973a; 1973b) is also of interest, though this sample was based on properties with known human cases and cannot be taken as a true random sample.

Nevertheless, there does appear to be a declining trend in the prevalence of *pomona* titres in cattle from the early 1950's to the middle 1970's (Table 1.1). Initial reports indicate that 10-25% of cattle had titres against *pomona* of 1:200 or more, whereas more recent reports based on generally much larger samples indicate that the current prevalence is more like 5-10%.

Since *Hebdomadis* serogroup antigens were not routinely employed in agglutination tests on bovine sera until the late 1960's (Table 1.2) and since *medanensis* was the serovar used in some of the first surveys, it is difficult to comment on possible changes in the prevalence of *hardjo* titres in cattle. Serovar *medanensis* was used routinely for some human serology conducted during the 1950's and 1960's (Josland *et al*, 1957; Tennent and Philip, 1964; Philip and Tennent, 1966). The first human *medanensis* titres were reported in 1957 and at that time they were much less prevalent than *pomona* titres (Josland *et al*, 1957). Since then there has been an increasing human prevalence reported (Philip and Tennent, 1966; Christmas *et al*, 1974b) and currently *hardjo* titres are twice as common as *pomona* titres (Christmas *et al*, 1974a; Robinson, 1975; Brockie, 1976), though this change may have resulted partly from improved diagnosis.

A reported 3% prevalence of *hardjo* titres, of 1:200 or more, in bovine clinical sera from the Manawatu region, for the period 1967-1969 (Marshall, 1978), is very low when compared with the prevalence of 45-55% of some more recent reports (Roach, 1973; de Lisle *et al*, 1975; Brockie, 1976). Also initial reports in 1967 and 1968 indicated that only 13% or 14% of dairy herds in the Hauraki Plains contained *Hebdomadis* reactors (Anon, 1968a; Jamieson *et al*, 1970) compared with 48.4% of 240 herds with

abortion problems in 1974 (Cordes, 1975). Internationally, *hardjo* titres are found at a prevalence in excess of 50% in many areas (Amatredjo and Campbell, 1975). The suggestion has also been made that the prevalence of *hardjo* infection has recently increased in parallel, in both cattle and man, in Australia (Stallman, 1972; Sullivan, 1974), where major outbreaks of *hardjo* infection occurred in 1969-70 (Hoare and Claxton, 1972).

Sullivan (1974) further commented that the prevalence of bovine serological reactions to *hardjo* appeared to be increasing in the United States and that *hardjo* had emerged as a disease of cattle more or less simultaneously in North America, Italy, Australia and New Zealand. On balance the evidence for a rising prevalence of *hardjo* infection in New Zealand is suggestive only. There is no doubt however, that it now constitutes the most prevalent serovar infecting cattle in this country.

The limited serological evidence available on the other two serovars known to infect New Zealand cattle, *ballum* and *copenhageni*, indicates that the prevalence of both is low, with much fewer than 1% of surveyed cattle having titres of 1:200 or more against either (Table 1.2).

Of the other serovars tested (Table 1.3) all except *tarassovi* have such a low prevalence of titres of 1:200 or more that they may be readily explained as cross-reactions with *pomona, hardjo, copenhageni* and *ballum* infections. However, *tarassovi* titres may represent actual infections. Serological reactions to *tarassovi* have been widely reported in Australian cattle, and in U.S.S.R. there have been a number of reports of bovine isolations (Amatredjo and Campbell, 1975).

Apart from the cultural and serological evidence, there have been a number of published clinical reports and impressions on the prevalence of leptospiral infection in New Zealand cattle. A 1952 survey in Northland based on a farmer questionnaire produced 82 farms reporting redwater in calves. Seventy-six of these were claimed to be due to leptospirosis though only 11

were laboratory-confirmed (Ensor and McClure, 1953). In 1953, the zoonotic risk of leptospirosis was considered sufficiently severe for the government veterinarian in Whangarei to advise cancellation of calf club shows that year (Anon, 1954b). McDonald and Rudge (1957) reported a personal communication from Ensor and McClure that up to 10% of Northland herds were experiencing outbreaks of redwater in calves each year. Harris (1977) reports, however, that clinical leptospirosis has markedly diminished in the Kaitaia district over recent years, with only 18 cases diagnosed from approximately 200 farms over the period 1972-77. His opinion agrees with that of practitioners in Hauraki Plains, Waikato and North and South Taranaki, who all regard redwater in calves as a now uncommon and declining disease (Dobinson, 1967; Cagienard, 1977; McPherson, 1977; Peterson, 1977).

Abortion due to leptospiral infection was first reported in 1953 when a number of abortion storms were attributed to *pomona* infection (Te Punga and Bishop, 1953). McClure (1961), though producing no supporting data, attributed 20% of bovine abortion in Waikato to leptospiral infection. Shortridge (1966) stated that 12% of abortions investigated at Ruakura over a two year period were caused by *pomona*. This figure agrees with the 12% of 266 aborting cattle, with titres of 1:5000 or more for "leptospirosis", reported by Moller (1967) from dairy cattle in the Huntly area, and the 142 of 1015 (14.0%) cases of bovine abortion investigated at Ruakura, in 1971, with *pomona* titres of 1:200 or more (Gardner, 1973).

Cagienard (1973) reported, from his practice data in North Taranaki, that leptospirosis was responsible for an increasing proportion of bovine abortion, rising from 11% of all abortions in 1969-70, to 29% in 1972-73, but his diagnostic criteria are not given. The overall abortion rate that he reported, of about 1% of susceptible cattle, agrees with the 1.2% incidence of abortions that Moller *et al* (1967) observed in 18,000 dairy cattle in the Huntly area, but is lower than the 2.8% of 255,891 cattle reported by the New Zealand Dairy Board (Anon, 1957b).

In the early 1970's nation-wide data on bovine abortion became available for the first time as an indirect result of the National Brucellosis Eradication Scheme. In 1974, 850 of 7422 herds, from all over New Zealand, reported four or more abortions and in 229 (26.9%) of these there was evidence of recent *pomona* or *hardjo* infection, though approximately one third of these (81 herds) were also infected with *Brucella abortus* (Cordes, 1975). Examination of results from 63 herds with suspected leptospiral abortion during 1976, in which sera and urines were taken from samples of 10 in-contact cows, indicated that in two cases abortion was probably associated with *hardjo* infection; only six herds were free of leptospirosis (Anon, 1976d).

During 1975-76 a study of sera from aborted foetuses indicated that 133 of 164 (81.1%) had experienced *in utero* infection, but only three had leptospiral agglutinating titres of 1:200 or more; two to *hardjo* and one to *pomona* (Kirkbride, Martinovich and Woodhouse, 1977). This finding is interesting in view of Fennestad and Borg-Peterson's (1962) report that calves will respond to *in utero* infection with leptospires, by producing agglutinating antibody as early as the 132nd day of gestation. In spite of more than 80% of Northland herds being under brucellosis test and thus subjected to compulsory abortion surveillance in 1976, only one leptospiral abortion storm, caused by *pomona*, was recorded in that year (Anon, 1976b).

With changes in laboratory criteria and Ministry of Agriculture policy, the rate of diagnosis of leptospiral abortion has fluctuated during the last few years. As *hardjo* titres in particular are so prevalent in New Zealand cattle, serological findings in cases of bovine abortion must be interpreted with great care (Anon, 1974c; Hodges, 1975; Lake, 1975; Anon, 1976c). At present serological reactions in the aborting animal, in the absence of other signs of infection, are considered insufficient evidence for a diagnosis of leptospiral abortion (Gardner, 1976).

The syndrome of acute kidney failure and sudden death in

calves, caused by *copenhageni* infection, was stated to be widespread in South Auckland and the Waikato during the period 1956-59 (Dodd and Brakenridge, 1960). More recently a *copenhageni*-induced syndrome of photosensitisation in calves was stated to be common in Waikato during the years 1965-1977 (Anon, 1974d; 1977d). But apart from Kirschner's initial reports of human farm-associated *copenhageni* infection, rat infections (Anon, 1950; Kirschner, 1960) and titres to *copenhageni* in pigs and cattle in the Dunedin district (Kirschner, Miller and Garlick, 1952; Kirschner, 1954), there have been no reports of *copenhageni* infection in other parts of New Zealand. It is therefore likely that nation-wide prevalence of *copenhageni* infection of cattle is very low.

There has been only one report of clinical disease in cattle attributable to *ballum*, a case of severe nephritis and haematuria in a calf (Anon, 1977d). The cultural and serological evidence also indicates a very low prevalence of bovine *ballum* infection, though some farms appear to experience sporadic outbreaks (Ris *et al*, 1973).

Although Blunt (1959) found in a survey in the Gisborne district that 2 of 45 (4.4%) beef cows that aborted had *pomona* titres of 1:200 or more, Fielden and McFarlane (1959) did not regard leptospirosis as a common cause of abortion in East Coast beef cattle. This opinion was supported by a substantial survey in the same region, which showed that 0.6% of those beef cows which failed to rear a calf had *pomona* titres (Young, 1965). More recently Hanly and Mossman (1977), conducting as investigation into the causes of beef cattle infertility in the Wairoa district, considered leptospirosis of little significance, based on extensive comparisons between those cows which failed to rear calves and those which succeeded. They found many low titres to *hardjo* and *pomona* and considered infection with both serovars was endemic in many herds in the district; they stated that the introduction of a new serovar into a susceptible herd is followed by an abortion rate of 2.5%.

Regional Distribution of Leptospirosis in New Zealand.

Ministry of Agriculture reports over the past 25 years probably

reflect the varying interest of state veterinarians in leptospiral infections but they state that the prevalence of *pomona* infection increased notably in 1952 (Anon, 1953) and that there were widespread outbreaks of *pomona* infection in cattle, sheep and calves in 1953 (Anon, 1954b). *Pomona* infection was also reported to be increasing in pigs and humans in 1954 and 1955 (Anon, 1955; 1956), though there were few livestock losses reported (Anon, 1955). The only region reporting an increased incidence in 1956 was Northland (Anon, 1957a). Outbreaks of leptospirosis in the South Island were first noted in 1962 (Anon, 1963). In 1964, large numbers of sheep kidneys showing lesions attributed to leptospirosis were reported from abattoirs all over the country (Anon, 1965). Abortion and economic loss caused by leptospirosis in both Islands were reported in 1967 (Anon, 1968b) and the number and severity of outbreaks of bovine leptospiral abortion increased in 1968 (Anon, 1969a). In 1970, sporadic outbreaks of *pomona* in cattle were first reported in Otago and Southland, following the introduction of infected calves from Golden Bay (Anon, 1971; Smith, 1973). Apart from these outbreaks, leptospirosis was reported to be rare south of Temuka at that time (Smith, 1973).

Even in 1954 human and bovine cases of *pomona* infection had been reported from all parts of New Zealand except Canterbury, though the prevalence appeared to be higher in the north of New Zealand (Salisbury, 1954). In Canterbury, during the years 1956-61, *pomona* infections of humans and cattle, though of low prevalence, were reported by Cook (1964). However, the prevalence of human and bovine *pomona* infections was still regarded as low in Canterbury , compared with Nelson and the West Coast, in 1970-71 (Porter, 1973). A later survey of mainly beef herds, in Otago-Southland, showed many herds with titres to *hardjo* and a few with *pomona* titres (Anon, 1974f) though Williams (1976) regarded *hardjo* infection as uncommon in his practice based on Invercargill.

In 1975, 1949 of the 45,546 cases submitted to Animal Health Laboratories for all reasons were diagnosed as leptospirosis; 4.2% from Northland and Central Auckland, 63.9% from

South Auckland, 12.3% from Taranaki, Wanganui and Manawatu, 4.4% from Gisborne, Hawkes Bay and Wairarapa, 12.4% from the upper half of the South Island and the West Coast and 2.9% from Otago and Southland (Anon, 1976a).

Epidemiology.

It has been stated that there is an increased incidence of clinical leptospirosis in New Zealand during wet spring and summer months (Anon, 1973b). Also a number of authors have commented on the association between periods of high rainfall in the spring and a high human (Kirschner *et al*, 1952; Tennent and Philip, 1964; Philip and Tennent, 1966; Brockie, 1976; Philip, 1976) and bovine (Kirschner *et al*, 1952; James, 1954; Philip, 1976) incidence. However the contrary opinion has also been expressed. Salisbury (1954) thought there was no real variation in seasonal incidence and that cases were only more apparent in the spring following the appearance of new susceptibles at calving. Blackmore, Marshall and Ingram (1976) observed a large outbreak during a period of hot dry weather. However, in at least one season (1968), a substantially increased bovine incidence (Anon, 1969b) followed a dry autumn and a particularly wet cold winter and spring (Anon, 1969a) and in 1972 a decline in bovine leptospirosis was attributed to a dry spring (Anon, 1973a).

Regarding the source of infection, various species of wild and domestic animals have been suggested as reservoirs for the different serovars. During the 1950's pigs were to be found on most New Zealand dairy farms and were considered to be the major source of *pomona* infection (Kirschner *et al*, 1952; Anon, 1953; Salisbury, 1954; Dodd, 1955). This belief was supported by reports of outbreaks where *pomona* infection in calves almost invariably followed direct contact with pigs (Bruere, 1952; Ensor and McClure, 1953). Subsequently there has been a rapid decline in mixed pig/dairy cattle farming resulting from the cessation of skim milk production on most dairy farms. As a consequence pigs have now been largely discounted as the source of bovine *pomona* infection (Tennent and Philip, 1964; Jamieson *et al*, 1970; Christmas *et al*, 1974a; Marshall, 1975). Also

pomona outbreaks have been reported where pigs were absent (Webster, 1957; Blackmore *et al*, 1976). However, it is still possible that pigs are indirectly responsible for *pomona* infection of cattle. A recent *pomona* epidemic on a calf-rearing property was attributed to the introduction of calves from a farm where there had been contact with pigs (Anon, 1974a). Nevertheless, there is no doubt that the transmission of *pomona* from cow to cow does occur (Dodd, 1955; Blackmore *et al*, 1976).

Wildlife do not appear to act as a reservoir for *pomona* in New Zealand as a number of workers have failed to detect any evidence of infection with this serovar in rodents, possums, hedgehogs, mustelids and birds (Salisbury, 1954; Smith, 1965; Anon, 1975a; Blackmore *et al*, 1976; Brockie, 1976; Anon, 1977a; Brockie and Till, 1977; Hathaway, 1978). The sole report of *pomona* infection in New Zealand hedgehogs (Webster, 1957) was not fully substantiated by isolation and typing nor subsequently supported by other workers. In another survey one of 100 red deer sera was reported to have a titre of 1:200 against *pomona* (Daniel, 1966).

While sheep in this country do experience *pomona* infection (Hartley, 1952; Te Punga and Bishop, 1953; Webster, 1955; Webster and Reynolds, 1955; Anon, 1956; 1965; Jamieson *et al*, 1970) the limited contact between sheep and dairy cattle on most farms, and the relatively low prevalence of *pomona* in beef cattle, would suggest that they are not a major reservoir for bovine infection. It has also been shown that sheep did not contribute to bovine outbreaks in one investigation (Blackmore *et al*, 1976).

With the exception of possums, no other wildlife species has been shown to have cultural or serological evidence of *hardjo* infection in this country in spite of extensive surveys (Blackmore *et al*,1976; Brockie,1976; Hathaway, 1978). The initial reports of widespread *hardjo* infection in possums (de Lisle *et al*, 1975; Brockie, 1976) and a postulated association between possum and bovine *hardjo* infection appear to be erroneous (Anon, 1977b). All the evidence to date indicates that it is *balcanica* infection which is widespread in the possum population (Marshall

et al, 1976; Hathaway, 1978) and it appears that earlier workers were misled by the almost total serological cross-reaction between *balcanica* and *hardjo*. Although there is convincing serological evidence of *hardjo* infection in New Zealand sheep (Ris, 1975), their role in the epidemiology of bovine *hardjo* infection is still not defined. It appears most likely that *hardjo* is spread, at least among dairy cattle, from cow to cow (Blackmore *et al*, 1976).

Apart from human and bovine infection, *copenhageni* has been isolated only from the Norwegian rat (*Rattus norvegicus*) in New Zealand (Anon, 1950; 1951b; Kirschner and Gray, 1951; Kirschner, 1960; Shortridge, 1960; Anon, 1961a; 1961b). There is strong evidence that cattle have been infected by rat contact (Dodd and Brakenridge, 1960; Anon, 1961b). Similarly it appears that the mouse (*Mus musculus*), the black rat (*Rattus rattus*), and the hedgehog (*Erinaceous europaeus*), serve as reservoirs for the sporadic bovine infections of *ballum* observed in this country (Brockie and Till, 1977; Hathaway, 1978). In two of the three recorded cases of bovine *ballum* infection were in calves being fed meal (Ris *et al*, 1973) and it is likely, therefore, that there could have been close contact with rodents.

Infected cattle have been shown to shed leptospires in milk (Anon, 1952) and urine. Experimentally-infected calves have been shown to shed *pomona* in their urine for six to nine weeks p.i. (Anon, 1952; 1958; 1968a) though it has been stated that naturally-infected cattle will shed *pomona* for 100 days or more (Salisbury, 1954). Salisbury (1954) suggested that clinically normal carriers were in fact responsible for carrying over infection on a property from one season to the next and other authors have reported infection and shedding in clinically normal calves and cows (Kirschner *et al*, 1952; Te Punga and Bishop, 1953; Webster and Reynolds, 1955; Lake, 1972; Anon, 1973b; Ris *et al*, 1973; Anon, 1976a) even when they have very low titres (McDonald and Rudge, 1958; Hodges and Ekdahl, 1973). However, Ensor and McClure (1953) stated that *pomona* epidemics in closed herds generally occurred only once every three to five years and very rarely in successive seasons.

Since *pomona* vaccines were not available in New Zealand at that time, each year's calves must have become susceptible following the loss of maternal immunity. This fact suggests that more frequent epidemics should have occurred in closed herds if asymptomatic adult carriers were present.

There is also the question of whether shed leptospires infect susceptible hosts by direct contact or via the environment. Direct contact is favoured by Blackmore *et al* (1976), Christmas *et al* (1974a), Jamieson *et al* (1970) and Brockie (1976), with the latter three authors particularly stressing the large herds, intensive management and high pasture stocking rates, which characterise New Zealand's dairy husbandry, as major factors in facilitating this spread. Blackmore *et al* (1976) suggested that the urine-licking involved in male bovine behaviour was the major transmission factor in the bull herd they studied.

Kirschner and McGuire (1957) felt that indirect spread via the environment was a major factor and conducted a series of studies to determine survival times of *pomona* organism in various physical environments. They were particularly concerned with the short survival time of *pomona* in undiluted bovine urine or milk, which they determined to be not more than 90 and 30 minutes respectively. De Jong (1968) reported *pomona*-infected urine to be still infective for guinea pigs six hours after collection and Marshall (1975) reported three day survival of *pomona* in whole milk. Kirschner and McGuire (1957) emphasised the major significance of rainfall in rapidly diluting urine and milk thereby greatly enhancing leptospiral survival. They experimentally demonstrated the survival of *pomona* for up to 35 days in urine diluted to 1 in 100 with rainwater, 10 days in diluted sewage and 60 days in milk diluted 1 in 80 with tap water (Faine and Kirschner, 1953; Kirschner and McGuire, 1957). They also demonstrated that *pomona* survived 21 days in a mixture of soil and water and remarked that many outbreaks occurred when soils were waterlogged (Kirschner and McGuire, 1957).

Both direct and indirect spread of *hardjo* and *pomona* have

been experimentally tested in this country without success. Three susceptible calves were kept with a *hardjo* shedder in a concrete pen which was hosed out every second day, yet they failed to become infected (Anon, 1975b). Because of concern at the risk of infection following the spraying of dairy shed effluent onto pastures, effluent was experimentally contaminated with cultures of *pomona* and *hardjo*. Pregnant heifers were grazed on the sprayed areas without infection occurring (Anon, 1974g). This experiment however, appears to have a number of weaknesses; the degree of pasture contamination, virulence of cultures used and the susceptibility and stocking rate of the intended hosts were all undetermined. There have been overseas reports of humans acquiring infection following contact with contaminated sewage (Wray, 1975). One outbreak of leptospiral haemoglobinuria in calves, in the Waikato, was attributed to grazing on a pasture which had been sprayed with piggery effluent (Anon, 1974a).

Summary

1. Of the four serovars infecting cattle in New Zealand only *hardjo* and *pomona* are widespread with the former being by far the most prevalent.
2. There is some evidence that the prevalence of *pomona* in cattle has declined since 1951 and that of *hardjo* has increased since the middle 1960's.
3. Within New Zealand it appears that leptospiral infection has spread more recently into the far south of the country and is still at a lower prevalence there and in Canterbury than in the West Coast of the South Island and in the North Island.
4. The four serovars isolated from cattle each appear to have their own reservoir hosts. Though cattle can undoubtedly transmit *pomona* to other cattle it is likely that the pig is the major host for this serovar and that bovine outbreaks are either directly or indirectly associated with porcine carriers. Cattle are the major hosts of *hardjo* though the role of sheep in the transmission of this serovar is still unknown. The black rat, house mouse and hedgehog are the major reservoirs of *ballum* and the

Norwegian rat the reservoir of *copenhageni*.

5. The main factors considered to contribute to the high prevalence of bovine leptospiral infection in this country are periods of high rainfall favouring transmission via the environment and the management practices adopted by the New Zealand dairy industry based on large herds and high stocking rates.

CHAPTER TWO

PREVALENCE STUDIES ON BOVINE LEPTOSPIROSIS

As was discussed in the previous chapter, the published information on the cultural and serological evidence of leptospirosis in New Zealand cattle fails to provide definitive data on a number of points. In particular, since no nation-wide random sample surveys have been conducted, the true national prevalence of bovine infection by various leptospiral serovars is still unknown. It was therefore decided to commence this study by defining the prevalence of serological reactions in bovines to the serovars known to occur in New Zealand. This study was followed by further surveys in the Manawatu region in preparation for the selection of suitable herds in which to undertake detailed investigations of the epidemiology of bovine leptospirosis.

Four surveys were undertaken: the first a national survey of leptospiral titres in a randomly-selected sample of beef and dairy cattle sera, the second and third, of leptospiral titres in dairy cattle from the Manawatu region at different times of the year, and the fourth a cultural survey of leptospiral infection in a randomly-selected group of typical Manawatu dairy cattle. In addition the three Massey University dairy herds were sampled to see if they conformed to the typical serological pattern observed in cattle in the surrounding Manawatu region.

Materials and Methods.

Random sample of sera from New Zealand dairy and beef cattle.

Sera were obtained from the bovine serum bank maintained by the Ministry of Agriculture and Fisheries at the Central Brucellosis Laboratory, Wallaceville. This serum bank contains sera which were randomly collected from dairy and beef herds at the time of whole herd brucellosis tests. Sera are held at -20°C and the sample selected for this study came from herds sampled during 1973 and 1974. The ages of cattle sampled were not individually specified but the serum bank sample was randomly selected from adult cattle more than 18 months old.

A sample of 480 sera was selected. The sample size was based on the previously-reported prevalences of bovine titres to the five serovars to be tested, the logistics of the microscopic agglutination tests (MAT) as conducted in the Massey University laboratory and the recommendations of the W.H.O. Study Group Report on Serological Surveys (Anon, 1959). The composition of the sample was based on the New Zealand distribution of cattle by statistical region (Anon, 1975e). As there were insufficient herds represented in the serum bank sample to provide one sample from each herd, samples of three sera were selected from each of 152 herds and samples of four sera from each of a further six herds. The numbers of herds and sera selected from each region as proportions of the national herd, compared with the actual distribution of New Zealand cattle in 1975 are given in Table 2.1.

Random samples of sera from Manawatu dairy herds.

Two stratified random samples, each of 480 sera, were obtained from Manawatu dairy cattle; the first in the winter of 1975 and the second in the spring of 1976. On each occasion 24 sera were collected by the use of random number tables (Remington and Schork, 1970) from each of 20 herds chosen during a 14 day period. The sample was stratified by selecting equal numbers of town-supply and factory-supply dairy herds from herds which had been submitted for whole herd blood sampling for brucellosis testing.

Abattoir sample for the cultural survey of the prevalence of leptospiral infection in Manawatu dairy cattle.

During the early winter of 1976, 113 cull dairy cattle from the Manawatu region sent to the Longburn abattoir, were bled prior to slaughter. Sera were immediately tested by the MAT and the cattle stratified according to level of titre. Kidneys, with their capsules intact, were collected from the first ten cattle slaughtered with each level of titre and taken to the laboratory for processing.

Survey of prevalence of leptospiral titres in the Massey University dairy herds.

Random serum samples were collected from 24 cattle in each

Table 2.1

Regional representation of herds and sera in the random sample of New Zealand bovine sera compared with the officially reported distribution* of New Zealand cattle in the same year (1974).

Statistical Region	No. of herds	% of total	No. of sera	% of total	Official % of total	Expected no. of sera +
Northland	18	11	54	11	11	52.8
Central Auckland	9	6	28	6	6	28.8
South Auckland	34	22	103	21	} 30	144.0
Bay of Plenty	19	12	57	12		
Taranaki	16	10	48	10	9	43.2
East Coast-Hawkes Bay	19	12	58	12	14	67.2
Wellington	18	11	55	11	14	67.2
Nelson-Marlborough	4	3	12	3	3	14.4
Canterbury	10	6	31	6	6	28.8
Otago	6	4	18	4	4	19.2
Southland	5	3	16	3	3	14.4
North Island	133	84	403	84	84	403.2
South Island	25	16	77	16	16	76.8
New Zealand	158	100	480	100	100	480.0

* Anon (1975e)

+ Expected number of sera based on official distribution of cattle by region.

of the three Massey University dairy herds. In each herd cows were assigned a rank number according to the sequence in which they were milked and 24 rank numbers were chosen by the use of random number tables (Remington and Schork, 1970).

Serological Procedures.

All serum samples were tested using a modification of the MAT described by Cole, Sulzer and Pursell (1973). Serial doubling dilutions of sera, which ranged from 1:24 to 1:3072 after the addition of antigen, were prepared in plastic, round-welled, microtitre plates (Microtitre*) using a semi-automated minidiluter (Dynatech). Each well contained 25 µl of diluted serum and 25 µl of antigen. Any serum reacting at a dilution of 1:3072 was further tested by taking out to titre. Known positive control antisera to each antigen being used were included in each set of tests. Plates were incubated at 37°C for 90 minutes.

The antigens used were living cultures of serovars *ballum, copenhageni, hardjo, pomona* and *tarassovi* grown in liquid EMJH medium. These cultures were grown for five to seven days to an estimated density of 10^8 organisms/ml.

Tests were read by transferring a drop from each well onto a glass microscope slide, using a multiple dipper designed by Ryan (1978). The slides were examined by dry dark-field microscopy at a magnification of 120x. A positive reaction was regarded as one in which 50% or more of the leptospires were agglutinated. The titre endpoint was either taken as the last well in which 50% or more agglutination was observed or, in those cases where there was much more than 50% agglutination in the penultimate well and much less than 50% agglutination in the final well, as half-way between those two wells.

Since titres are geometric measurements, for example doubling dilutions in this study, some appropriate mathematical transformation must be applied to them to allow statistical analysis. Titres can be averaged to determine a geometric mean titre (GMT) (Paul and White, 1973) but there can be no standard deviation of a GMT. Thus all titres in this study were recorded

* Details of all manufacturers adresses are given in Appendix I.

and processed in a logarithmic coded form where the code is the rank of the well in which the endpoint occurred. In this form titres can be subjected to all normal statistical procedures such as comparison of means and regression analysis and can be presented graphically. Since the actual titre of the first well is 1:24, coded titres can be converted to true titres by the expression $\text{Titre} = \frac{1}{12 \times (10^{\{0 \cdot 301 \times \text{code}\}})}$.

The relationship between true titres and coded titres is illustrated in Table 2.2. In those cases where the endpoint occurred between two wells the coded titre was recorded as a half unit more than the penultimate well. For the sake of clarity, titres are given in conventional notation whenever comparisons are made with other workers' data. Data on the repeatability of the MAT procedure used in this study are given in Chapter Five.

Cultural Procedures.

Kidneys were processed within four hours of the animal being slaughtered. The capsule was removed from each kidney and a 20 to 30 gram portion was taken aseptically and placed in a gamma-sterilised plastic bag containing 100 ml of sterile Stuart's Basal Medium (B.B.L.). The portion of kidney was homogenised in this medium by placing the bag in a stomacher (Colworth) 400) until it was completely disrupted. This usually took three to five minutes. Three serial tenfold dilutions of this homogenate were made immediately in 15 ml glass bottles containing sterile Stuart's Basal Medium. Aliquots of 0.5 ml of each dilution were inoculated into culture tubes containing each of two media: EMJH (Difco), 0.15% agar (Difco) and 1.0% bovine serum albumin (Difco) either with or without 200 µg/ml 5-Flourouracil (5FU) (Sigma). These media will be referred to as EMJH or EMJH plus 5FU respectively.

The tubes of culture media were incubated at 30°C for 12 weeks and examined by dark-field microscopy at the 14th, 28th, 56th and 84th days after inoculation and then discarded. Isolates were subcultured into a liquid medium (liquid EMJH) which

Table 2.2

The relationship between coded titres and true titres.

Coded Titre	True Titre
0	< 1:12
½	1:17
1	1:24
1½	1:34
2	1:48
2½	1:68
3	1:96
3½	1:136
4	1:192
4½	1:272
5	1:384
5½	1:543
6	1:768
6½	1:1086
7	1:1536
7½	1:2172
8	1:3072
8½	1:4344
9	1:6144
9½	1:8689

$$T = 12 \times (10^{\{.301c\}})$$

where T = reciprocal of true titre

c = coded titre

consisted of EMJH (Difco) basal media and 1% bovine serum albumin (Difco) and passaged weekly into fresh tubes of liquid EMJH until they were at a sufficient density to be serologically typed, generally in the order of 1 x 10^8 leptospires/ml.

Serological Typing.

Serogroup typing was conducted using sera of known serogroup specificity supplied to this laboratory from the Centre for Disease Control (CDC), Atlanta, Georgia. Microtitre plates were set up using these standard antisera against the following serovars: *australis, autumnalis, ballum, bataviae, biflexa, canicola, copenhageni, grippotyphosa, hardjo, pomona* and *pyrogenes*.

Each isolate was tested against serial dilutions of all 12 antisera and classified as belonging to the serogroup whose antiserum reacted to the highest titre. Since the isolation of serovar *balcanica* at this laboratory (Marshall *et al*, 1976) it has become apparent that isolates belonging to the *Hebdomadis* serogroup require further testing to distinguish between *hardjo* and *balcanica*. Absorbed antisera were prepared by exhaustively absorbing high-titre anti-*hardjo* sera with *balcanica* and anti-*balcanica* sera with *hardjo*. These absorbed antisera react monospecifically with *hardjo* and *balcanica* respectively (Hathaway, 1978). All *Hebdomadis* serogroup isolates were typed against these absorbed antisera to distinguish between isolates of these two serovars.

Statistical Methods.

The statistical methods used in the analysis of the results obtained in this chapter are given in Appendix II.

Results.

Random sample survey of the prevalence of leptospiral titres in New Zealand cattle.

A comparison between the number of herds and animals sampled in each statistical region and the census figures for cattle in those regions in 1974 (Anon, 1975e) is given in Table 2.1. Inspection of this table indicates that the sample closely follows the official census distribution of livestock in those regions. The small differences between the observed (sample)

distribution and the expected (official) distribution are not significant ($P > 0.50$).

The proportions of cattle reacting serologically against the five serovars tested are given in Table 2.3. The data presented in this table indicate that 60% of New Zealand cattle sera had titres to *hardjo*, 18% to *pomona*, 9% to *tarassovi*, 2% to *copenhageni* and 4% to *ballum*. Inspection of the table suggests that *hardjo*, *copenhageni* and *ballum* were more prevalent in the North Island while *pomona* and *tarassovi* were equally prevalent in both North and South Islands. When these differences were tested statistically it was found that significantly more cattle in the North Island had *hardjo* titres ($P < 0.01$) and that the prevalence of *copenhageni* and *ballum* titres combined was approaching a significantly greater level in the North Island ($P < 0.10$).

Confidence limits for the prevalence of titres were calculated using the expression that the standard error of the percentage of serological positives in a population, based on a large random sample, is $100\sqrt{(\frac{pq}{n})}$. In this expression p = proportion positive, q = proportion negative and n = sample size (Anon, 1959). These 95% confidence limits were 56.0% to 65.0% for *hardjo* titres, 14.0% to 21.0% for *pomona* titres, 6.5% to 11.5% for *tarassovi* titres, 1.0% to 3.5% for *copenhageni* titres and 2.0% to 5.0% for *ballum* titres.

To allow the results of this survey to be compared with other published data on the prevalence of titres to those serovars tested in this study, the proportions of animals with titres ≥ 3 and ≥ 4, which are approximately equivalent to 1:100 and 1:200 respectively, are presented in Table 2.4. The table shows the numbers of animals reacting at different titres. The mean positive titres, together with their standard errors, are also given. The significance of the differences between these mean titres was tested by a one-way analysis of variance. Highly significant differences were found between all means except those of *ballum* and *copenhageni*. Reactors to *pomona* had the highest mean titre, then in descending order *hardjo*, *tarassovi*,

Table 2.3

Proportions of herds and animals from different statistical regions with MAT titres ≥ 1:17 to serovars *hardjo, pomona, tarassovi, copenhageni* and *ballum* in a random sample of New Zealand cattle sera collected in 1973-74.

Statistical Region	*hardjo*		*pomona*		*tarassovi*		*copenhageni*		*ballum*	
	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals
Northland	16/18=89%	35/54=65%	7/18 =39%	9/54 =17%	6/18 =33%	7/54 =13%	2/18 =11%	2/54 =4%	2/18 =11%	2/54 =4%
Central Auckland	9/9=100%	23/28=82%	3/9 =33%	3/28 =11%	3/9 =33%	6/28 =21%	0/9 =0%	0/28 =0%	1/9 =11%	1/28 =4%
South Auckland	31/34=91%	82/103=80%	19/34 =56%	30/103 =29%	3/34 =9%	5/103 =5%	4/34 =12%	5/103 =5%	4/34 =12%	4/103 =4%
Bay of Plenty	16/19=84%	35/57=61%	9/19 =47%	13/57 =23%	8/19 =42%	11/57 =19%	1/19 =5%	1/57 =2%	2/19 =11%	3/57 =5%
Taranaki	14/16=88%	32/48=67%	5/16 =31%	7/48 =15%	0/16 =0%	0/48 =0%	1/16 =6%	2/48 =4%	1/16=6%	1/48 =2%
East Coast-Hawkes Bay	17/19=89%	33/58=57%	5/19 -26%	7/58 =12%	5/19 =26%	6/58 =10%	1/19 =5%	1/58 =2%	2/19=11%	2/58 =3%
Wellington	11/18=61%	20/55=36%	3/18 =17%	4/55 =7%	1/18 =6%	1/55 =2%	0/18 =0%	0/55 =0%	3/18=17%	3/55 =5%
Nelson-Marlborough	1/4=25%	2/12=17%	0/4 =0%	0/12 =0%	2/4 =50%	2/12 =17%	0/4 =0%	0/12 =0%	0/4=0%	0/12 =0%
Canterbury	8/10=80%	17/31=55%	3/10 =30%	5/31 =16%	2/10 =20%	2/31 =6%	0/10 =0%	0/31 =0%	1/10=10%	1/31 =3%
Otago	0/6=C%	0/18=0%	0/6 =0%	0/18 =0%	2/6 =33%	2/18 =11%	0/6 =0%	0/18 =0%	0/6 =0%	0/18 =0%
Southland	5/5=100%	11/16=69%	3/5 =60%	7/16 =44%	1/5 =20%	1/16 =6%	0/5 =0%	0/16 =0%	0/5 =0%	0/16 =0%
North Island	114/133=86%	260/403=65%	51/133=38%	73/403 =18%	26/133 =20%	36/403 =9%	9/133 =7%	11/403 =3%	15/133 =11%	16/403 =4%
South Island	14/25=56%	30/77=39%	6/25 =24%	12/77 =16%	7/25 =28%	7/77 =9%	0/25 =0%	0/77 =0%	1/25 =4%	1/77 =1%
New Zealand	128/158=81%	290/480=60%	57/158 =36%	85/480 =18%	33/158 =21%	43/480 =9%	9/158 =6%	11/480 =2%	16/158 =10%	17/480 =4%

Table 2.4

The numbers and proportions of sera from a random sample of New Zealand bovine sera reacting to serovars *hardjo*, *pomona*, *tarassovi*, *copenhageni* and *ballum* at various titres (n = 480).

Titre	*hardjo*	*pomona*	*tarassovi*	*copenhageni*	*ballum*
0	190	395	437	469	462
½	20		6	3	8
1	31	11	11	6	5
1½	25	3	3	1	2
2	45	10	7		2
2½	23	7	3		
3	44	16	8	1	
3½	22	1	1		
4	38	14	3		
4½	14	6	1		
5	14	9			
5½	7	1			
6	5	3			
6½	1				
7	1	1			
7½					
8		2			
8½					
9		1			
> 0	$\frac{290}{480}$ = 60%	$\frac{85}{480}$ = 18%	$\frac{43}{480}$ = 9%	$\frac{11}{480}$ = 2%	$\frac{17}{480}$ = 4%
$\geq 3^{\alpha}$	$\frac{146}{480}$ = 30%	$\frac{54}{480}$ = 11%	$\frac{13}{480}$ = 3%	$\frac{1}{480}$ = 0%	$\frac{0}{480}$ = 0%
$\geq 4^{\beta}$	$\frac{80}{480}$ = 17%	$\frac{37}{480}$ = 8%	$\frac{4}{480}$ = 1%	$\frac{0}{480}$ = 0%	$\frac{0}{480}$ = 0%
Mean titre of +ves	2.77B$^{\psi}$	3.39A	1.95C	1.09Dd	0.94Dd
S.E.$^{\phi}$	0.08	0.19	0.17	0.21	0.13
G.M.T.$^{\lambda}$ +ves	1:82	1:126	1:46	1:26	1:23

α ≥ 3 is approximately equivalent to a titre of 1:100

β ≥ 4 " " " " " " " 1:200

ψ Notation: Duncan's lettering; mean values with a capital letter in common are not significantly different at the 1% level of probability and those with lower case letters in common do not significantly differ at the 5% level.

ϕ Standard error of the mean.

λ G.M.T. = Geometric mean titre.

copenhageni and *ballum* reactors.

The association between serological reactions occurring in individual animals to two or more serovars was examined. The distribution of single and multiple reactions to the five serovars is given in Table 2.5. Of the 480 animals tested 316 (66%) reacted to at least one serovar. The proportion of all animals tested reacting to only one serovar was 42%, to two serovars 20%, to three serovars 4% and to four serovars 0.4%. Using the data from Table 2.5, 95% confidence limits were established for each serovar, which show the expected proportions of reactors to one serovar reacting to other serovars, based on chance alone. These confidence limits and the observed proportions of multiple reactions occurring in cattle reacting to each serovar are shown in Table 2.6. In each case the observed proportions of multiple reactions lie just within or beyond the upper limit of each confidence interval, indicating near-significant or significant differences between the observed levels and those expected by chance alone.

The sizes of multiple reaction titres are presented in Table 2.7. This table was constructed by considering each group of reactors to a given serovar as a sub-sample from the population of 480 sera tested. The means and standard errors of the titres to the other serovars occurring in those samples were then compared with those calculated for all 480 sera. Inspection of this data (Table 2.7) indicates that *tarassovi, ballum* and *copenhageni* reactors had mean *hardjo* and *pomona* titres which were greater than those observed in the whole population. Statistically significant differences are indicated; all other differences were non-significant at the 5% level of probability.

Random sample of sera from dairy cattle in the Manawatu region.

The numbers of sera in each sample reacting at different titres to the five serovars tested are shown in Table 2.8. A higher proportion of sera from the spring 1976 sample were positive against all serovars tested. The numbers and proportions of sera reacting against one or more serovars are shown in Table 2.9. The proportion of sera with multiple titres was

Table 2.5

The numbers of animals tested in a random sample of New Zealand bovine sera with titres to one or more serovars (n = 480).

Reactions to one serovar.

hardjo	165
pomona	23
tarassovi	9
ballum	2
copenhageni	1

Reactions to two serovars.

hardjo & pomona	60
hardjo & tarassovi	21
hardjo & ballum	8
hardjo & copenhageni	4
pomona & tarassovi	1
pomona & ballum	1

Reactions to three serovars.

hardjo, pomona & tarassovi	9
hardjo, pomona & ballum	5
hardjo, pomona & copenhageni	4
hardjo, tarassovi & ballum	1

Reactions to four serovars.

hardjo, pomona, tarassovi & copenhageni 2

Total number of animals with leptospiral titres $\frac{316}{480}$ = 66%

Number of animals with titres to one serovar $\frac{200}{480}$ = 42%

" " " " " " two serovars $\frac{95}{480}$ = 20%

" " " " " " three " $\frac{19}{480}$ = 4%

" " " " " " four " $\frac{2}{480}$ = 0%

Table 2.6

Multiple reactions occurring in a randomly selected sample of New Zealand bovine sera showing the expected (95% CL*) and observed proportions of reactors to each serovar reacting to one or more additional serovars.

Serovar tested	95% CL for expected proportion of multiple reactions	Observed proportion of multiple reactions
hardjo	26 - 37%	43%
pomona	51 - 72%	73%
tarassovi	48 - 78%	77%
ballum	39 - 87%	89%
copenhageni	33 - 91%	91%

* 95% CL = 95% Confidence Limits

Table 2.7

Multiple reactions: mean coded titres to other serovars occurring in reactors to *hardjo*, *pomona*, *tarassovi*, *copenhageni* and *ballum* from a random sample of New Zealand cattle.

Sample	*hardjo*	*pomona*	*tarassovi*	*ballum*	*copenhageni*	n^{α}
population	1·67±0·08$^{\beta}$	0·60±0,07	0·18±0·03	0·03±0·01	0·03±0·01	480
hardjo reactors	-	0·72±0·10	0·22±0·04	0·04±0·01	0·04±0·01	290
pomona reactors	1·91±0·08$^{*\psi}$	-	0·21±0·06	0·07±0·03	0·08±0·04	85
tarassovi reactors	2·22±0·08***	0·95±0·26	-	0·04±0·03	0·05±0·08	43
ballum reactors	2·32±0·38	1·32±0·49	0·27±0·26	-	0·00±0·00	17
copenhageni reactors	3·46±0·10***	3·18±1·09*	0·32±0·24	0·00±0·00	-	11

α = sample size

β = mean ± standard error

ψ notation: * = P<0.05 significance level compared with population mean

***= P<0.001 " " " " " "

Table 2.8

The numbers and proportions of sera in two random samples of Manawatu dairy cattle conducted in winter 1975 and spring 1976 reacting to the serovars indicated (for each sample n = 480).

	hardjo		*pomona*		*tarassovi*		*copenhageni*		*ballum*	
Titre	July 1975	October 1976	July 1975	October 1976	July 1975	October 1976	July 1975	October 1976	July 1975	October 1976
0	128	115	456	435	470	426	474	460	474	448
1	74	57	3	16	7	47	5	18	5	29
2	78	73	6	8	1	5	1	3	-	3
3	81	92	6	6	-	1	-	-	2	-
4	71	67	6	4	1	1	-	-	-	-
5	33	46	1	8	-	-	-	-	-	-
6	10	23	-	2	-	-	-	-	-	-
7	2	6	1	1	-	-	-	-	-	-
8	-	1	-	-	-	-	-	-	-	-
9	3	-	-	-	-	-	-	-	-	-
> 0	$\frac{352}{480}$ =73%	$\frac{365}{480}$ =76%	$\frac{23}{480}$ =5%	$\frac{45}{480}$ =9%	$\frac{9}{480}$ =2%	$\frac{54}{480}$ =11%	$\frac{6}{480}$ =1%	$\frac{21}{480}$ =4%	$\frac{7}{480}$ =1%	$\frac{32}{480}$ =7%
$\geq 3^{\alpha}$	$\frac{200}{480}$ =42%	$\frac{234}{480}$ =49%	$\frac{14}{480}$ =3%	$\frac{21}{480}$ =4%	$\frac{1}{480}$ =0%	$\frac{2}{480}$ =0%	$\frac{0}{480}$ =0%	$\frac{0}{480}$ =0%	$\frac{2}{480}$ =0%	$\frac{0}{480}$ =0%
$\geq 4^{\beta}$	$\frac{119}{480}$ =25%	$\frac{143}{480}$ =30%	$\frac{8}{480}$ =2%	$\frac{15}{480}$ =3%	$\frac{1}{480}$ =0%	$\frac{1}{480}$ =0%	$\frac{0}{480}$ =0%	$\frac{0}{480}$ =0%	$\frac{0}{480}$ =0%	$\frac{0}{480}$ =0%
GMT$^{\psi}$	1:53	1:65	1:13	1:14	1:12	1:13	1:12	1:13	1:12	1:12

α ≥ 3 = $\geq$ 1:96
β ≥ 4 = $\geq$ 1:192
ψ GMT = geometric mean titre

Table 2.9

The numbers of animals tested in two random samples of Manawatu dairy cattle with titres to one or more serovars (n = 480 for each sample).

	Winter 1975	Spring 1976
	Reactions to one serovar	
hardjo	326	273
pomona	8	-
tarassovi	2	2
copenhageni	2	3
ballum	1	-
	Reactions to two serovars	
hardjo/pomona	10	19
hardjo/tarassovi	4	32
hardjo/ballum	5	9
hardjo/copenhageni	3	5
pomona/tarassovi	-	1
pomona/ballum	1	2
pomona/copenhageni	-	-
tarassovi/ballum	-	-
tarassovi/copenhageni	-	-
ballum/copenhageni	-	-
	Reactions to three serovars	
hardjo/pomona/tarassovi	3	1
hardjo/pomona/copenhageni	1	2
hardjo/pomona/ballum	-	5
hardjo/tarassovi/copenhageni	-	1
hardjo/tarassovi/ballum	-	3
pomona/tarassovi/ballum	-	2
	Reactions to four serovars	
hardjo/pomona/tarassovi/ballum	-	9
hardjo/pomona/copenhageni/ballum	-	3
hardjo/tarassovi/copenhageni/ballum	-	2
pomona/tarassovi/copenhageni/ballum	-	1
Total number of sera with titres	$\frac{366}{480}$ = 76%	$\frac{376}{480}$ = 78%
Reactions to one serovar	$\frac{339}{480}$ = 71%	$\frac{278}{480}$ = 58%
" " two serovars	$\frac{23}{480}$ = 5%	$\frac{68}{480}$ = 14%
" " three serovars	$\frac{4}{480}$ = 1%	$\frac{14}{480}$ = 3%
" " four serovars	$\frac{0}{480}$ = 0%	$\frac{15}{480}$ = 3%

significantly greater ($P < 0.001$) in spring 1976 (20%) when compared with winter 1975 (6%). The proportions of reactors to each serovar expected to have multiple reactions and the proportions of such reactions observed for both samples are given in Table 2.10. The proportions of multiple reactions observed in winter 1975 all lay within the expected range (95% CL) but this was not the case in spring 1976.

Table 2.10

Multiple reactions occurring in sera collected in two random samples of Manawatu dairy cattle conducted in winter 1975 and spring 1976, showing the expected (95% CL) and observed proportions of reactors to each serovar reacting to one or more additional serovars.

Winter 1975		
Serovar Tested	95% CL's for expected proportions of multiple reactors.	Observed proportions of multiple reactors
hardjo	6% - 13%	7%
pomona	53% - 91%	65%
tarassovi	37% - 96%	78%
copenhageni	30% - 98%	67%
ballum	32% - 97%	86%
Spring 1976		
Serovar Tested	95% CL's for expected proportions of multiple reactors.	Observed Proportions of multiple reactors
hardjo	17% - 27%	25%
pomona	63% - 89%	100%
tarassovi	64% - 88%	96%
copenhageni	55% - 93%	86%
ballum	60% - 91%	100%

The mean titres to each of the other serovars observed in reactors to *hardjo*, *pomona*, *tarassovi*, *copenhageni* and *ballum* are shown for both samples in Table 2.11. Significantly lower *hardjo* titres ($P < 0.05$) occurred in *pomona* and *copenhageni* reactors when compared with the mean *hardjo* titre of the winter 1975 sample, but all other mean titres in that sample were not significantly different from the population means. In the spring

Table 2.11

Manawatu dairy cattle random samples: mean coded titres to other serovars occurring in reactors to *hardjo*, *pomona*, *tarassovi*, *copenhageni* and *ballum*.

Sample	Serovar tested for multiple reaction: *hardjo*	*pomona*	*tarassovi*	*ballum*	*copenhageni*	Sample Size	Season
Population	2·13 ± 0·08[α]	0·14 ± 0·03	0·03 ± 0·01	0·02 ± 0·01	0·01 ± 0·01	480	Winter 1975
hardjo Reactors	–	0·13 ± 0·04	0·03 ± 0·01	0·02 ± 0·01	0·01 ± 0·01	352	" "
pomona Reactors	1·39 ± 0·31*[β]	–	0·17 ± 0·10	0·04 ± 0·04	0·04 ± 0·04	23	" "
tarassovi Reactors	2·44 ± 0·58	1·00 ± 0·58	–	0·00 ± 0·00	0·00 ± 0·00	9	" "
ballum Reactors	2·00 ± 0·41	0·50 ± 0·43	0·00 ± 0·60	–	0·00 ± 0·00	7	" "
copenhageni Reactors	1·17 ± 0·39*	0·50 ± 0·50	0·00 ± 0·00	0·00 ± 0·00	–	6	" "
Population	2·43 ± 0·09	0·26 ± 0·04	0·13 ± 0·02	0·07 ± 0·01	0·05 ± 0·01	480	Spring 1976
hardjo Reactors	–	0·30 ± 0·05	0·16 ± 0·02	0·08 ± 0·02	0·05 ± 0·01	365	" "
pomona Reactors	2·51 ± 0·22	–	0·36 ± 0·09**	0·53 ± 0·09***	0·13 ± 0·10	45	" "
tarassovi Reactors	3·22 ± 0·24**	0·57 ± 0·18	–	0·37 ± 0·08***	0·09 ± 0·05	54	" "
ballum Reactors	3·00 ± 0·31	1·78 ± 0·26***	0·72 ± 0·16***	–	0·33 ± 0·13*	32	" "
copenhageni Reactors	3·38 ± 0·51*	1·10 ± 0·46**	0·38 ± 0·21	0·33 ± 0·13*	–	21	" "

α = Mean ± standard error.

β = Significant differences from population means in each season are indicated by * = P < 0.05, ** = P < 0.01, *** = P < 0.001

1976 sample there were a number of significant differences. Notably, higher mean *hardjo* titres were observed in *tarassovi* ($P < 0.01$) and *copenhageni* ($P < 0.05$) reactors, higher mean *pomona* titres in *ballum* ($P < 0.001$) and *copenhageni* ($P < 0.01$) reactors and higher mean *tarassovi* titres in *ballum* reactors ($P < 0.001$) when compared with the mean *hardjo, pomona* and *tarassovi* titres occurring in the whole sample of 480 sera. There was also a significant association between *ballum* *copenhageni* titres ($P < 0.05$) in this sample.

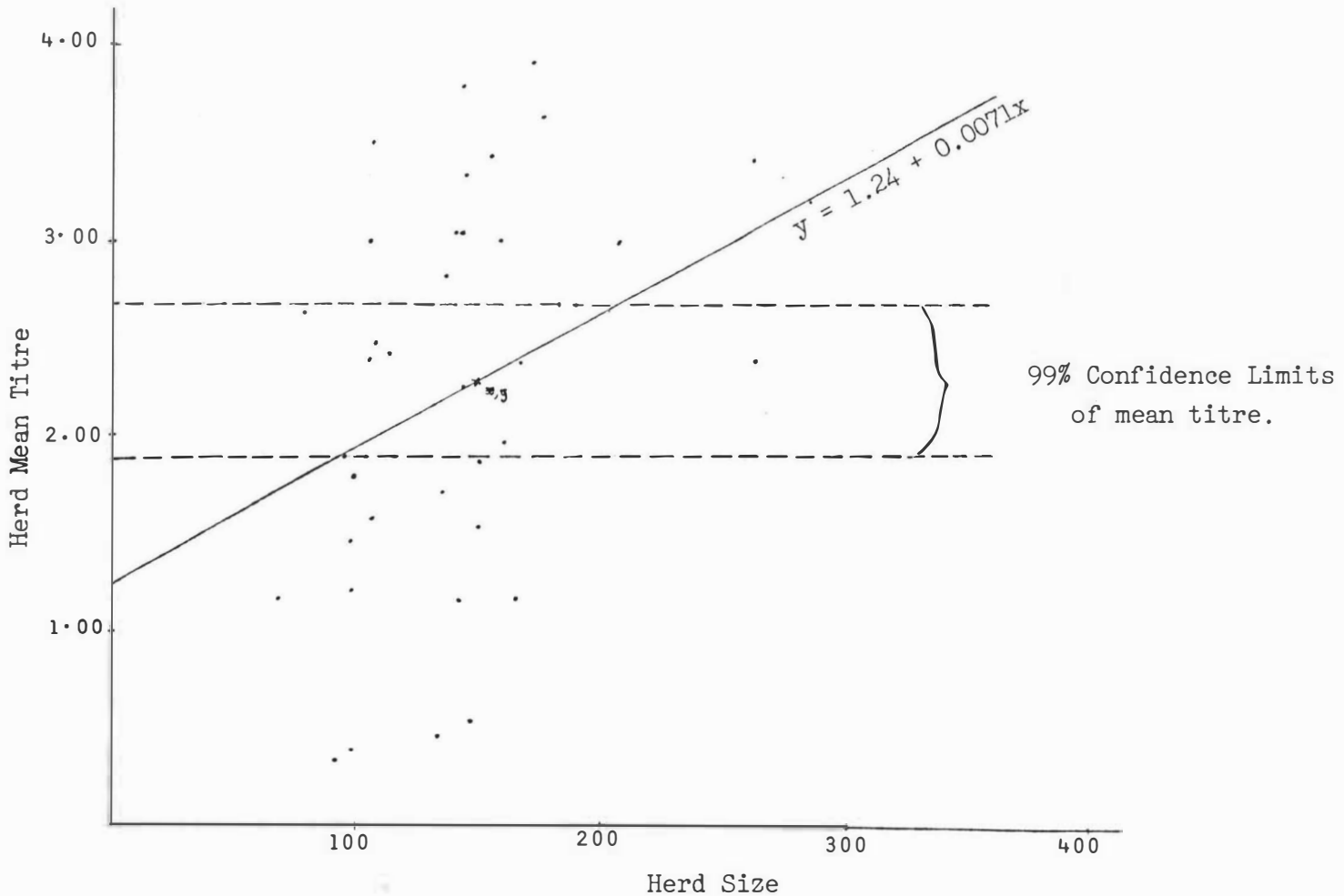
Mean titres against all serovars were significantly higher in the spring 1976 sample when compared with the one taken in the winter of 1975: *hardjo, pomona* and *copenhageni* at the 5% level of probability, *ballum* at the 1% level and *tarassovi* at the 0.1% level.

Both samples of Manawatu dairy herds had significantly more ($P < 0.01$) and significantly higher ($P < 0.001$) mean *hardjo* titres and significantly less ($P < 0.01$) and significantly lower ($P < 0.001$) mean *pomona* titres than observed in the random sample of New Zealand cattle. The mean *tarassovi* titre in the winter 1975 sample of Manawatu dairy cattle was also significantly lower ($P < 0.001$) than that of the national sample.

Each sample consisted of ten town supply and ten factory supply herds. In both there was no significant difference between the numbers of reactors or their mean titres in either class of herd. The sizes of the herds sampled ranged from 68 to 303 cows (mean 146.5 ± 13.2) in winter 1975 and 78 to 262 cows (mean 149.2 ± 11.3) in spring 1976. In both years there was no significant difference between the mean sizes of the town and factory supply herds nor were there significant differences in herd sizes between the two samples. Therefore the results from all 40 herds were pooled and the correlation between herd size and mean *hardjo* titre was calculated. The corrected correlation coefficient, $r = 0.41$, was significant ($P < 0.01$) indicating that larger herds tend to have higher mean *hardjo* titres, and that approximately 17% of the variation in

Figure III

Association Between Herd Size and Mean Titre.



mean *hardjo* titres may be explained by variation in herd size. The mean coded titres and the herd sizes are shown graphically in Figure III with the fitted regression line ($y = 1.24 + 0.0071x$). The 99% confidence limits of the mean herd titre are also shown and it can be seen that approximately one third of the herd mean titres were below these limits and approximately one third above, indicating that there are significant differences between herds.

Prevalence of leptospiral infection in Manawatu cattle sampled at an abattoir.

One hundred and thirteen cull dairy cows were available for sampling during the study period from 26th May to 6th June 1976. The *hardjo* titres of these cattle are given in Table 2.12.

Table 2.12

Numbers of cows from an abattoir survey with different *hardjo* coded titres (n=113).

Titres	0	1	2	3	4	5	6	7	8
No. of cows	22	17	31	23	10	9	0	1	0

The distribution of titres in this sample was significantly different ($P < 0.05$) from that observed in the random sample of Manawatu dairy cattle conducted in July 1975 (Table 2.8), there being relatively fewer serologically negative animals and relatively more with titres of 3 and 4 in the abattoir sample. However, the geometric mean *hardjo* titre of the animals in the abattoir sample (1:52) was not significantly different from that observed in the earlier survey (1:53).

Four groups of ten animals with coded titres of zero, one, two and three respectively, eight with a titre of four, nine with a titre of five and one with a titre of seven, making a total of 58 cows, were sampled for kidney culture and isolation. Two cows (3.4%) yielded isolates which were provisionally typed as *hardjo* by their reaction with absorbed antisera. One of these isolates was submitted to the W.H.O. Laboratory at the C.D.C. and was confirmed as *hardjo* on the basis of its reaction with absorbed antisera to all members of the *Hebdomadis* serogroup.

The isolates came from cows with titres of 1:96 and 1:192. Both isolates were obtained in pure culture in plain EMJH and EMJH plus 5FU inoculated with 1:100 dilutions of kidney homogenate but not in any tubes inoculated with the 1:10 or 1:1000 dilutions. There were 27 of 348 (8%) contaminated tubes; 21 of 116 (18%) inoculated with 1:10 dilutions of kidney homogenate, 4 (3%) of 116 with 1:100 and 2 (2%) with 1:1000. There was no significant difference between the numbers of contaminated tubes in plain EMJH or EMJH plus 5FU and in no case were more than two of the six tubes inoculated with homogenate from each kidney contaminated.

Prevalence of leptospiral titres in the Massey University herds.

Twenty-four cows were sampled at random from each of the three Massey dairy herds in spring 1975. The *hardjo* and *pomona* titres of these cows are given in Table 2.13. All cows were negative to serovars *tarassovi, ballum* and *copenhageni*.

The No. 1 and No. 4 herds had mean *hardjo* titres which were not significantly different from those observed in the random samples of Manawatu dairy herds but the No. 3 dairy herd had a significantly lower mean *hardjo* titre ($P < 0.001$). The mean *pomona* titre of the No. 1 dairy herd was significantly greater ($P < 0.001$) than that of the Manawatu sample but those of the No. 3 and No. 4 dairy herds were not.

Table 2.13

Numbers of cows in random samples from the three Massey University dairy herds with different *hardjo* and *pomona* coded tires (n = 24).

	Herd					
	No. 1 Dairy		No. 3 Dairy		No. 4 Dairy	
Titre	*hardjo*	*pomona*	*hardjo*	*pomona*	*hardjo*	*pomona*
0	-	8	5	21	-	23
1	3	5	9	-	7	-
2	9	1	5	2	5	-
3	5	6	3	1	5	1
4	4	2	2	-	4	-
5	3	2	-	-	3	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-

Discussion.

It has been suggested, by the W.H.O., that the ideal sampling method for determining the level of infection or immunity in the population of a whole country is to select individuals from all localities within that country in proportion to the population in each locality on a random basis (Anon, 1959). In this study the sample of sera studied complies with the first condition of locality representation (Table 2.1) though not in the strictest sense with the second condition of randomness. Herds were selected at random from each region for inclusion in the serum bank sample and sera from within those herds were also randomly selected. But once the herds were selected for the present study three samples were taken from each herd. This survey was undertaken to investigate the prevalence of serological titres to an infectious disease. The presence of one reactor in a herd implies an increased probability of other members within the herd also possessing titres. Thus it should be appreciated that the results of this survey may overestimate the true prevalence of leptospiral titres in New Zealand cattle. However, the results summarised in Table 2.3 indicate the widespread and common occurrence of titres to *hardjo, pomona* and *tarassovi* in this country. It is likely that the results reported in Tables 2.3 and 2.4 provide far more accurate estimates of the prevalence of titres to the five serovars tested than have been previously reported (Tables 1.1 to 1.3). The proportion of cattle with titres $\geq$ 1:200 to *hardjo* (17%) is lower than in most previous reports though the proportion with any titres (60%) agrees closely with the 56% of sera tested for export in 1972 which had titres $\geq$ 1:20 (Roach, 1973). The proportions of cattle with titres $\geq$ 1:200 against *pomona* (8%), *tarassovi* (1%), *copenhageni* (0%) and *ballum* (0%) observed in the present study are similar to those summarised in Tables 1.1 to 1.3.

Because of variation in the application and interpretation of MAT results in different countries, any comparison with the results obtained in this survey should be interpreted with caution. However, the prevalence of *hardjo* titres in the present survey appears to be similar to those reported to *hebdomadis* and

sejroe (now attributed to *hardjo*) in Florida (Galton *et al*, 1956; Alexander and Evans, 1962), Scotland, Northern Ireland (Coghlan and Norval, 1967; Ellis and Michna, 1976a) and Panama (Murnane *et al*, 1963) and to *hardjo* in Italy (Farina *et al*, 1972), New South Wales (Hoare and Claxton, 1972), Argentina and Bolivia (Anon, 1975f). They are higher than those reported to *hebdomadis* in U.S.S.R. (Lyubashenko *et al*, 1966) and Malaysia (Arunsalem, 1975) and to *hardjo* in Queensland (Spradbrow, 1964), the U.K. (Twigg, Hughes and McDiarmid, 1972) and U.S.A. (Anon, 1974e). Prevalences of *pomona* titres similar to those detected in the present study have been reported in Queensland, Peru, U.S.S.R. and U.S.A. (Spradbrow, 1964; Fernandez and Acosta, 1966; Lyubashenko *et al*, 1966; Anon, 1974e). Similar prevalences of *tarassovi* have been reported in Queensland and U.S.S.R.; of *copenhageni* titres in Panama, Scotland, U.K. and U.S.A.; and of *ballum* titres in Peru, Scotland and U.K. (Murnane *et al*, 1963; Spradbrow, 1964; Fernandez and Acosta, 1966; Lyubashenko *et al*, 1966; Twigg *et al*, 1972; Anon, 1974e; Ellis and Michna, 1976a). However, Winks (1962) reported a higher prevalence of *pomona* and *tarassovi* titres in Queensland than those detected in the present survey.

The sample was designed to estimate the national prevalences of titres to the serovars tested, rather than to assess regional differences. However, the data presented in Table 2.3 does indicate the possibility of real differences existing between some regions. Apparently higher prevalences of titres to most serovars were detected in sera from the more northern regions. There were significantly ($P < 0.01$) more *hardjo* reactors in the North Island (65%) compared with the South (39%) while *pomona* (18%) and *tarassovi* (9%) reactors were equally prevalent in both Islands. There was a non-significant ($P < 0.10$) tendency, reflecting their low overall prevalence, for *copenhageni* (2%) and *ballum* (4%) reactors to be more prevalent in the North Island.

The detection of significant differences between the mean titres of serological reactors to each serovar (Table 2.4) is in agreement with reports that animals respond to *pomona* infection with a more marked serological response than they do to *hardjo*

infection (Hodges, 1975; Anon, 1975c). The low mean titres of reactors to the other three serovars suggests the possibility that many of these titres represent low cross-reaction titres. This possibility, that most of the titres to *tarassovi, ballum* and *copenhageni* were caused by cross-reactions, is corroborated by the data presented in Tables 2.5 to 2.7. The associations of titres against *tarassovi, ballum* and *copenhageni* with higher than average *hardjo* and *pomona* titres does not prove that titres to the former three serovars are entirely due to cross-reaction.

It could be argued that causal associated factors shared by any, or all, of these serovars would equally explain the excessive occurrence of cows with multiple titres. However, only one of the 480 sera tested in the present survey had a titre $\geq$ 1:100 to either *copenhageni* or *ballum*. Also Ryan (1978) observed that certain strains of *pomona* produced high titre cross-reacticns to *copenhageni* in pigs. Consideration of these facts in conjunction with the earlier evidence for bovine infection by these two serovars in New Zealand (Chapter One) appears to establish a strong case for regarding all the titres to *ballum* and *copenhageni* in this survey as cross-reactions. The situation with *tarassovi* is less clear. Since both *tarassovi* and *pomona* occur in pigs in this country and would be expected to present a common source of bovine infection, it was expected that these titres would be associated, yet this was not so. Instead *tarassovi* titres are very strongly associated with *hardjo* titres (Table 2.7). It is suggested that *tarassovi* titres represent two populations: a population of low titres representing cross-reactions with high *hardjo* titres and a population of moderate and low titres representing true convalescent *tarassovi* reactions.

The higher prevalence and mean level of *hardjo* titres in the two Manawatu dairy cow samples (Tables 2.8 and 2.11) when compared with the random sample of New Zealand sera (Tables 2.3 and 2.7) is not unexpected. The lower figures obtained for New Zealand cattle reflect the lower prevalence of *hardjo* in the South Island and possibly a lower prevalence in beef cattle in some areas. Also the selection of 24 samples per herd may have introduced a herd bias into the Manawatu samples. The lower *pomona* and *tarassovi* titres

in the Manawatu samples may indicate an actual lower prevalence of infection with these serovars in this region. There is also strong evidence that all of the *tarassovi* titres detected in Manawatu dairy cattle may in fact represent cross-reactions.

Those Manawatu dairy cows sampled in spring 1976 had significantly higher mean titres, against all five serovars, than the winter 1975 sample, and it is likely that these increases are associated. The prevalences of multiple titres (Table 2.9) were substantially higher in the spring sample and in general exceeded their expected values (Table 2.10), suggesting that an increased amount of cross-reactivity was occurring in the sera collected in the spring. This suggestion is further strengthened by the data presented in Table 2.11. This table shows that there was a considerable increase in the degree of asssociation between titres to different serovars in the spring sample. It is likely therefore, that an increased incidence of *hardjo* and *pomona* infection in the spring accounts for the increased prevalence of titres to all five serovars. This hypothesis is in agreement with the observation that there were relatively greater proportions of higher titres to both *pomona* and *hardjo* in the spring sample.

The fact that there were no differences between the prevalence of titres to each serovar detected in town or factory supply herds nor between mean titres is interesting. The management practices applied in these two types of herds differ markedly. Town supply herds have two main calving periods each year, generally in the spring and autumn, with some movement of cows between these groups, to maintain a relatively constant level of milk production. Factory supply herds, on the other hand, have a highly seasonal calving pattern, with most cows calving in a six to eight week period in the spring following an approximately three months long period when none are milked. Town supply herds also have at least twice as many groups of young animals being reared as replacements, with a resultant greater opportunity for contact between different age groups of cattle than occurs in factory supply herds, which tend to be managed on an "all-in all-out" basis, with separate age-groups generally being reared in isolation. It would therefore seem likely that there is a greater opportunity for transmission

of infection within town supply herds, favouring the establishment of endemic leptospirosis. Yet the absence of a difference between the prevalence of infection in these two types of herds indicates that similar causal associated factors are acting in both.

The significant association between herd mean titre and herd size (Figure III), supports the impression of Jamieson *et al* (1970), Christmas *et al* (1973) and Brockie (1974b)that the large dairy herds which occur in this country are one reason for the high prevalence of bovine leptospirosis observed. It is likely that there is an increased opportunity for transmission of infection in larger herds.

A large number of herds in the present survey had mean titres which lay outside the 99% confidence limits for herd mean titre (Figure III). This indicates that there are real differences between individual herds which require further investigation to add to the knowledge of the epidemiology of bovine leptospirosis in the Manawatu.

The significant difference in the distribution of *hardjo* in the abattoir samples of dairy cows, when compared with Manawatu dairy cattle, in the absence of a significant difference between mean titres, may reflect the age structure of this sample which contained few young cows. The prevalence of convalescent titres would be expected to increase with age, providing the majority of cows retain these titres for life.

The recovery of two *hardjo* isolates from the 58 cows sampled (3.4%) agrees with the report of Lyubashenko *et al* (1966), who reported that 2% to 5% of those cattle, with titres to a variety of serovars, were shedding leptospires. Marshall (1976), in a cultural survey of two-year-old beef cattle, obtained a much higher proportion of *hardjo* isolates (47%) though these animals had probably been infected much more recently than the cattle sampled in the present survey.

The two isolates were obtained from cows with titres which,

on normally accepted criteria, would be considered to have no diagnostic significance, and which were possibly of many months or even years duration. Their recovery indicates the probability that a proportion of adult cattle may carry leptospires, and constitute a reservoir, from which new susceptibles brought into the herd may become infected. The combined evidence of the present survey, and that of Marshall (1976), suggests that either a small proportion of cows remain infected with *hardjo* for a much greater period than their peers, or that some cows may become reinfected in later life. Both these possibilities were investigated in the course of the present study. However, it can be argued that the source of the infection detected in adult cows is of considerably less epidemiological significance than the fact that adult cow infection exists.

The question of the sensitivity of the cultural methods used in this survey is difficult to resolve. It is considered that the contamination rate was so low that it is unlikely that isolates were missed for this reason. Since only two isolates were obtained, and these in both media, it is not possible to assess the superiority of either. Similarly, though both isolates were obtained from only the 1:100 dilutions of kidney homogenate, there is insufficient evidence from this study to exclude either the 1:10 or 1:1000 dilutions from further surveys. Ryan (1978), using similar isolation techniques for a cultural survey of *pomona* infection in porcine kidneys, concluded that the 1:1000 dilution could be omitted without a loss of sensitivity, but Hathaway (1978), working with possum kidneys infected with *balcanica,* obtained some isolates from the 1:1000 dilution only. The experiences of these two workers and those of Marshall (1976), when considered in association with the facts discussed above, indicate that the sensitivity of the technique used is sufficiently great to suggest that the observed prevalence of leptospiral kidney infection in adult cows is not substantially different from the true prevalence.

The results obtained from random samples obtained from the three Massey dairy herds (Table 2.13) indicated that both the No. 1 and No. 4 herds had prevalences of *hardjo* titres similar to the average occurring in the Manawatu, though the prevalence of *pomona*

titres was significantly greater in the No. 1 herd. Since the No. 1 Dairy Farm is a town supply herd, with an associated piggery, it was decided to investigate in greater detail the epidemiology of leptospirosis on this unit.

Summary.

1. In a random sample of sera from beef and dairy cattle selected to allow equal representation from all regions of New Zealand, 81% of herds and 60% of animals had *hardjo* titres, 36% and 18% *pomona* titres, 21% and 9% *tarassovi* titres, 6% and 2% *copenhageni* titres and 10% and 4% *ballum* titres $\geq$ 1:17.
2. These prevalences are equivalent to or greater than those reported from most other parts of the world.
3. Significantly more North Island cattle had *hardjo* titres, but there was no difference in the prevalence of both *pomona* and *tarassovi* titres in either the North or South Islands.
4. Evidence is presented that most *ballum* and *copenhageni* titres, and probably a proportion of *tarassovi* titres, represent cross-reactions occurring in sera with high titres against either *hardjo* or *pomona*.
5. In two random samples of Manawatu dairy cattle an average of 75% had *hardjo* titres, 8% *pomona* titres, 7% *tarassovi* titres, 6% *copenhageni* titres and 4% *ballum* titres $\geq$ 1:17. The prevalences of *hardjo* and *pomona* titres were significantly different from those observed in the national sample.
6. The prevalence, in Manawatu dairy cattle, of titres $\geq$ 1:17 against all five of these serovars was higher in spring 1976 than in winter 1975. It was considered that this was due to an increased incidence of *hardjo* and *pomona* infection with an accompanying rise in cross-reactions to *tarassovi*, *copenhageni* and *ballum*.
7. In spite of major differences in the management of town and factory supply herds, there was no difference between the prevalence of leptospiral titres in these types of herds.
8. There was a significant trend ($P < 0.01$) for larger herds to have higher mean *hardjo* titres.
9. In a cultural survey in the Manawatu, *hardjo* isolates were obtained from 2 of 58 (3.4%) cull dairy cows. These cows had low, presumably long-standing, titres.

10. A comparison between samples obtained from the three Massey University dairy herds and the Manawatu dairy cow samples indicated the suitability of the No. 1 dairy herd for a detailed investigation into the epidemiology of bovine leptospriosis.

CHAPTER THREE

SEROLOGICAL OBSERVATIONS IN THE NEONATE.

Having established that there was a high prevalence of *hardjo* MAT titres in the Massey University No. 1 dairy herd, comparable with other surveyed herds in the Manawatu region, a long-term surveillance of titres in various classes of cattle in the herd was undertaken. The observations made during a two and a half year period of surveillance are presented in Chapters Three, Four and Five. Each chapter covers the serological observations made in different age groupings of cattle; Chapter Three is concerned with neonates, Chapter Four with young adults and Chapter Five deals with adult animals.

Introduction.

Calves which have experienced no antigenic stimulation *in utero* are born virtually agammaglobulinaemic and passively acquire immunoglobulins from their dams' colostrum (Butler, 1969; Brambell, 1970). Absorption of the colostral immunoglobulins ingested by the calf is restricted to the first 24 to 48 hours of life (Lascelles, 1963; Kruse, 1970b). These immunoglobulins appear in the serum within one to three hours of the first meal of colostrum, and reach a peak level from six to twenty-four hours later (Butler, 1969; Husband *et al*, 1972). This peak level declines logarithmically with a half-life of approximately 20 days, but increasing production of autogenous immunoglobulins ensures that relatively constant serum levels are established by three to six weeks of age (Brambell, 1970; Husband *et al*, 1972; Porter, 1972). Absorbed immunoglobulin has been reported to remain detectable in serum for up to 67 days (Butler, 1969). However, passively acquired antibodies against *Brucella abortus* have been shown to persist in calves for up to six months (McDiarmid, 1946) and against rinderpest persistance for up to 10.9 months has been calculated (Brown, 1958). Passively acquired antibody titres in calves will sometimes exceed those of their dams but very rarely those of colostrum (Brambell, 1970).

Many factors influence the level of immunoglobulins obtained by calves including their age at first feeding, the length of time

for which they suckle, the volume of colostrum ingested, the colostrum immunoglobulin concentration, calf weight, season, calving management and breed (Butler, 1969; Kruse, 1970a; 1970b; Selman *et al*, 1970; 1971). An analysis of the total variation between individual calf immunoglobulin levels demonstrated that 55% could be accounted for by variation in the actual mass of immunoglobulin fed, a further 10 to 15% by variation in the age of the calf at first feeding and 2 to 3% by variation in the birth weight of the calf (Kruse, 1970b). This still leaves a residual unexplained variation of approximately 30%. Computer predictions based on this and other data suggest that a theoretical 10% of new-born calves will be hypogammaglobulinaemic even after receiving a colostrum meal (Kruse, 1970c). This figure is in close agreement with experimental observations and survey reports (Klaus *et al*, 1969; Selman *et al*, 1970; 1971; Bailey and McLean, 1972; McGuire *et al*, 1976).

The passive acquisition of agglutinins against leptospirae was first documented by van der Hoeden (1955), in calves born to dams experiencing a natural outbreak of *canicola* infection. This observation has been confirmed in calves born to vaccinated, and naturally, or experimentally infected dams (Fennestad and Borg-Petersen, 1956; McDonald and Rudge, 1957; Kiesel and Dacres, 1959; Hanson *et al*, 1964). Similar observations have also been reported for horses, pigs and mice (Bryans,1955; Chaudhary *et al*,1966; Kemenes and Szecky, 1966; Mitchell *et al*, 1966; Birnbaum *et al*, 1972).

Calves born to dams with antileptospiral titres are seronegative before suckling (Bohl *et al*, 1954; Fennestad and Borg-Petersen, 1956; Cacchione *et al*, 1968; Saski and Arima, 1971; Ellis and Michna, 1977). After suckling they rapidly develop titres which reach peak levels by 48 hours (Fennestad and Borg-Petersen, 1956). Peak antileptospiral titres in the newborn calf are generally equivalent to, or greater than, maternal serum titres though both are lower than colostral titres (Fennestad and Borg-Petersen, 1956; Hanson *et al*, 1964; Mitchell *et al*, 1966). Titres decline logarithmically from birth and are reported to persist for two to three months in horses, pigs and mice (Bryans,

1955; Chaudhary *et al*, 1966; Mitchell *et al*, 1966; Birnbaum *et al*, 1972) and three to six months in cattle (Fennestad and Borg-Petersen, 1956; McDonald and Rudge, 1957; Hanson *et al*, 1964; Mitchell *et al*, 1966).

These agglutinating titres appear to be associated with protection. Cook (1964) claimed that vaccination of sows with a *pomona* bacterin reduced perinatal losses of piglets caused by *pomona*. Mitchell *et al* (1966) observed that piglets with passively acquired titres against *pomona* failed to become infected, even though their in-contact dams were experiencing leptospiruria, while Chaudhary *et al* (1966) found that passively-acquired antibody protected piglets against experimental challenge with *pomona*. Rudge (1956) demonstrated that the passive administration of an antiserum against *pomona* gave calves substantial protection against challenge with that serovar. Passively-acquired agglutinating titres to *pomona* in calves born to vaccinated dams protected them against *pomona* challenge (McDonald and Rudge, 1957; Kiesel and Dacres, 1959). Hanson *et al* (1964) noted that calves possessing colostral titres against *hardjo* and *pomona* did not become infected with these serovars, even though they were grazing with their actively-infected dams, and Farina *et al* (1972) found that calves born to *hardjo*-infected dams obtained antibody against this serovar from colostrum and were protected against experimental challenge with *hardjo* for two to two and a half months. Birnbaum *et al* (1972) could not experimentally infect mice born to *grippotyphosa*-infected dams, even though controls born to non-infected dams were fully susceptible. They attributed this result to the passive acquisition of immunity, *in utero* or via colostrum, but their experimental design did not rule out the possibility that these mice were protected by actively-acquired immunity. Actively-acquired titres have been demonstrated in calves following experimental or natural infection *in utero* (Fennestad and Borg-Petersen, 1962; Kirkbride *et al*, 1977).

Hanson (1977) has also stated that passively-acquired immunity in calves will cause a poor response to vaccination. Gillespie and Kenzy (1958) failed to produce a vaccinal response in a calf which had a titre which may well have been passively-

acquired. They also reported a generally poor antibody response to a vaccine used in calves less than three months old when compared to that of calves six to eight months old, a fact which may have been due to low levels of passive immunity in the younger calves.

Aims of this Investigation.

A series of serum samples were taken from calves born each spring and autumn from spring 1975 to autumn 1977. In some instances their dams were also sampled with the aim of answering the following questions:

1. Do new-born calves in a herd with a high prevalence of adult serological reactors have antileptospiral antibody?
2. Is this antibody acquired actively or passively?
3. What is the titre of this antibody and how long does it remain?
4. What is the significance of some specific factors on the passive acquisition of antibody by neonates; namely maternal titre, colostral titre, suckling time, calf weight and age of dam?

Materials and Methods.

The Herd.

The herd consists of approximately 200 Friesian cows of which 100 to 130 are lactating at any given time. Cows calve during two periods each year: spring (July to September) and summer/autumn (January to May). The length of lactation and length of dry period are flexible to meet the commercial requirements of a milk production quota and some animals change from the spring to the autumn calving groups each year and vice versa. Mating is either by natural service or artificial insemination, the latter method being used on the bulk of adult animals while most maiden animals are mated naturally. Teaser bulls are run with the milking herd during each mating period to aid in the detection of cows in oestrus.

The herd is basically run as a commercial unit which is also used for teaching purposes. Some grazing experiments are run from time to time which result in the herd being divided into sub-groups for management by different grazing practices. All animals

are run at pasture on the main farm throughout the year and calves are born outdoors. Some groups of younger animals are sent to other properties when pastures cannot cope with feeding all age groups.

Calves are reared in pens for the first three to four weeks of life. They are then run in small groups on pasture while still being fed milk until the whole calf crop from one calving period is formed into a single group. Each group is then run at pasture in isolation from other animals for approximately six months. After this time mixing with other cattle six to eighteen months older may occur as regrouping takes place on basis of body weight. These animals are generally mated at between fifteen and eighteen months of age and calve down into the herd at 24 to 27 months of age.

Sampling Procedure.

Calves born at each calving period were sampled at regular intervals ranging from weekly to every three months. A more detailed study in the spring of 1976 and the autumn of 1977 was conducted on a sample of approximately 50% of calf/dam pairs. Blood samples were taken from each calf when its dam was brought in from the calving paddock for the first milking. At this time the calf was separated from its dam and penned without feeding for a further 36 hours before being fed whole milk, which generally consisted of pooled colostrum from freshly calved cows. The calf's birth date, weight and sampling dates were recorded.

During the more detailed study both calf and dam were bled when the dam was brought in for her first milking and a 40ml sample of colostrum was collected from a proportion of dams. In these cases the calf was weighed and the period for which the calf had been suckled was recorded. Cows were milked into individual buckets and the colostrum sample was taken after mixing the contents of the bucket.

Two groups, each of ten dams, were selected from the spring 1976 and autumn 1977 calving groups and kept under close observation until they calved. Presuckling blood samples were taken from their calves, in addition to the range of samples described above.

Sample Processing.

All blood samples were collected into 10ml, evacuated, rubber-stoppered glass tubes (Vacutainer) using 20 guage, 25mm, double-ended needles (Vacutainer). Cows were bled from the middle coccygeal vein and calves from the jugular vein until three months old and thereafter from the middle coccygeal vein. Blood samples were left to clot at room temperature for 24 hours, the clot removed and the serum centrifuged at 2000G for five minutes. Serum samples were decanted into sterile bijoux bottles and stored at -20°C. Aliquots were removed before freezing for serological examination.

Colostrum samples were held at 4°C for 12 hours before being centrifuged at 3000G for 15 minutes to remove milk fat and cellular material. Aliquots were then taken for serological examination before the colostrum was decanted into screw-capped, 20ml, glass containers and stored at -20°C.

Serological Examination.

Sera and colostral samples were subjected to serological examination against serovars *hardjo, pomona, copenhageni, ballum* and *tarassovi* as described in Chapter Two.

Cellulose-Acetate Electrophoresis.

Serum samples from a number of calves with suspected hypogammaglobulinaemia following suckling, and from a number of calves thought to have normal levels of serum immunoglobulins, were tested by cellulose-acetate electrophoresis. Serum samples were tested using cellulose-acetate strips (Titan Zipzone, Helena Laboratories) and analyses were run in Sodium Barbitone buffer, pH 6.8 (Appendix III) and read at 500nm in a densitometer (Atago).

Statistical Methods.

The statistical procedures applied to test observations are given in Appendix II.

Results.

1. *Prevalence of antileptospiral antibody titres in new-born calves.*

Titres were detected to *hardjo* and *pomona* but not to

copenhageni, tarassovi or *ballum* in new-born calves tested over four consecutive calving periods (Table 3.1). Over the first two calving periods a large proportion of animals tested were three to six weeks old when first tested and hence the mean ages at sampling were 29 and 35 days respectively but during the last two calving periods most calves were sampled from one to three days after birth. Overall 109 of 136 calves tested (80%) were seropositive to *hardjo* and 33 (24%) to *pomona*.

Table 3.1

Prevalence of MAT titres ≧ 1:24 to *hardjo* and *pomona* in new-born calves.

Calving Period	No. calves Sampled	No. calves seropositive *hardjo*	No. calves seropositive *pomona*	% calves seropositive *hardjo*	% calves seropositive *pomona*	Mean age at Sampling(days)
26/7/75 -16/9/75	38	31	17	82%	45%	29
16/2/76 -20/4/76	17	9	0	53%	0%	35
30/7/76 -21/9/76	38	34	14	89%	37%	2
26/12/76 -4/5/77	43	35	2	81%	5%	5
Total	136	109	33	80%	24%	14

2. *Magnitude and duration of titres in new-born calves.*

Mean coded titres, coded standard deviations of the means, geometric mean titres and mean ages at sampling for the four calving periods are presented in Table 3.2. These statistics refer to all members of each calving group, including the seronegatives. Individual titres at first sampling varied considerably ranging from less that 1:24 to 1:3072.

All *hardjo* titres for those calves which were sequentially tested and their ages when sampled are given in Tables 3.3, 3.4, 3.5 and 3.6. In every case titres declined as the calves grew older and the periods for which the titres persisted were strongly correlated with the titre at the commencement of the period

($r = 0.85$, $r = 0.75$, $r = 0.76$ for spring 1975, spring 1976 and autumn 1977 respectively, $P < 0.001$ in each case). Plots of titre versus duration in days with their fitted regression lines and 99% confidence limits are shown for each set of observations in Figures IV, V and VI. The coefficients calculated for the three regression lines are not significantly different ($P > 0.4$).

Table 3.2

Mean *hardjo* and *pomona* MAT titres at initial test in new-born calves.

Calving period	No. calves sampled	Mean coded titre *hardjo*	Mean coded titre *pomona*	S.E.* coded MAT *hardjo*	S.E.* coded MAT *pomona*	Geometric mean titre *hardjo*	Geometric mean titre *pomona*	Mean age (days)
26/7/75 -16/9/75	38	3.76	0.94	0.39	0.21	1:163	1:23	29
16/2/76 -20/4/76	17	0.79	0	0.17	N.A.+	1:21	0	35
30/7/76 -21/9/76	38	4.05	1.05	0.18	0.27	1:199	1:25	2
26/12/76 -4/5/77	43	3.52	0.14	0.35	0.10	1:138	1:13	5

* S.E. = standard error of the mean.

\+ N.A. = not applicable

A histogram showing the combined proportion of calves which were still sero-positive (titre $\geq$ 1:17) at various times after birth is presented in Figure VII. This shows that 75% of calves were still sero-positive at 50 days of age, 50% by 100 to 110 days of age and 25% by 130 to 140 days of age. No calves more than 190 days old still had detectable titres.

Pomona titres were generally lower than *hardjo* titres and persisted for shorter periods; none of the calves had a *pomona* titre of $\geq$ 1:24 more than 80 days after birth.

3. *Effect of first suckling on appearance of MAT titres in new-born calves.*

Ten calves were sampled immediately following birth, but prior

Table 3.3

1975 spring calves: coded *hardjo* titres at various ages.

Calf Identification	Birth Date	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)
32M	26/7/75	0	42	0	74	0	107	0	130	-	α	-	
33M	31/7/75	4	37	3	67	2	100	1	123	0	176	-	
33	23/7/75	2	45	1	77	0	110	0	133	0	186	0	218
34	26/7/75	2	42	2	74	0	107	0	130	0	183	0	215
35	26/7/75	4	42	3	74	2	107	0	130	0	183	0	215
36	28/7/75	5	40	3	72	1	105	0	128	0	181	0	213
37	30/7/75	2	38	1	70	0	103	0	126	0	179	0	211
38	1/8/75	5	36	4	68	2	101	2	124	0	177	0	209
39	3/8/75	5	34	3	66	1	99	0	122	0	175	0	207
40	5/8/75	3	32	-		1	97	0	120	0	173	0	205
41	6/8/75	4	31	2	63	1	96	1	119	0	172	0	204
42	6/8/75	0	31	0	63	0	96	0	119	0	172	0	204
43	7/8/75	2	30	½	62	0	95	0	118	-		-	
45	9/8/75	2	28	0	60	0	93	0	116	0	169	0	201
46	10/8/75	6	27	4	59	½	92	½	115	0	168	0	200
47	10/8/75	0	27	0	59	0	92	0	115	0	168	0	200
48	11/8/75	2	26	½	58	0	91	0	114	0	167	0	199
49	12/8/75	3	25	2	57	3	90	0	113	0	166	0	198
50	14/8/75	6	23	-		3	88	1	111	0	164	0	196
51	15/8/75	6	22	5	54	3	87	1	110	0	163	0	195
52	17/8/75	3	20	4	52	2	85	1	108	0	161	0	193
53	19/8/75	5	18	3	50	2	83	1	106	0	159	0	191
54	28/8/75	7	9	5	41	3	74	3	97	1	150	0	182
55	29/8/75	7	8	7	40	5	73	4	96	½	149	0	181
56	31/8/75	7	6	6	38	4	71	3	94	1	147	0	178
57	4/9/75	8	2	6	34	5	67	2	90	1	143	0	175
58	7/9/75	4	31	3	64	2	87	0	140	0	172	-	
59	8/9/75	0	30	0	63	0	86	0	139	0	171	-	
60	9/9/75	0	29	0	62	0	85	0	138	0	169	-	
61	13/9/75	6	25	3	58	1	81	0	134	0	165	-	
62	17/9/75	6	21	4	54	3	77	1	130	0	161	0	191
63	16/9/75	5	22	4	55	3	78	½	131	0	162	0	192

α - = not tested.

Figure IV

Persistence of Colostral Titres in Spring 1975 Calves.

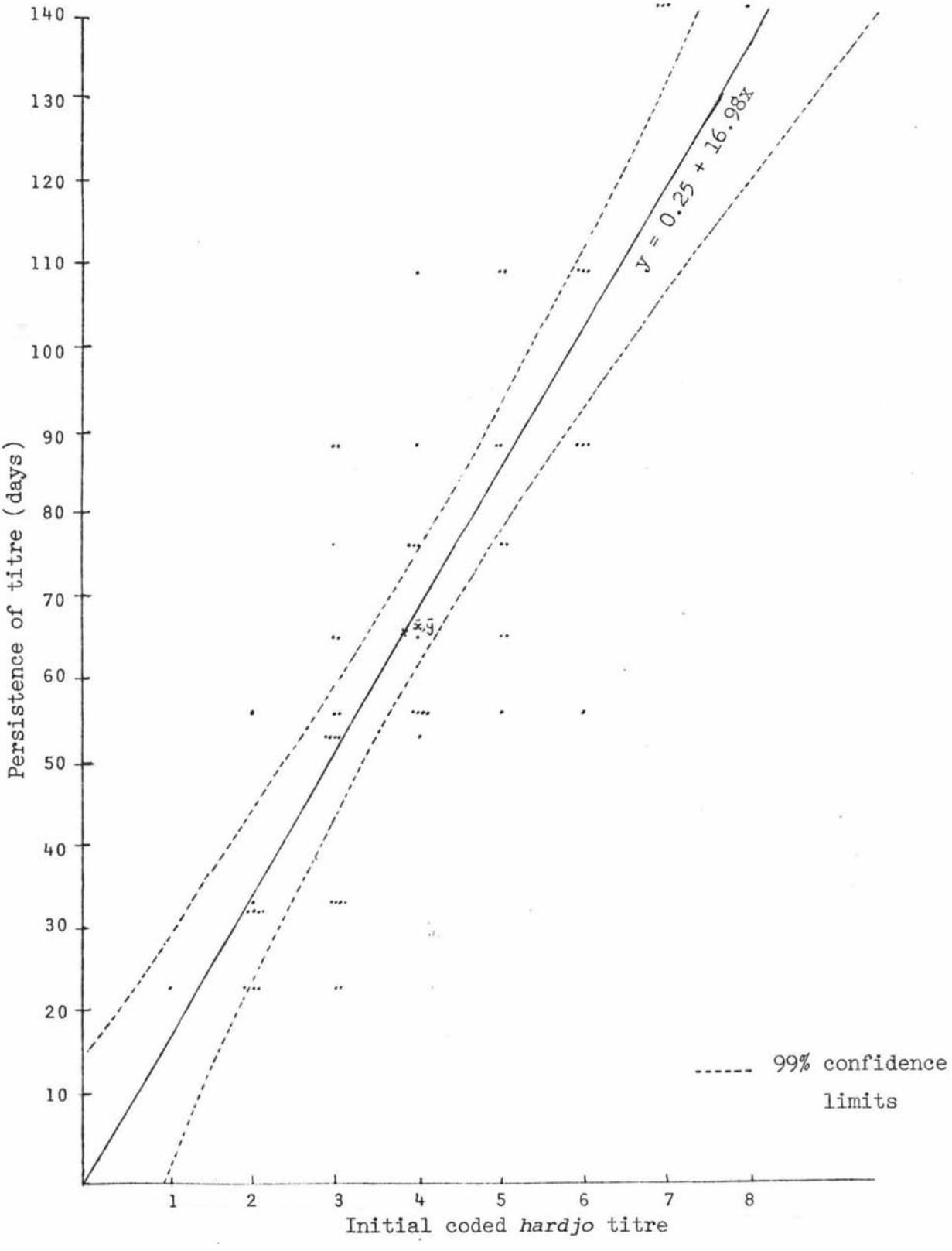


Table 3.4

1976 autumn calves: coded *hardjo* titres at various ages.

Calf Identification	Birth Date	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)
6	10/3/76	0	47	0	84	0	141
7	11/3/76	1	46	½	83	0	140
8	12/3/76	0	45	½	82	0	139
9	13/3/76	0	44	0	81	0	138
10	14/3/76	0	43	0	80	0	137
11	14/3/76	1½	43	½	80	0	137
12	14/3/76	2	43	1	80	0	137
13	20/3/76	1	37	½	74	0	131
14	21/3/76	2	36	0	73	0	130
15	23/3/76	0	34	0	71	0	128
16	25/3/76	0	32	0	69	0	126
17	29/3/76	1	28	0	65	0	122
20	16/4/76	2	10	½	47	0	94
21	17/4/76	3	9	1	46	0	93
22	20/4/76	0	6	0	43	0	90

Table 3.5

1976 spring calves: coded *hardjo* titres at various ages.

Calf Identification	Birth Date	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)
23	29/7/76	6	1	4	28	3	47	2	76	½	110	0	144		
25	5/8/76	2	4	2	21	1	41	1½	69	0	103	0	134		
27	8/8/76	3	1	3	18	2	38	2	66	½	100	0	131		
28	7/8/76	5	2	4	19	2	39	½	67	0	101	0	132		
29	8/8/76	4	1	3	18	1	38	0	66	0	100	0	131		
0F	8/8/76	5	1	3	22	1	66	0	100	0	131	0	171		
0J	10/8/76	4	4	3	16	1	63	0	97	0	128	0	168		
30	10/8/76	1	4	0	20	0	36	0	64	0	98	0	129		
31	11/8/76	4	3	3	19	2	35	0	63	0	97	0	128		
32	11/8/76	0	3	0	19	0	35	0	63	0	97	0	128		
34	13/8/76	2	12	1	33	1	61	0	95	0	126	0	166		
35	22/8/76	6	3	4	24	2	52	1	86	1	117	0	157		
36	21/8/76	5	4	4	25	3	53	1	87	0	118	0	158		
37	24/8/76	7	1	6	22	6	50	3	84	2	115	1	155	0	195
38	27/8/76	3	3	4	19	3	47	0	81	0	112	0	152		
39	1/9/76	4	1	4	14	3	42	½	76	0	107	0	147		
40	4/9/76	5	1	5	11	2½	40	2	74	-		0	135		
41	4/9/76	6	1	4	23	4	40	2	74	1	105	0	135		
42	7/9/76	5	1	5	20	4	36	1	70	1	101	0	131		
43	9/9/76	5	1	4	18	3	34	½	67	0	98	0	128		

Figure V

Persistence of Colostral Titres in Spring 1976 Calves.

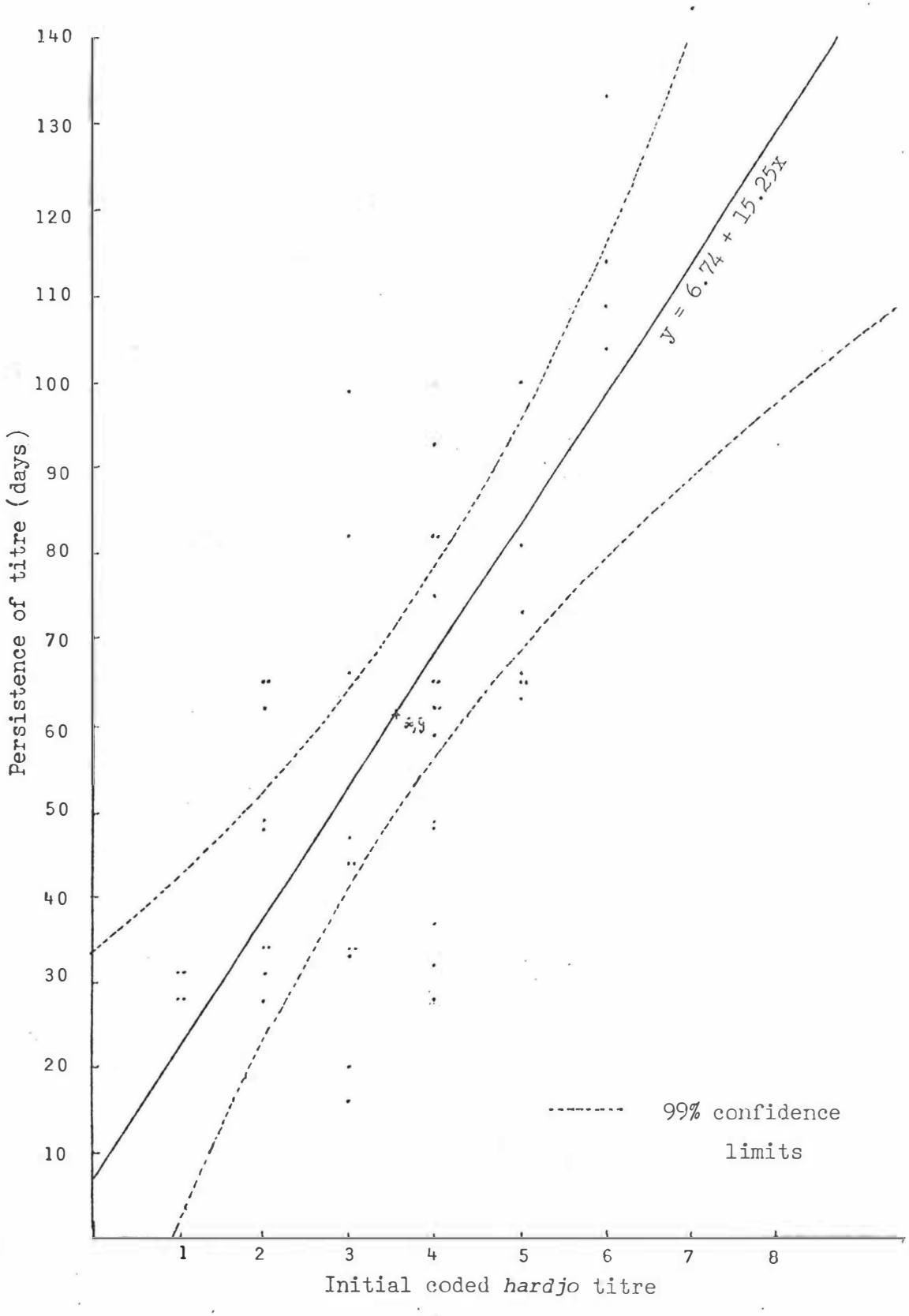


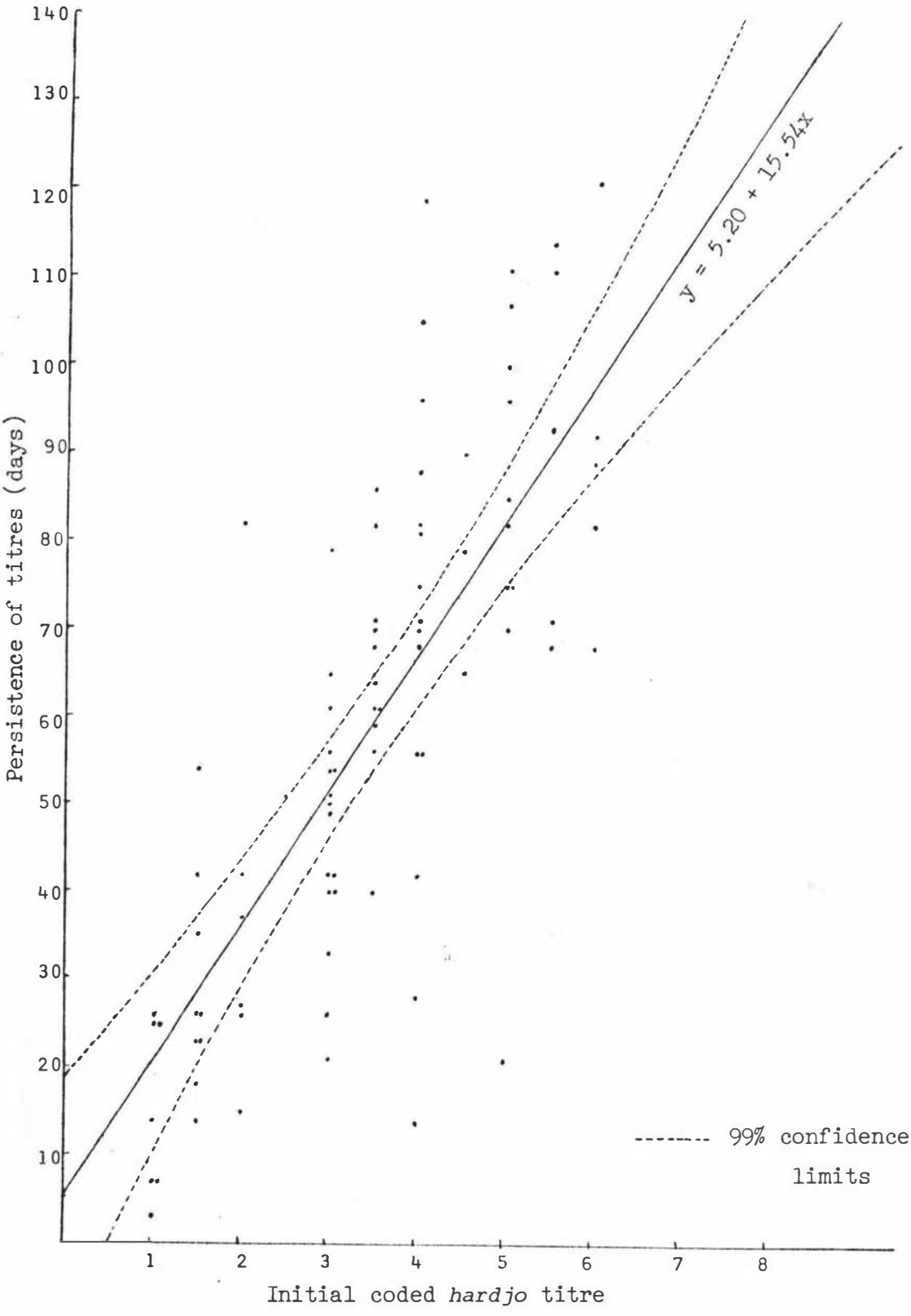
Table 3.6

1977 autumn calves: coded *hardjo* titres at various ages.

Calf Identification	Birth Date	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)	Titre	Age (days)
W3	26/2/77	0	2	0	11	0	53	0	76	0	118	0	144	0	188										
W4	28/2/77	5	0	4½	10	3	52	2	75	0	117	0	143	0	187										
W5	1/3/77	0	1	0	15	0	50	0	73	0	115	0	141	0	185										
W6	1/3/77	2	1	½	15	0	50	0	73	0	115	0	141	0	185										
W7	28/2/77	3	2	1½	16	½	51	0	74	0	116	0	142	0	186										
W8	3/3/77	3½	6	1½	48	½	71	0	113	0	139	0	183	-											
W9	5/3/77	5	4	4	23	3½	46	2	69	½	111	0	137	0	181										
W10	7/3/77	4	2	3	21	2	44	1	71	0	113	0	139	0	183										
W11	10/3/77	3½	6	2	27	1½	41	1	64	0	106	0	136	0	180										
W12	23/3/77	4	5	4	14	4	23	3½	37	3½	51	1½	93	½	119	0	163								
W13	26/3/77	4½	2	4½	11	4	20	4	34	3	48	½	90	0	116	0	160								
W14	31/3/77	0	1	0	16	0	30	0	44	0	86	0	112	0	156										
W15	31/3/77	5½	1	5	15	2	29	4	43	2	85	½	111	0	155										
W16	3/4/77	6	1	5	12	4	26	4	40	1½	82	0	108	0	152										
W17	4/4/77	4	1	3½	11	3	25	1½	39	1	81	0	107	0	151										
VG1	13/4/77	6	2	5½	9	5	16	5	23	5½	30	3½	37	3	44	3	58	2½	72	1	98	½	123	0	150
VG2	15/4/77	3	0	1	7	1	14	1½	21	0	28	0	35	0	42	0	56	0	70	0	96	0	121	0	148
VG3	17/4/77	6	2	6	5	5	12	5	19	5½	26	3½	33	3	40	3	54	3	68	1	94	0	119	0	146
VG4	19/4/77	0	0	0	3	0	10	0	17	0	24	0	31	0	38	0	52	0	66	0	92	0	117	0	144
VG5	22/4/77	4	7	5	14	4	21	1	28	½	35	0	49	0	63	0	89	0	114	0	141				
VG6	24/4/77	0	2	0	5	0	12	0	19	0	26	0	33	0	47	0	61	0	87	0	112	0	139		
VG7	25/4/77	5	1	4	4	4	11	4	18	3	25	1½	32	3	46	1	60	½	86	0	111	0	138		
VG8	26/4/77	3½	0	3½	3	3	10	3	17	1	24	1½	31	1½	45	1	59	0	85	0	110	0	137		
VG9	24/4/77	1	2	1½	5	0	12	0	19	0	26	0	33	0	47	0	61	0	87	0	112	0	139		
VG10	8/5/77	5½	2	6	5	3½	12	3	19	3½	33	1½	47	½	73	0	98	0	125						

Figure VI

Persistence of Colostral Titres in Autumn 1977 Calves.



to first suckling, in spring 1976 and again in autumn 1977. Table 3.7 shows MAT titres, before and after suckling, for the calves in these two groups. Prior to suckling no calves had detectable titres at a dilution of 1:24 but after suckling nine of the ten calves, in spring 1976, and eight of the ten calves, in autumn 1977, had developed titres. The calf born to cow 28 on 22/4/77 had not suckled when it was taken from its dam and bled prior to being fed two litres of its dam's colostrum through an artificial teat. It was sero-negative prior to artificial feeding but developed a titre of 1:384 following ingestion of the artificially-fed colostrum.

4. Factors influencing the acquisition of MAT titres by new-born calves.

Thirty-eight calves were sampled within a few days of birth during spring 1976; 34 had titres of 1:24 or greater and four were sero-negative. The geometric mean titre (GMT) of the sero-positives was 1:277 with 95% confidence limits (95% CL) of 1:183 - 1:419 and for all 38 calves the GMT was 1:199 (1:123 - 1:323, 95% CL). Forty-one calves were sampled within a few days of birth during autumn 1977 and of these 33 had titres of 1:24 or greater. The GMT of the sero-positives was 1:302 (1:208 - 1:438, 95% CL) and of all 41 calves 1:161 (1:98 - 1:264, 95% CL). Detailed information for both groups is given in Tables 3.8 and 3.9.

A total of 12 calves were sero-negative after suckling, four born in spring 1976 and eight in autumn 1977. Five of these were born to sero-negative dams (43, 116, 120, 147 and 158) and seven failed to develop titres even though born to, and apparently suckling sero-positive dams (70, 89, 111, 139, 146, 178 and 193). Cellulose-acetate strip electrophoresis carried out on sera from these seven calves showed six to be deficient in gammaglobulins and the seventh, born to dam 178, to have a level of 11.50g/l, equivalent to the bottom of the normal range of 11.22 - 36.20 g/l which is the 99% CL range based on the data of Williams *et al* (1975). These levels are given in Table 3.10 along with those of four positive control sera (from calves born to dams 134, 160, 171 and 173) which had titres following suckling. The sera from positive controls had levels of gammaglobulins which lay within the normal range.

Table 3.7

Coded *hardjo* titres in new-born calves before and after suckling.

Spring 1976

Dam	Birthdate	Presuckle titre	Postsuckle titre
30	1/9/76	0	5
93	1/9/76	0	4
219	3/9/76	0	4
165	4/9/76	0	5
124	4/9/76	0	6
95	6/9/76	0	2
106	7/9/76	0	2
1	9/9/76	0	5
120	9/9/76	0	0
223	21/9/76	0	6

Autumn 1977

Dam	Birthdate	Presuckle titre	Postsuckle titre
139	3/3/77	0	0
72	9/3/77	0	5
115	5/4/77	0	6
121	5/4/77	0	8
124	6/4/77	0	6½
26	7/4/77	0	3
147	19/4/77	0	0
28	22/4/77	0	5
207	24/4/77	0	1
172	25/4/77	0	5

Figure VII

Proportion of Calves with Colostral Titres at Various Times After Birth.

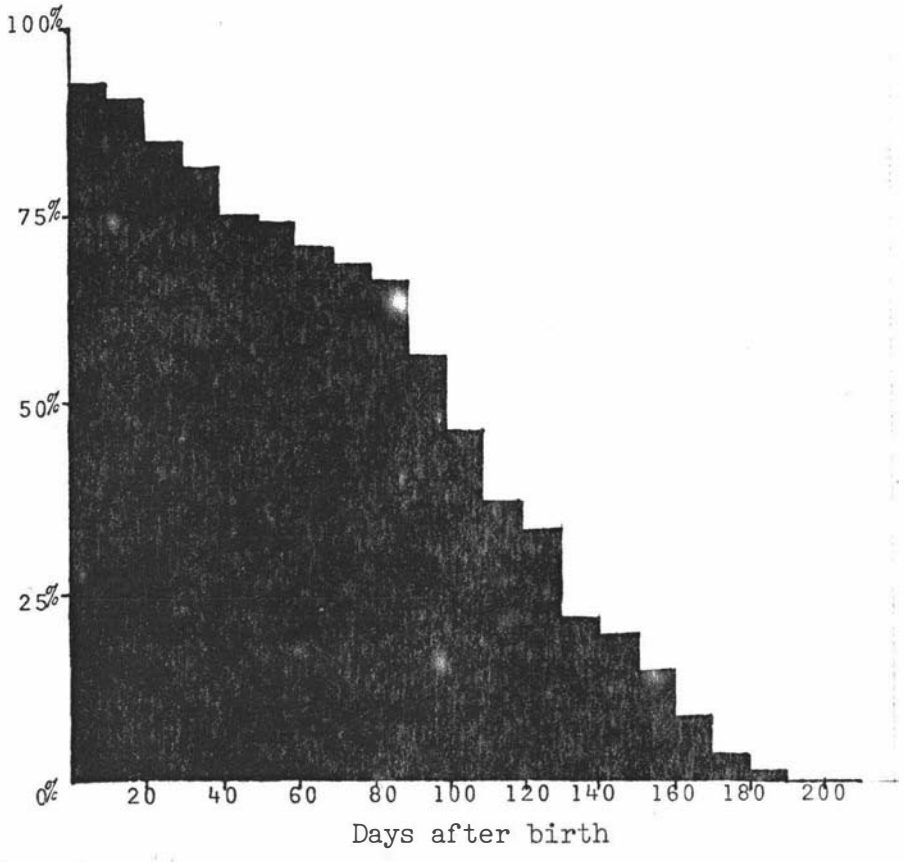


Table 3.8

Spring calves 1976: sampling data and coded *hardjo* titres of dam and calf.

Dam No.	Calving Date	Dam Sample Date	Calf Sample Date	Dam Titre	Calf Titre	Colostral Titre	Age of Dam July 1976
52	23.7	30.7	30.7	4	7	-	2
10	24.7	30.7	30.7	6	8	-	2
46	26.7	30.7	30.7	5	8	-	2
4	29.7	30.7	30.7	5	6	-	3
39	2.8	5.8	5.8	4	5	-	2
213	2.8	5.8	5.8	2	3	-	5
166	3.8	5.8	5.8	3	5	-	4
2	4.8	5.8	5.8	1	3	-	5
37	4.8	5.8	5.8	3	7	-	4
89	4.8	5.8	5.8	2*	0*	-	9*
88	5.8	9.8	9.8	2	3	-	6
112	7.8	9.8	9.8	3	5	-	4
5	8.8	9.8	9.8	2	3	-	9
61	8.8	9.8	9.8	3	4	-	7
129	8.8	9.8	9.8	1	3	-	8
21	8.8	9.8	9.8	3	4	-	10
171	10.8	18.8	14.8	3	4	-	5
180	10.8	18.8	14.8	2*	1*	-	6*
111	11.8	18.8	14.8	1*	0*	-	12*
221	11.8	14.8	14.8	2	4	-	4
76	13.8	2.9	25.8	1	2	-	5
70	14.8	18.8	14.8	4*	0*+	-	9*
222	21.8	25.8	25.8	2	5	-	4
191	22.8	25.8	25.8	3	6	-	5
152	24.8	25.8	25.8	5	7	-	5
195	27.8	30.8	30.8	1	3	-	7
127	30.8	1.9	1.9	2	3	2	8
30	1.9	2.9	1.9	4	5	6	3
93	1.9	2.9	2.9	2	4	5	9
219	3.9	4.9	3.9	2	4	5	4
165	4.9	5.9	5.9	2	5	6	7
124	4.9	5.9	5.9	4	6	4	12
95	6.9	6.9	6.9	2	2	>8	9
106	7.9	7.9	7.9	0	2	6	6
1	9.9	10.9	10.9	3	5	6	7
120	9.9	10.9	10.9	0	0	0	9
223	21.9	22.9	22.9	2	6	5	4
29	20.9	22.9	22.9	5	6	5	2

* These data omitted from correlation and regression analyses.

\+ Calf died after 72 hours.

Figure VIII

Association Between Post-Suckle Titres in Spring 1976 Calves and Titres of Their Dams.

(Circled points obtained from hypogammaglobulinaemic calves omitted from regression analyses.)

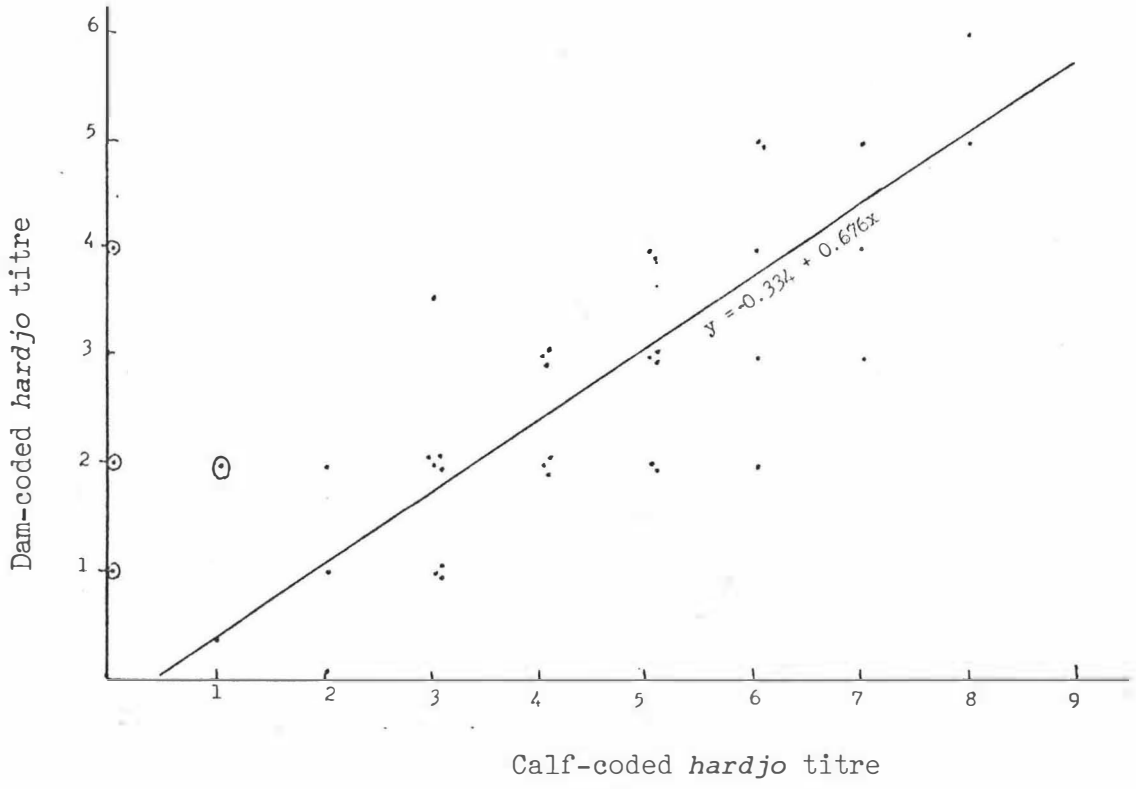


Table 3.9

Autumn calves 1977: sampling data and coded *hardjo* titres of dam and calf.

Dam No.	Calving Date	Dam Sample Date	Calf Sample Date	Dam Titre	Calf Titre	Colostral Titre	Age of Dam July 1977
146	26.2	28.2	28.2	2*	0*	2½	9*
19	28.2	28.2	28.2	4	5½	5	6
41	28.2	1.3	1.3	2	4	5½	5
116	28.2	28.2	28.2	0	0	0	5
173	28.2	28.2	28.2	3	5	6	8
188	1.3	2.3	2.3	4	3	3½	6
193	1.3	1.3	1.3	4*	0*	6	5*
206	1.3	1.3	1.3	2	3	4½	6
215	1.3	2.3	2.3	3	5	4	6
43	2.3	2.3	2.3	0	0	0	5
117	2.3	2.3	2.3	2	2	6	9
11	3.3	9.3	7.3	4	3½	-	7
139	3.3	4.3	4.3	½*	0*	3½	9*
160	5.3	9.3	7.3	2	5	-	7
134	7.3	9.3	9.3	1	4	-	9
72	9.3	9.3	9.3	2	5	6	5
68	10.3	16.3	16.3	2	3½	-	5
181	23.3	28.3	28.3	3	4	-	7
157	26.3	28.3	28.3	3	4½	-	5
178	31.3	6.4	1.4	2½*	0*	-	8*
218	31.3	6.4	1.4	3½	5½	-	5
27	3.4	4.4	4.4	3	6	-	8
65	4.4	6.4	4.4	1	4	-	5
113	4.4	6.6	6.6	5½	6	5	2
115	5.4	6.4	6.4	3	6	5	2
121	5.4	6.4	6.4	4	8	5	2
124	6.4	7.4	7.4	4½	6½	4½	2
26	7.4	7.4	7.4	1	3	4½	7
96	8.4	15.4	15.4	1½	2½	-	7
156	13.4	15.4	15.4	3	6	4	6
174	15.4	15.4	15.4	1	3	5	9
20	17.4	19.4	19.4	4½	6	4½	3
147	19.4	19.4	19.4	0	0	0	9
130	20.4	21.4	21.4	2½	5	5	9
28	22.4	22.4	22.4	2	5	5½	8
158	24.4	25.4	26.4	0	0	0	8
6	26.4	26.4	26.4	2	4	7	13
207	24.4	25.4	26.4	½	1	2	6
172	25.4	25.4	26.4	2	5	8	7
84	3.5	4.5	4.5	4½	8	6	6
78	8.5	10.5	10.5	4	6	3	12

* These data omitted from correlation and regression analysis.

Figure IX

Association Between Post-Suckle Titres in Autumn 1977 Calves and Titres of Their Dams.

(Circled points obtained for hypogammaglobulinaemic calves omitted from regression analyses.)

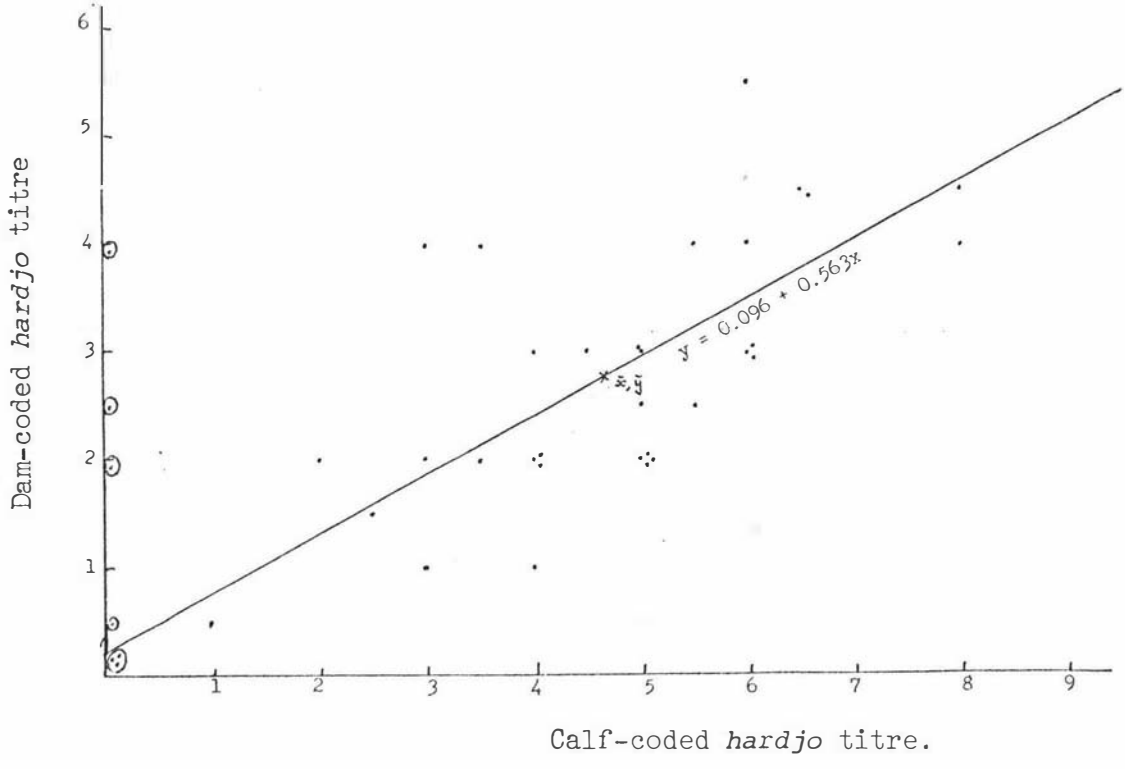


Table 3.10

Serum gammaglobulin levels determined by cellulose-acetate strip electrophoresis.

Dam	Dam titre	Calf titre	Calf immuno-globulin level*
11	4	3½	12.00
70	4	0	0.75
89	2	0	6.75
111	1	0	1.50
134	1	4	20.50
139	½	0	3.50
146	2	0	2.75
160	2	5	29.50
171	3	4	17.50
173	3	5	18.50
178	2½	0	11.50
180	2	1	7.50
188	4	3	12.50
193	4	0	1.50

* Units grams per litre. Normal range = 11.22 - 36.20 g/l (Williams *et al*, 1975).

With the exception of three calves, all others had titres equivalent to or greater than those of their dams. The exceptions were calves born to dams 11, 180 and 188 and sera from them were also submitted to cellulose-acetate strip electrophoresis. One, the calf born to dam 180, was hypogammaglobulinaemic and the other two had levels of immunoglobulins near the bottom of the normal range (Table 3.10).

A comparison of calf post-suckle titres and dam titres is given in Figures VIII and IX for the spring 1976 and autumn 1977 calves respectively. In both groups correlation coefficients between the titres of sero-positive calves and their dams were calculated. The data for dams 70, 89, 111 and 180 were omitted from the calculations for spring 1976 and for dams 139, 140, 178 and 193 from the calculations for autumn 1977 since their calves

were hypogammaglobulinaemic (Table 3.10) and were thus considered to represent a separate population from the rest of the calves in each group. The correlation coefficients of 0.85 and 0.81 for spring 1976 and autumn 1977 respectively are highly significant ($P < 0.001$). The calculated regression lines, fitted to the data for both groups of calves, are $y = -0.334 + 0.676x$ and $y = 0.096 + 0.563x$ respectively. The regression coefficients of these lines are not significantly different ($P > 0.60$).

Correlation coefficients were also calculated to test the association between dam's age and dam's titre or calf's titre. For the spring 1976 data the correlation coefficient between dam's age and dam's titre was -0.54 ($P < 0.001$) and between dam's age and calf's titre was -0.47 ($P < 0.01$). For the autumn 1977 data dam's age was significantly correlated with dam's titre ($r = -0.34$, $P < 0.05$) but dam's age was not significantly correlated with calf's titre ($r = 0.27$, $P > 0.10$).

Data from both samplings was pooled for those cases where the calf's titre and weight, the dam's titre and age, colostral titre and suckling time were all recorded (Table 3.11) and a multiple regression analysis conducted using the BAR 3 programme on an IBM 1620. This showed that although there were significant correlations between the titre of the calf and that of the dam ($r = 0.735$, $P < 0.001$), the age of the dam ($r = -0.336$, $P < 0.05$) and suckling time ($r = 0.569$, $P < 0.001$), the small negative correlations between the titre of the calf and colostral titre ($r = -0.057$) and calf's birth weight ($r = -0.197$) were not significant. In the multiple regression analysis 52.5% of the variation in calf's titre was predicted by variation in dam's titre and the addition of any other factor into the analysis reduced the strength of this prediction.

Discussion.

In agreement with the findings of Bohl *et al* (1954), Fennestad and Borg-Petersen (1956), Cacchione *et al* (1968) and Saski and Arima (1971), it was found that calves born to sero-positive dams are sero-negative prior to suckling (Table 3.7),

Table 3.11

Data used in a multiple regression analysis of the factors involved in the acquisition of *hardjo* titres in newborn calves.

Dam ID	Calf titre	Dam titre	Colostral titre	Dam's age (years)	Suckle time (hours)	Calf weight (kilos)
30	5	4	6	3	24	34.5
93	4	2	5	9	24	29.9
219	4	2	5	4	20	32.7
124	6	4	4	12	36	36.3
165	5	2	6	7	27	34.0
95	2	2	9	9	12	39.5
106	2	0	6	6	11	38.6
1	5	3	6	7	32	31.8
223	6	2	5	4	15	30.4
29	6	5	5	2	32	34.0
41	4	2	5½	5	27	40.8
19	5½	4	5	6	14	44.5
173	5	3	6	8	14	39.0
188	3	4	3½	6	38	34.0
206	3	2	4½	6	15	41.3
215	5	3	4	6	26	36.3
117	2	2	6	9	14	39.5
72	5	2	6	5	14	44.0
113	6	5½	5	2	48	31.3
121	8	4	5	2	36	36.7
115	6	3	5	2	36	33.6
1124	6½	4½	4½	2	24	33.1
26	3	1	4½	7	18	44.0
156	6	3	4	6	48	33.6
174	3	1	5	9	14	42.6
20	6	4½	4½	3	52	34.5
130	5	2½	5	9	14	36.3
1128	5	2	5½	8	16	34.5
6	4	2	7	13	12	37.6
207	1	½	2	6	14	34.0
172	5	2	8	7	15	34.9
78	6	4	3	12	30	40.8
84	8	4½	6	6	36	41.3

but develop peak titres after suckling as soon as 11 hours after birth (Table 3.11). A single feed of colostrum is all that is necessary for the development of the usual level of agglutinating antibody found in new-born calves. This was shown when two litres of colostrum, with a *hardjo* titre of 1:543, were fed to a calf resulting in the development of a titre of 1:384.

The finding that 80% of new-born calves had *hardjo* titres and 24% had *pomona* titres of 1:24 or greater (Table 3.1) probably underestimates the true situation. A proportion of the calves sampled, particularly from the first two calvings, were up to 47 days old when first sampled, and may have lost low titres present at birth. The regression lines fitted to the data for spring 1975, spring 1976 and autumn 1977 predict that, on average, titres of 2.5 to 2.7 (1:68 - 1:92) at birth will be lost by about 45 days (Figures IV, V and VI). The more detailed data from the last two calvings (Tables 3.8 and 3.9) shows that of 79 calves sampled within a few days of birth, 67 (85%) had titres of 1:24 or more against *hardjo* and of those born to sero-positive dams 67 of 74 (91%) had such titres. This result is in close agreement with that of Cacchione *et al* (1968), who observed *hardjo* titres in 90% of three-day-old calves born to 103 sero-positive dams and is similar to the report that 95% to 100% of approximately 100 calves in a herd with endemic *hardjo* and *pomona* infection had passively-acquired titres (Hanson *et al*, 1964). Passively-acquired titres have also been reported in 16 out of 16 calves (100%) born to dams experimentally-infected with various serovars (Fennestad and Borg-Petersen, 1956) and 25 of 26 calves (96%) born to dams vaccinated with a *pomona* bacterin (McDonald and Rudge, 1957).

A number of studies have shown that approximately 10% of new-born calves are hypogammaglobulinaemic after suckling is completed (Klaus *et al*, 1969; Selman *et al*, 1970; 1971; Bailey and McLean, 1972; Mohamad, 1975; McGuire *et al*, 1976). A similar result was obtained in this study where seven calves failed to develop titres following suckling from sero-positive dams and another calf developed only a very low titre. All these animals were hypogamma-globulinaemic, making a total of eight out of 74 calves (11%) where there was an apparent failure of colostral transfer of

immunoglobulin.

A number of explanations have been given by different authors for the failure of calves to acquire colostral immunoglobulins or specific antibody. Kruse (1970b) demonstrated that a low level of immunoglobulin in colostrum was a major factor, and Cacchione *et al* (1968) believed that this was the reason for 10% of 103 calves failing to acquire *hardjo* titres from sero-positive dams. Matching colostrum samples were taken from three of the eight dams of hypogammaglobulinaemic calves detected in the course of the present study (dams 146, 193 and 139). In these three cases, colostral titres were similar to those which produced titres in many other calves (Table 3.9), indicating that the failure of these calves to acquire passive titres was not associated with colostral levels of antibody.

The premature loss of absorptive capacity of immunoglobulins by the calf has also been reported as the cause of hypogammaglobulinaemia (Kruse, 1970b; Selman *et al*, 1970). In contrast recent studies on hypogammaglobulinaemic calves in the Massey No. 1 dairy herd showed that their absorptive capacity was normal (Mohamad, 1975). Mohamad (1975) considered that neonatal calf hypogammaglobulinaemia resulted from a low intake of colostrum and was caused by such factors as parent rejection, environmental conditions and udder conformation, and McGuire *et al* (1976) claimed delayed suckling, which could result from these factors, was the major cause. A calf examined in the present study was sero-negative when removed from its dam 16 hours after birth and appeared not to have suckled. When colostrum was taken from the dam and fed to the calf it sero-converted. This case supports the conclusions of Mohamad (1975) and McGuire *et al* (1976).

Geometric mean titres against *hardjo* at time of first sampling ranged from 1:138 - 1:199 for the three calvings spring 1975, spring 1976 and autumn 1977 (Table 3.2) with the GMT for autumn 1976 of 1:21 being very substantially less than observed at the other calvings. This is probably explained by the greater mean age at sampling of this group as no other factor could be found to explain this difference.

The strong positive correlation between dam titre and calf titre observed in this study confirms the impressions of Fennestad and Borg-Petersen (1956) and Hanson *et al* (1964) that new-born calf titres are generally equal to or greater than maternal titres. Similarly, the fact that the small negative correlation between colostral titre and calf titre is non-significant agrees with both the observation of Fennestad and Borg-Petersen (1956) that calf's serum generally has a lower titre than does the corresponding colostral whey, and the finding of McGuire *et al* (1976) that colostral immunoglobulin levels were not correlated with calf serum immunoglobulin levels. Since the dam's colostral levels of specific antibody and immunoglobulin decline very rapidly after calving (Porter, 1972) and since there is also considerable variation in the amount of colostrum ingested by individual calves (Mohamad, 1975), sampling a dam's colostrum after a variable suckling period, as occurred in this study (Table 3.11) is likely to produce highly variable results and explain the non-significant correlation obtained.

Although both dam titre and calf titre are significantly correlated with dam age, the multiple regression analysis showed that maternal titre alone is the best predictor of calf titre. This suggests that the age effect results from the fact that younger dams generally have higher titres.

Passively-acquired titres in calves decline logarithmically from birth (Tables 3.3, 3.4, 3.5 and 3.6 and Figures IV, V and VI). The length of time over which a calf remains sero-positive is positively correlated with the size of the initial titre. The fitted regression lines (Figures IV, V and VI) indicate that a calf with a titre of 1:3072 will on average remain sero-positive for 125 to 135 days and that the half-life of a titre is 15 to 17 days. Since the sampling of calves at that age has been carried out only at approximately monthly intervals the actual day on which individual calves became sero-negative cannot be determined. However, on average at least 50% of those calves serially sampled are still sero-positive at 100 days of age and approximately 25% at 140 days; all are sero-negative by 190 days of age (Figure VII). These results agree very closely with those previously reported

for calves by Fennestad and Borg-Petersen (1956), McDonald and Rudge (1957), Hanson *et al* (1964) and Cacchione *et al* (1968).

The prevalence of *pomona* titres in new-born calves was always less than that of *hardjo* titres (Tables 3.1 and 3.2), and declined over the two year study period, particularly between spring 1976 and autumn 1977. The reason for this is discussed more fully in Chapter Five but is a direct result of the removal of *pomona* positive dams from the herd. Persistance of *pomona* MAT titres in young calves was also shorter than for *hardjo* reflecting their initial substantially lower titres.

Summary.

1. New-born calves passively acquired MAT titres from their dams.
2. Initially these titres were higher than those of their dams but lower than those of their dams' colostrum.
3. The titres declined logarithmically from the first days of life, with a half-life of 15 to 17 days.
4. Half of the calves had lost their titres by 100 days of age and all by 190 days of age.
5. Although dam titre, dam age and suckling time were all significantly correlated with calf's initial post-suckle titre, only dam titre had any value as a predictor of calf titre in this study and variation in dam titre accounted for 52.5% of the observed variation in calf titre.

CHAPTER FOUR

SEROLOGICAL RESPONSE OF CATTLE TO NATURALLY-OCCURRING INFECTION WITH *Leptospira interrogans* SEROVAR *hardjo*.

Introduction.

Cattle have been shown to develop titres 4 to 23 days after experimental infection with *hardjo* though in most cases they are first detected 7 to 14 days post infection (p.i.)(Roth and Galton, 1960; Sullivan, 1970a; 1970b; 1972; Sullivan and Callan, 1970; Farina *et al*, 1972; Hodges and Ris, 1974; Ellis and Michna, 1977). Peak titres, generally in the range of 1:1000 to 1:30,000, occur within 10 to 23 days p.i., though peak titres as low as 1:30 (Ellis and Michna, 1977), 1:100 (Hodges and Ris, 1974) and 1:300 (Sullivan, 1970a) and as high as 1:100,000 (Ellis and Michna, 1977), 1:300,000 (Sullivan, 1972) and 1:500,000 (Farina *et al*, 1972) have been reported in some individual animals.

All these authors report that after only a few days titres decline. Titres ranging from 1:30 to 1:1000 have been reported as early as 37 days p.i. (Sullivan, 1970a). Almost invariably experimentally-infected cattle were still sero-positive when the various studies were terminated at times ranging from 37 to 174 days p.i. Two authors reported a diphasic serological response. Secondary peaks occurred 77 to 133 days p.i. in four heifers experimentally infected by Ellis and Michna (1977) and 77 to 98 days p.i. in six cows experimentally infected by Sullivan (1972). In the latter report secondary peak titres exceeded initial peak titres in two animals.

In naturally-occurring outbreaks of bovine *hardjo* infection peak titres as high as 1:300,000 have been reported in some animals (Sullivan, 1972; Ellis *et al*, 1976), though most develop peak titres of 1:1000 to 1:10,000 (Sullivan and Callan, 1970; Sullivan, 1972; Johnson *et al*, 1974; Hoare and Claxton, 1972; Ellis *et al*, 1976). Titres then fall though persisting for at least 126 to 322 days when titres ranging from 1:30 to 1:1000 have been observed

(Sulzer *et al*, 1964; Sullivan, 1972; Johnson *et al*, 1974). Convalescent titres in the range of 1:1000 to 1:10,000 have been reported (Robertson *et al*, 1964; Cacchione *et al*, 1968; Sullivan and Callan, 1970), though the actual ages of these titres (i.e. the times elapsed since the animals sero-converted) were not known.

During the course of the two and a half year surveillance of the Massey No. 1 dairy herd a number of outbreaks of *hardjo* infection were observed. This chapter reports the serological changes occurring in cattle becoming infected during these outbreaks as assessed by the microscopic agglutination test (MAT). The epidemiological factors involved in these outbreaks are discussed in Chapter Seven.

Materials and Methods.

The Animals.

Two crops of calves are reared in the herd each year, one born in spring and the other in autumn. Each group is generally reared in isolation from all other stock until about six months of age when some contact occurs with older stock, initially with the immediately preceding calf crop and later with in-calf heifers and a few older dry cows. Each of the groups of calves in this study experienced outbreaks of *hardjo* infection at between six months and one year of age while at the No. 1 dairy farm, Best's farm or Mathew's farm.

Sample Collection and Processing.

Blood sampling and serological testing were conducted as described in Chapter Two.

Sampling Frequency.

Blood sampling was generally carried out at monthly intervals when calves were available for testing, and then following sero-conversion at monthly and later approximately three-monthly intervals. Frequently, for management reasons, animals were not available for testing on a particular day, and on some occasions were not tested for two to three months while grazing away from the main farm. Two groups of calves were sampled repetitively at

seven-day intervals before, during and after sero-conversion.

Statistical Analysis.

Statistical analyses were conducted as detailed in Appendix II.

Results.

Serological response to hardjo.

For the 16 calves sampled at seven-day intervals, peak titres ranged from 1:768 to 1:8689 (Table 4.1) with a geometric mean titre (GMT) of 1:2528 (1:1603 to 1:3987, 95% CL). In all cases the peak titre was detected within 14 days of sero-conversion and for ten animals the peak titre was the first detected. Titres declined rapidly for the first three months after infection and then at a slower rate over the following six to twelve months. All 16 calves were still sero-positive when finally sampled. Mean titres, with standard errors, for these calves are shown graphically in Figure X.

The titres observed at various times in six other groups of calves which experienced outbreaks of *hardjo* infection are given in Tables 4.2 to 4.7 and are summarised graphically in Figure XI. Peak titres ranged from 1:48 to 1:6144, and although mean titres at different times after infection varied between the six groups of calves, they generally declined as time elapsed. The only exception occurred in the group which sero-converted while at Mathew's farm (Table 4.4). This group had a higher mean titre (1:416) 241 days p.i. than at 157 days p.i. (1:310) but this rise was not significant ($P > 0.10$). This declining trend in convalescent titres is emphasised by the data presented in Table 4.8 which shows titres recorded from four calves over 13 approximately one-monthly intervals.

During the study period 38 calves were finally sampled 11 to 14 months (mean 404 days) after infection (Tables 4.1 to 4.5). By this time only three had become sero-negative (Table 4.5) and the GMT of all 38 calves was 1:102. The possibility of an association between the initial and final titres of these calves was investigated. Final titres were strongly correlated with initial titres and the correlation coefficient ($r = 0.674$, $P < 0.001$) indicated

Table 4.1

MAT reponse of calves naturally infected with *hardjo*.

Coded titres.

Calf	Estimated time since sero-conversion (days). 0	7	14	25	35	51	91	188	357	mean
	0	7-8	13-15	20-29	34-36	42-60	75-106	170-206	342-371	range
G39	0	7	7	6	7	7	5½	6	5	
G40	0	6	5	4	4	- α	4	4½	2½	
G42	0	8	9	7	5	6	5½	5	4	
G54	1	8	5½	7	6	7	5½	4	3	
G55	0	8	8	6½	5½	6	6	4	3	
G56	0	9	7	8	6	-	5½	5	-	
G57	0	8	7	-	-	-	5	7	5½	
G62	0	9½	9	-	-	-	5	6	4	
G63	1	7	8	7	7	6	4½	5	2½	
024	½	6	6	5	5	4½	5	3		
028	1	-	5	-	-	3½	2½	4½		
029	0	8	6	8	5½	-	6	3		
031	1½	6	-	4½	-	5	3½	3		
035	0	9	-	7½	-	6	4½	2½		
037	0	8	-	6	-	5	5	4		
039	0	9	-	6½	-	5	5	3½		
number	16	15	12	13	9	11	16	16	8	
mean	0.3	7.8	6.9	6.4	5.7	5.5	4.9	4.4	3.7	
standard error	0.13	0.30	0.41	0.35	0.32	0.32	0.23	0.32	0.40	
GMT	1:15	1:2528	1:1409	1:1003	1:610	1:560	1:352	1:249	1:155	

α = not tested.

Figure X

Mean Coded *hardjo* Titres ± S.E. of 16 Calves at Various Times After Sero-Conversion.

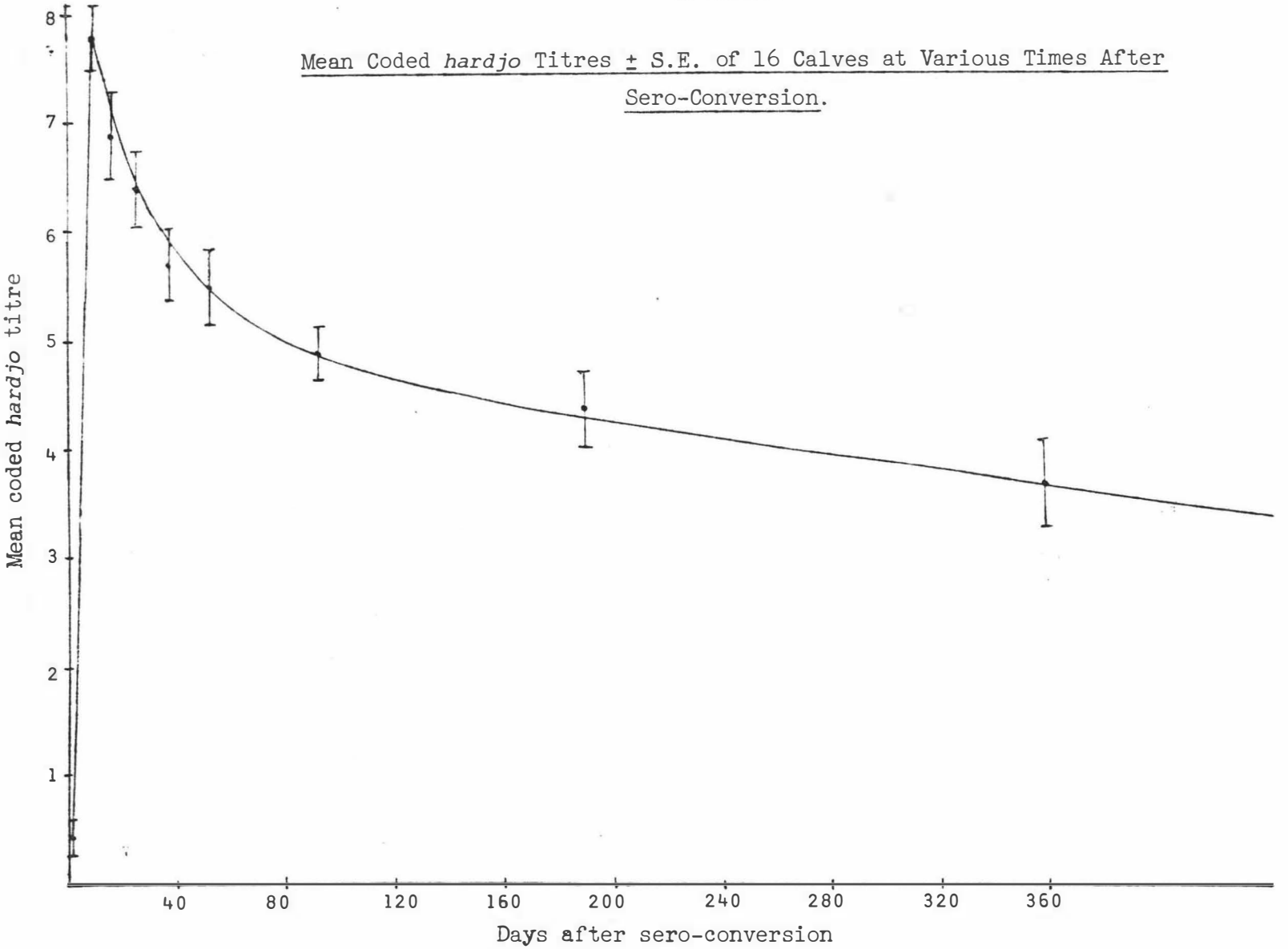


Table 4.2

MAT response of autumn calves born 1975 which sero-converted during 1975-76 at Massey Dairy Farm No. 1.

Coded titres.

Calf	Estimated time since sero-conversion (days). 21 1-41	53 36-69	96 62-129	112 92-132	141 111-171	190 168-228	221 205-238	351 332-365	mean range
G1	9	-	7	7	-	-	-	-	
G2	5	6	6	-	5	6	3	2	
G22	6	6	7	-	6	5	5	5	
GNT	6	-	5	5	6	5	-	-	
number	4	2	4	2	3	3	2	2	
mean	6.5	6.0	6.3	6.0	5.7	5.3	4.0	3.5	
standard error	0.87	0.00	0.48	1.00	0.33	0.33	0.78	1.50	
GMT	1:1086	1:768	1:913	1:768	1:610	1:484	1:192	1:136	

Table 4.3

MAT response of autumn calves born 1975 which sero-converted at Best's farm.

Coded titres.

Calf	Estimated time since sero-conversion (days). 34 1-67	78 37-119	134 101-167	210 168-257	295 258-363	407 364-434	mean range
G3	4	5	5	5½	4½	4	
G4	5	6	5	4½	5	4½	
G6	3	3	3	3	2	1	
G7	6	5	5	5	5	4	
G9	5	4	5	3½	5	4	
G12	6	5	5	5½	4½	4	
G28	6	6	6	6½	5	4	
number	7	7	7	7	7	7	
mean	5	4.9	4.9	4.8	4.4	3.6	
standard error	0.44	0.40	0.34	0.46	0.41	0.45	
GMT	1:384	1:348	1:348	1:331	1:258	1:150	

Table 4.4

Calves born 1975 which sero-converted at P. Mathew's farm.

Coded titres.

Calf	Estimated time since sero-conversion (days). 36 1-70	93 58-127	157 122-191	241 192-289	424 389-458	mean range
G10	4	5	4	3½	2	
G13	9	6	5	6	5	
G21	9	6	5	5	4	
G29	8	5	6	8	5½	
G32	8	5	5	6	4½	
G33	7	7	6	5	4	
G36	7	5	5	5	4	
G37	8	6	5	6	4	
G38	4	4	3	3½	2	
G45	7	6	5	4½	3	
G46	7	6	4	5	1½	
G47	5	6	6	5	3	
G52	4	2	2	4	1	
number	13	13	13	13	13	
mean	6.7	5.3	4.7	5.1	3.3	
standard error	0.51	0.35	0.32	0.34	0.39	
GMT	1:1241	1:475	1:310	1:416	1:122	

Table 4.5

Autumn calves born 1976 which sero-converted at Massey Dairy Farm No. 1.

Coded titres.

Calf	Estimated time since sero-conversion (days). 35 1-68	123 88-163	236 200-275	315 277-352	441 403-478	mean range
01	5	4	4	3½	-	
02	6	7	6	4½	-	
03	3	2½	2½	1½	2	
04	5	5½	5	3½	½	
06	5	6	5	4	-	
07	2	1	2	0	-	
08	6	4½	4	4	3	
09	2	1	0	½	0	
011	4	3	4	4	1	
012	4	6	4	4	-	
013	4	4	3	4	1½	
014	5	4	4	-	-	
015	5	4	4	4	-	
016	5	5½	4	4	3	
017	3	3	2	1½	-	
018	4	1	0	0	-	
021	7½	5	5½	4½	-	
022	5	4½	3½	3	1	
number	18	18	18	17	8	
mean	4.5	4.0	3.5	3.0	1.5	
standard error	0.33	0.42	0.39	0.39	0.39	
GMT	1:266	1:188	1:133	1:94	1:34	

Table 4.6

Spring calves born 1976 which sero-converted at Massey Dairy Farm No.1.

Coded Titres.

Calf	Estimated time since sero-conversion (days) 21 1-40	86 15-55	134 118-157	mean range
023	6½	5	4	
027	6½	5	3	
040	4	4	3	
041	6½	6	4½	
042	5½	5	3½	
043	5½	5	4	
number	6	6	6	
mean	5.8	5.0	3.7	
standard error	0.40	0.26	0.25	
GMT	1:646	1:384	1:152	

Table 4.7

Autumn calves born 1977 which sero-converted at Massey Dairy Farm No.1.

Coded Titres.

Calf	Estimated time since sero-conversion (days). 31 1-60	46 16-75	116 86-145	mean range
W1	7	5½	5	
W2	5	3½	4	
W3	6	5	5	
W4	4½	3	3	
W5	4	5	3	
W6	5	4	4	
W7	4½	4	5	
W8	5½	5	4	
W9	6½	4	3	
W10	5	4	4½	
W11	4½	4½	3	
W14	4	3½	3	
number	12	12	12	
mean	5.1	4.3	3.9	
standard error	0.28	0.21	0.25	
GMT	1:419	1:228	1:176	

Figure XI

Mean Coded *hardjo* Titres ± S.E. of Calves at Various Times After Sero-Conversion.

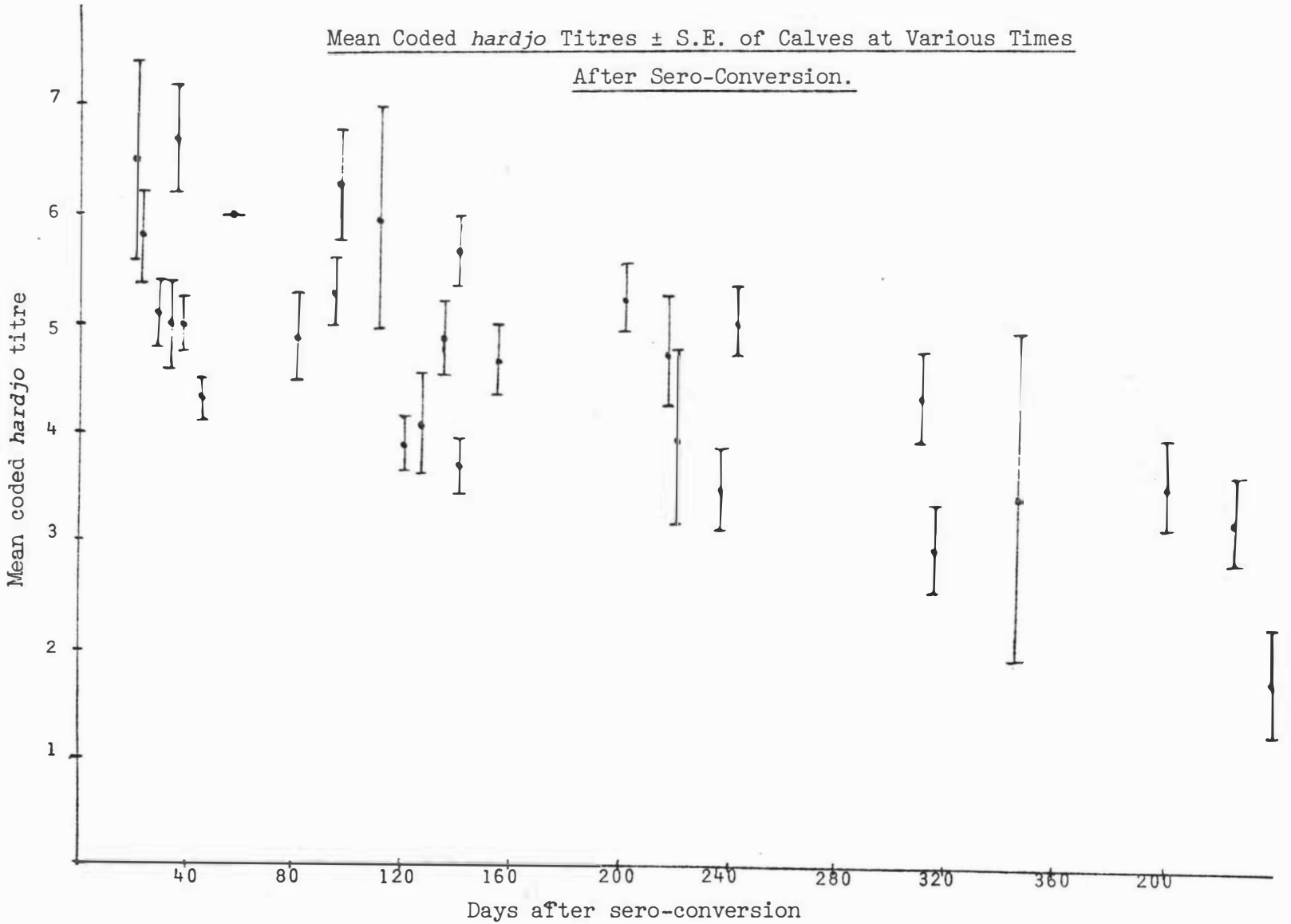


Table 4.8

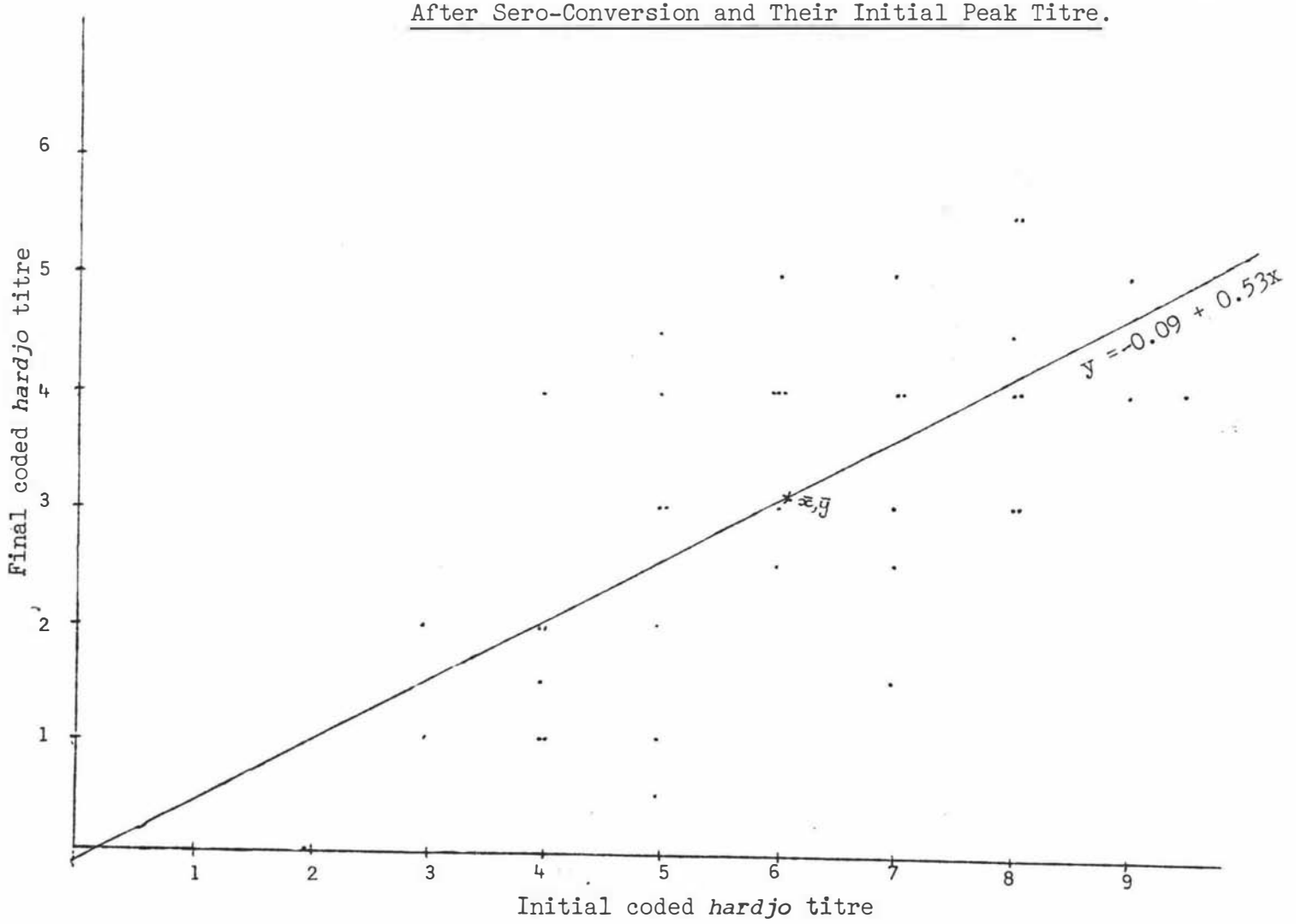
Reciprocal *hardjo* MAT titres of 4 calves sampled at frequent intervals.

Test Dates.

Calf	13/12/76	26/1/77	10/3/77	20/4/77	12/5/77	27/5/77	10/6/77	24/6/77	8/7/77	20/8/77	3/9/77	23/9/77	27/1/78
08	768	384	272	384	272	136	96	192	192	-	96	192	96
016	384	543	543	768	768	192	192	192	192	-	-	192	96
021	12	12	2172	768	543	272	384	384	384	96	192	543	272
022	384	136	272	384	384	136	96	96	136	96	-	96	24

Figure XII

Association Between Coded *hardjo* Titres of 38 Calves 11 - 16 Months After Sero-Conversion and Their Initial Peak Titre.



that 45% of the variation in final titres could be explained by the variation in initial titres. This association is shown graphically in Figure XII with the fitted regression line ($y = -0.09 + 0.53x$).

At the time that serological studies commenced on the Massey No. 1 dairy herd, there was a group of yearlings on the farm which had apparently experienced an outbreak of *hardjo* shortly before testing commenced. The serological findings for this group are presented in Table 4.9. Over the two years that the members of this group were under test the group mean titre showed a gradual decline though rising non-significantly ($P > 0.10$) in August/September 1976 and again rising non-significantly ($P > 0.05$) in May/June 1977. Their mean titres at each sampling with their standard errors plotted against the estimated mean age of the titres are shown graphically in Figure XIII. This figure again shows the declining trend in group mean titres as time from sero-conversion increases. The only animal which was sero-negative at the final sampling had never possessed a titre greater than 1:48. The GMT for these animals, observed an estimated 780 days after the group had sero-converted, was 1:89.

Seventeen animals sampled in December 1975 were sampled again in September 1977 and analysis of these titre-pairs measured 639 days apart again shows a strong correlation between initial and final titres ($r = 0.714$, $P < 0.001$) and indicates that 51% of the variation in final titre can be explained by variation in initial titre. Figure XIV shows final coded titre versus initial coded titre and the fitted regression line ($y = -0.24 + 0.65x$) for these data.

Cross-reactions observed in calves infected with hardjo.

All sera collected from eight calves which were sampled weekly during an outbreak of *hardjo* infection in September 1976 were tested against nine serovars: *australis, ballum, canicola, copenhageni, grippotyphosa, hardjo, pomona, pyrogenes* and *tarassovi*, and some positive reactions were observed to all serovars except *australis, grippotyphosa* and *pyrogenes*. These results are recorded in Table 4.10.

Figure XIII

Mean Coded *hardjo* Titres ± S.E. of Heifers at Various Times After Sero-Conversion.

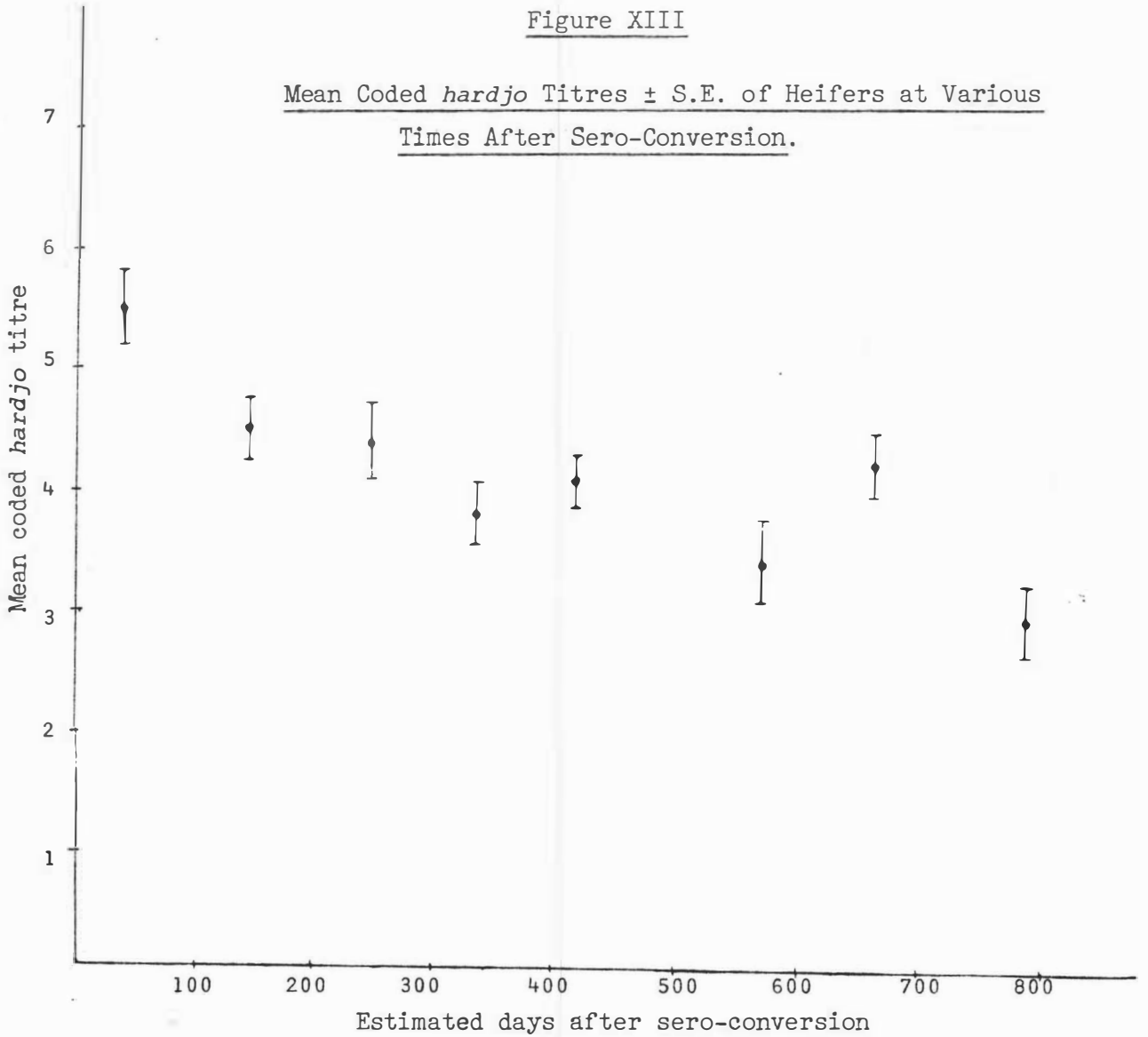


Table 4.9

Spring and autumn calves born 1974 which sero-converted winter 1975.

Coded MAT titres.

Calf	Month of test. Sept 75	Dec 75	March 76	June 76	Sept 76	Feb 77	May 77	Sept 77
B2	5½	5	3½	3	4	-	-	3½
B3	-	6	-	-	6	4½	6	5
B5	-	5½	5	-	5	-	4½	3½
B8	-	6	6	4	4	-	5	4
B9	-	4½	3	2½	3	-	3½	1½
B10	-	-	4	5	3	-	3	2
B11	-	4	5	3	6	-	5	3½
B12	7	3½	4	4½	4	-	4½	2
B13	4½	4½	5½	5	4½	4	5	3
B14	-	6	6½	5	4½	-	-	4
B22	5	3	4	4	5	-	3½	3
B23	-	4	5	4	-	2½	3½	2
B28	-	-	-	5½	4	3	5	3½
B29	4	3	3½	3	4½	-	-	1
B39	5	3½	4	4	4	3½	4	-
B41	-	-	6	6	5	5	5½	4
B43	-	4½	2½	4	3	2½	3	-
B50	7	5½	5	5	3	-	4	-
B52	-	6	4½	2½	3½	-	-	2
B54	-	-	6	5	5	4½	5	3½
B55	5½	5	4	3	4	4	5	2½
B56	6	4	5½	5	5	-	-	3
B63	5	4	4	3	4	3	3	-
B64	-	1	0	0	1	1	2	0
B65	4½	5	-	3	-	-	3	2
B71	7	-	-	-	-	-	6	5
est.mean age of titre(days)	40	141	243	328	411	562	653	780
number	12	21	22	23	23	11	21	22
mean	5.5	4.5	4.4	3.8	4.1	3.4	4.2	2.9
standard error	0.30	0.27	0.30	0.27	0.23	0.35	0.24	0.26
GMT	1:543	1:263	1:251	1:170	1:210	1:127	1:226	1:89

Figure XIV

Association Between Coded *hardjo* Titres in 17 Heifers Observed at an Interval of 639 Days.

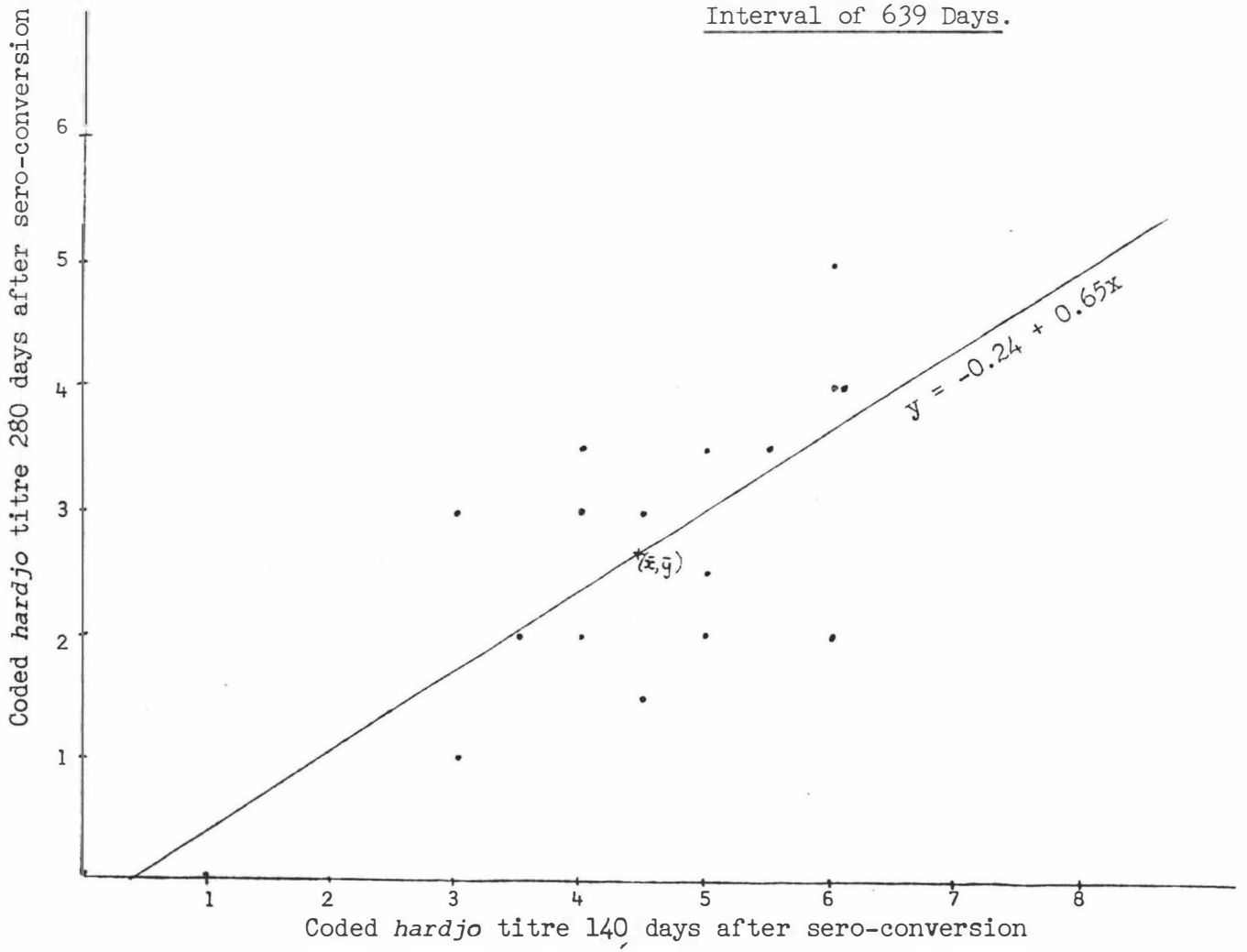


Table 4.10

Cross-Reaction Pattern - Coded MAT Titres.

ID	Serovar	Test Date 29/7/76	26/8/76	2/9/76	9/9/76	16/9/76	30/9/76	7/10/76	10/12/76
G39	*hardjo*	0	0	7	7	6	7	7	5½
	pomona	0	0	2	1	1	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	2	0	0	0	0	0
	ballum	0	0	1	0	0	0	0	0
	canicola	0	0	0	0	0	0	0	0
G42	*hardjo*	0	0	8	9	7	5	6	5½
	pomona	0	0	2	1	2	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	3	2	2	0	0	0
	ballum	0	0	0	0	1	1	0	0
	canicola	0	0	0	0	0	0	0	0
G54	*hardjo*	0	1	8	5½	7	6	7	5½
	pomona	0	0	3	3	0	0	0	0
	copenhageni	0	0	2	1	0	0	0	0
	tarassovi	0	0	3	1	2	0	0	0
	ballum	0	0	0	0	0	0	0	0
	canicola	0	1	4	1	0	0	0	0
G55	*hardjo*	0	0	8	8	6½	5½	6	6
	pomona	0	0	2	0	2	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	2	0	0	0	0	0
	ballum	0	0	1	0	0	0	0	0
	canicola	0	0	0	0	0	0	0	0
G56	*hardjo*	0	0	0	9	7	8	6	5½
	pomona	0	0	2	2	2	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	0	1	1	0	0	0
	ballum	0	0	0	0	0	0	0	0
	canicola	0	0	0	0	0	0	0	0
G57	*hardjo*	0	0	0	0	0	8	7	5
	pomona	0	2	3	2	2	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	0	0	0	3	2	0
	ballum	0	0	0	0	0	1	2	0
	canicola	0	0	0	0	0	2	1	0
G62	*hardjo*	0	0	0	0	0	9½	9	5
	pomona	0	0	2	2	2	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	0	0	0	3	2	0
	ballum	0	0	0	0	0	1	1	0
	canicola	0	0	0	0	0	2	2	0
G63	*hardjo*	0	1	7	8	7	7	6	4½
	pomona	0	0	1	2	3	0	0	0
	copenhageni	0	0	0	0	0	0	0	0
	tarassovi	0	0	2	2	0	0	0	0
	ballum	0	0	0	0	0	0	0	0
	canicola	0	0	0	1	0	0	0	0

All sera were negative to *australis*, *grippotyphosa* and *pyrogenes*.

Discussion.

Monitoring the serological parameters of animals experiencing a series of natural outbreaks of leptospiral infections presents considerable difficulties. An outbreak can only be detected once some or all the animals within the group have sero-converted, and the date of onset of infection of an individual within the group can only be estimated. The infection will have occurred not earlier than a few days before the previous negative test and not later than a few days before the first positive test. Thus in order to obtain more precise estimates of the average serological response, all susceptible animals must be tested at frequent intervals, ideally every few days, not only until all the animals have sero-converted but until all titres have reached a stable level.

Unfortunately, there were commercial and logistical restrictions which prevented repetitive testing at very short intervals in this dairy herd and testing of most animals was carried out at approximately monthly intervals. Also groups of susceptible young cattle were removed for periods of up to four months for grazing on areas where blood samples could not be taken and some of these groups experienced outbreaks before returning to the home farm. Therefore much of the data which is recorded and discussed in this chapter was obtained from animals which sero-converted whilst not accessible for testing. These animals had titres of unknown duration, possibly some months, when first detected. Therefore, to detect any trends in these groups' serological parameters, the approach taken in the analysis of the data obtained from them has been to compare group GMT's for each occasion on which the groups were tested.

On two occasions, in spring 1976 and again in spring 1977, there were opportunities to sample groups of calves at weekly intervals, before, during, and for a short period after, each had experienced an outbreak of *hardjo* infection. The day on which any animal sero-converted in these two outbreaks can thus be estimated with a maximum error of only seven days. The ages of these animals' titres at subsequent tests can also be defined accurately. One group, consisting of eight calves, had been run in isolation for some months prior to mixing with a larger group of recently-infected calves in spring 1976. Two of these eight

calves, G54 and G63, had low titres 17 days later and five of the eight had developed titres by 24 days. Although the actual days on which individuals became infected cannot be known, the range of 17 to 24 days to sero-conversion lies at the upper end of the range observed in experimental *hardjo* infections by Roth and Galton (1960), Sullivan (1970a, 1970b and 1972) and Ellis and Michna (1977).

In order to provide the estimate in Table 4.1 of the mean serological response, at various stages of infection, of individuals naturally infected with *hardjo*, it was necessary to make some approximations. In a naturally propagating epidemic, where new cases occur at different times, some individuals will have rising titres whilst others have constant or declining titres depending on their stage of infection. It is therefore inappropriate to determine group mean titres at various times during the outbreak. Instead a method of analysis is required in which all individuals that are to be compared are at nearly the same stage of infection.

As the 16 calves studied in Table 4.1 were usually being sampled at seven-day intervals the day on which they sero-converted was known with a maximum error of seven days and the first titre observed was generally at near peak levels. Five of these calves had titres of up to 1:34 on the first occasion on which sero-conversion was detected and did not have near peak titres until seven days later. These findings indicate that the phase of rapidly climbing titres occupies less than seven days of the serological response. Therefore Table 4.1 was constructed aligning the low pre-peak titres along with the negative pre-peak titres for all the other 11 calves on day zero. This presents the data as though all the calves experienced their infection on the same or nearly the same day.

Thus the mean data in Table 4.1 and Figure X are approximations which still suffer to some extent from the fact that mean peak titres will be masked. Nevertheless, it can be argued that without daily testing for an extended period of a group of animals, which may be expected to experience an outbreak, they represent the best estimate of the response of cattle to a natural *hardjo* infection that is logistically feasible.

The data in Table 4.1 and Figure X indicates that the mean peak MAT response to *hardjo* occurred seven to eight days after sero-conversion had commenced and that an initial rapid decline of GMT from a peak of 1:2613 (1:1789 - 1:3998, 95% CL) to 1:610 (1:402 - 1:967, 95% CL) occurred in only three to four weeks and was followed by a gradual decline to 1:155 (1:91 - 1:269, 95% CL) over the next 11 months.

The serological data from the other six groups of calves experiencing *hardjo* outbreaks (Tables 4.2 to 4.7 inclusive) summarised in Figure XI suffers to a far greater extent the problems discussed above, since they were tested at less frequent intervals and were therefore aligned with less precision. Thus it is not unexpected that the mean peak titres are lower for these groups than that presented in Figure X nor that mean titres at various times show some variation. Nevertheless, the same trends are apparent in both Figures X and XI; titres decline relatively quickly from peak levels over the first 50 days from sero-conversion and then tend to decline more gradually, with the range of mean titres in Figure XI being distributed symmetrically about the means presented in Figure X. Thus the data from these latter six outbreaks confirm the relevance of the titre decay curve presented in Figure X.

The relatively short period of rising titre observed in this study is in agreement with the range of 3 to 14 days in experimentally-infected cattle reported by most workers (Roth and Galton, 1960; Fennestad, 1963; Sullivan, 1970a; 1970b; 1972; Farina *et al*, 1972; Hodges and Ris, 1974; Ellis and Michna, 1977). The peak titres generally lie nearer to the lower limits of the range most frequently reported for animals experiencing both experimental and natural *hardjo* infection (Roth and Galton, 1960; Sullivan, 1970a; Sullivan and Callan, 1970; Hoare and Claxton, 1972; Hodges and Ris, 1974; Johnson *et al*, 1974; Ellis and Michna, 1977) and none developed titres as high as those occurring in some individuals observed by Sullivan (1970b), Farina *et al* (1972) Sullivan (1972), Ellis *et al* (1976) and Ellis and Michna (1977).

It had been observed in studies involving experimental

infections of calves with various leptospiral serovars that members of the *Hebdomadis* serogroup provoke lower peak agglutinating titres than members of other serogroups (Fennestad, 1963; Robertson and Boulanger, 1963; Hodges and Ris, 1974). It has also been reported that peak homologous titres in *hardjo* infections are generally lower than those occurring in natural infections with other serovars in New Zealand (Hodges, 1975; Anon, 1975c). Data presented by the Ruakura Animal Health Laboratory from two studies also show that a much higher proportion of *pomona* reactors (34% and 44%) than *hardjo* reactors (16% and 16%) have titres in excess of 1:2000 (Anon, 1974a; 1974b) and similar results were obtained from the random surveys reported in Chapter Two. In this study, of the 76 calves observed to sero-convert to *hardjo* only 18 (24%) developed peak titres greater than 1:2000 and 41 (54%) had peak titres lower than 1:1000. This fact demonstrates that the use of an MAT titre of 1:2000 or even 1:1000 as a diagnostic level for bovine *hardjo* infection is inappropriate. It is strongly believed that these facts indicate that under New Zealand conditions the use of the MAT on either single sera, or paired sera taken a few days apart, is of little use in the detection of recent cases of *hardjo* infection. Additional support for this arguement can be found in the data presented in Tables 4.1 to 4.9 which show that although high titres (greater than a coded titre of seven or 1:1536) generally occur in recently-infected animals, they occasionally occur more than 200 days after infection, and that peak titres are reached so rapidly that it is rare to observe a rising titre. Coded titres in the range of four to six (1:384 - 1:768) commonly occur in animals at all stages of infection. These observations are in conflict with the opinion of Ellis and Michna (1976b) that bovine *sejroe* titres $\geq$ 1:300 indicated recent infection.

Very few animals became sero-negative during the study period and the persistance of *hardjo* titres for as long as an estimated 780 days (Table 4.9) considerably exceeds the previous maximum of 322 days reported by Johnson *et al* (1974). The estimated age of titres in Table 4.9 is based on observations on the epidemiology of *hardjo* infection in this herd (Chapter Seven) which indicate that virtually all new cases occurred in the winter months of July to September and also by extrapolating back from the group mean

titres observed in this group of cattle by comparing their titre decay curve (Figure XIII) with the standard curve presented in Figure X. Thus it can be estimated that the mean time of sero-conversion for these animals was about 40 days earlier than their initial test in September 1975. Even if this estimated date for the onset of infection is not taken into account, some of these animals remained sero-positive for 740 days.

Although there are statistically non-significant fluctuations in the mean titre of these animals, their titre decay curve (Figure XIII) shows a continuing declining trend and is hyperbolic over the period studied. It would appear reasonable to extrapolate onwards and suggest that most animals will remain sero-positive, with low declining titres and possibly with minor fluctuations for many years. Since only 30% of New Zealand dairy cattle are more than five years old (Anon, 1951c) it is likely that many cows infected at about one year of age will remain sero-positive for life. The question of the biological significance of the fluctuations of titre observed is discussed in Chapter Five, but it may be noted here, that apart from fluctuations in group mean titres, individual animals show small fluctuations at different times (e.g. Table 4.8). It is likely that much of this fluctuation is attributable to the precision of the MAT.

The highly significant correlations between initial and final titres for calves sero-converting shortly before or during the study period indicate that the degree of the initial serological response also influences the magnitude of the residual titre. The fitted regression lines, shown in Figures XII and XIV, relating initial and final titres in two groups of calves, indicate that mean coded titres in these groups declined by different amounts. The coded titres shown in Figure XII declined by an average 62% over the 404 days study period while those shown in Figure XIV had declined by an average 89% over 639 days. These observations indicate that the overall rates of titre decay were different in these two groups and this comparison further suggests that titres decline relatively more slowly in the second year after sero-conversion. It should be noted that these rates of decay refer to logarithmically coded titres, not true titres, and that a

decline of 50% in say a coded titre of eight to a coded titre of four represents a 94% decline in true titres from 1:3072 to 1:192. Rates of decay cannot be determined for true titres without a logarithmic transformation as true titres decline logarithmically.

Thus both time from infection and the degree of initial serological response are major factors in determining the residual titre in a given individual at a given time. The range of host and organism factors influencing this latter component were not investigated in this study. However, the investigation does confirm their importance in influencing the length of the seropositive phase of convalescence as previously discussed by Turner (1968).

Cross-reactions were not subjected to a detailed investigation in this study, but were observed in eight calves intensively studied at the time leading up to and immediately subsequent to sero-conversion (Table 4.10). Low titres in the range of one to four (1:24 to 1:192) to serovars *pomona, copenhageni, tarassovi, ballum,* or *canicola*, never lasted longer than 21 days. Also two calves, G62 and G57, developed low titres to *pomona*, lasting two and four weeks respectively, immediately prior to sero-converting to *hardjo*. Alexander and Evans (1962) demonstrated that although calves with *hardjo* titres at unknown stages of infection cross-reacted strongly with other members of the *hebdomadis* subgroup they did not appear to cross-react with members of other serogroups. Sullivan (1970b and 1972) did not observe cross-reactions to members of other serogroups in *hardjo*-infected calves. In contrast serogroup cross-reactions occurring in the early stages of infection have been reported in calves experimentally-infected with *sejroe* and *hardjo* (Fennestad, 1963; Hodges and Ris, 1974). These cross-reactions may explain in part the association of low titres to serovars *copenhageni, tarassovi* and *ballum* with higher *hardjo* titres observed in the surveys reported in Chapter Two. They indicate that the detection of a small number of low titres to some serovars in serological surveys of cattle populations with a high prevalence of *hardjo* titres should be interpreted with considerable caution.

Summary.

1. Calves naturally infected with *hardjo* commence sero-converting within 17 to 24 days of exposure to infection.
2. Peak titres in the range of 1:768 to 1:8689 appear within 7 to 14 days after sero-converting though it is likely that some animals develop peak titres even lower than 1:768.
3. It is considered that the MAT has very little value in the detection of new cases of *hardjo* infection except in those few animals detected with titres of more than 1:2000.
4. An initially logarithmic rate of decline in *hardjo* titres during the first few months after sero-conversion appeared to slow by the end of the second year after sero-conversion and tended to become linear.
5. Titres persist at low and declining levels for at least two years and probably for life in the majority of cows becoming infected during the first two years of life.
6. Both the level of the initial titre and the length of time since infection have major influence on the level of a convalescent titre in a given individual at a given time.
7. During the first few weeks of bovine *hardjo* infection low MAT cross-reaction titres to members of unrelated serogroups occur.

CHAPTER FIVE

SEROLOGICAL OBSERVATIONS IN ADULT CATTLE.

Introduction.

Leptospiral agglutinins have been reported to persist in some cattle for four to ten years after sero-conversion (Morse *et al*, 1955; Roberts, 1958; Hanson, Mansfield and Andrews, 1964). Hanson (1977) attributed this persistance for lengthy periods to the possible stimulation of IgM production by related antigens. Considerable variation in the length of persistance of *pomona* titres was reported by Morse *et al* (1955) who observed titres ranging from 1:100 to 1:10,000 29 months after infection. However, it appears that persistant titres are usually substantially lower than those initially observed (Morse *et al*, 1955; Roberts, 1958).

In order to determine if changes occurred in the serological status of adult cattle with persistant *hardjo* titres a surveillance programme was undertaken at the Massey No. 1 Dairy Farm. In addition, serological changes occurring at calving and the relationship of titre to age were examined.

Materials and Methods.

The Herd.

The Massey No. 1 dairy herd has been fully described in previous chapters. In this study cattle were selected to test possible differences between spring and autumn calving groups. Initially two groups of adult cattle were selected from the milking herd to provide random samples of those cattle calving in spring and in autumn, and as animals were culled from each group they were replaced by random selection from other members of their groups. These cattle were sampled at approximately three-monthly intervals, though these intervals varied during calving periods, when some animals calved up to two and a half months before others.

Before the spring 1976 and autumn 1977 calving periods samples of cattle were selected, and they were sampled six weeks prior to calving, at calving and approximately six weeks after calving.

These sampling times were chosen in order to determine whether serological changes in the dam accompanied the production of colostral immunoglobulin.

Serological Examination.

The serological procedures used were those described in Chapter Two. A proportion of sera tested at a previous test were included with those examined at each new test, in an attempt to ensure that the serological changes observed were real changes and not artefacts of poor test precision.

Statistical Methods.

The statistical methods used in this study are described in Appendix II.

Results.

Comparisons between cows calving in spring and autumn.

The mean coded *hardjo* titres of sera collected from cows which had calved in the spring, or in autumn, were compared in September 1975, October 1976 and September 1977 (Table 5.1). On each occasion there was no significant difference between the two groups ($P > 0.30$, $P > 0.20$, $P > 0.30$ respectively).

Table 5.1

Mean coded *hardjo* titres of cows calving in spring or in autumn.

Test Period	Calving Group	Mean Titre	S.E. of Mean	Sample Size
September -	Spring	2.96	0.33	24
October 1975	Autumn	2.75	0.26	24
October 1976	Spring	3.25	0.23	36
	Autumn	3.07	0.21	28
September 1977	Spring	1.96	0.18	42
	Autumn	2.07	0.20	41

Prevalence of titres to leptospiral serovars in adult cattle at the No. 1 Dairy Farm.

All sera collected from cattle throughout the study period were tested against *hardjo, pomona, ballum, copenhageni* and *tarassovi*. Over the two year surveillance period approximately 700 sera were collected from 133 different cows. Seven sera reacted at a titre of 1:24 to *ballum*, three to *tarassovi* and one to *copenhageni*. In no case were these low titres detected in more than one serum sample of a series taken from any individual cow.

Titres to *hardjo* were detected in almost every cow at some time. Only four cows (3.0%) were consistantly sero-negative to *hardjo*, but a further eight cows (6.0%) had low fluctuating titres which were undetectable at various times during the study period. Any sample of adult cow sera, taken at any time during the study period, had well in excess of 95% sero-positives to *hardjo* (Table 5.2). Titres to *pomona* were found in 40 (30.1%) of the cows sampled throughout the study period, though their prevalence declined as the study advanced and only 15 of 83 cows (18.1%) had *pomona* titres in September 1977, when this part of the study was terminated (Table 5.3). There was also a relatively higher proportion of cows with *pomona* titres that had low fluctuating titres that became undetectable at various times (43%).

Apart from these animals, with low level fluctuating titres to *hardjo* and *pomona*, no adult cow was observed to sero-convert throughout the study period, and none developed a rising titre indicative of reinfection or an anamnestic response.

Prevalence of hardjo and pomona titres in different age groups.

Sera were collected from 133 cows which had experienced one or more gestations (Table 5.2). The cows sampled represented all age groups present in the herd. The numbers of animals sampled in each age group and the proportions which were sero-positive and sero-negative are shown in Table 5.4. Titres to *hardjo* were evenly distributed amongst all age groups but *pomona* titres only occurred in cows aged five years, or older, in July 1976. This distribution is significantly different from one which would be expected by chance alone ($P < 0.001$). However, the distribution

Table 5.2.

Coded *hardjo* titres by month of sampling in cows from the No. 1 dairy farm.

		Month in which serum sample was collected																							Calving Date		
	1976				'76												'77										
ID	Age	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	75	76	77
1	6		3			4			4						4	3	4		2					3		2	13/9	9/9	6/8
2	5												1		2	2	3		2					3		1½	-	4/8	25/8
3	2												3									3				2	-	19/7	3/9
4	4											5			5	4	5		3½							3½	-	24/7	1/7
5	8		1			2							2		2	1	2		1							1	7/8	8/8	10/8
6	12										2	2			3	3	3				2	4	3			2	-	4/3	26/4
10	3											6												3		3½	-	24/7	13/8
11	6																			4		4½		3		3	-	-	3/3
12	4	2	K																3*			5				3½	-	-	29/1
13	2																		2½			3½				2	-	-	22/1
17	8											5			4	3	4		K								-	14/4	-
18	2											6	5														-	8/7	-
20	10	4			5						3		K														-	28/4	-
21	9		4			6			6				3		4	4	4		3					3		3	3/8	8/8	2/8
25	6	3			5						3				4	K											-	27/4	-
27	7	5			7						3				5	4	5				3	3½	3			3	-	15/4	3/4
28	2								3				5		4	3	4				2	3	2½			2½	-	-	22/4
29	2													5												3	-	20/9	13/8
30	3												5	4	4	4	5		3					5	K		-	1/9	-
31	2																		4			5				2½	-	-	19/1
32	11	1			4				3		1	3			2	K			1*			2				0	-	13/3	24/1
34	2												5														-	16/7	-
36	2												3											3½		1½	-	4/7	11/9
37	4												3														-	4/8	-
38	6	3			2				3		2	4			4	3	4					4	3			1	-	31/3	26/2
39	8		5			7			K				4*													1	15/8	3/8	28/8
42	11				3				3		1	2			2	K											-	9/3	-
43	4																			0		0	0			0	-	-	2/3
44	2												4											5		2	-	19/8	12/9
45	2												4													3½	-	16/7	1/8
46	13	2			3	K						5*												3½		3	-	26/7	5/9
49	2												5									K					-	16/7	-
47	8	5			6						3				2	K											-	21/5	-

K = culled

* = replacement 2 year old.

Table 5.2 (continued)

ID	1976 Age	Month in which serum sample was collected: 1976 S	O	N	D	'76 J	F	M	A	M	J	J	A	S	O	N	D	'77 J	F	M	A	M	J	J	A	S	Calving Date 75	76	77
51	2												4											5		4	-	17/7	16/8
52	2												4													2	-	23/7	?
53	7		4			5			5				5		5	5	5		4							4	31/8	40/8	1/3
54	7	1			1	K							5*											4½		3½	-	7/8	13/8
57	9	1			1							K															-	-	-
58	6		2			4							2		3	3	3		2½					3		1½	28/8	14/8	2/8
60	8	2			2				2			1			2	2	2½		2	1½	K						-	25/3	-
61	7												3		4	3	4		2	K							-	8/8	-
67	4		5			6							3		4	4	4½		3	K							10/8	11/8	-
68	3														4	3	4			2		3½	2½			2	-	12/1	10/3
69	3	5			4						3				4	4	4					4	4	K			-	6/3	21/3
70	8		4			6							4		4	4	5		4			5				4	5/8	14/8	8/8
71	3		3										3		5	3½	4		3							2	8/9	5/8	10/8
72	3															3	4			2		3				1	-	9/1	9/3
73	11								6			5	K														-	12/3	-
75	7								3			2	K														-	15/4	-
76	5													1	2	1	2		2	K							-	13/8	-
77	2																		3			4				2½	-	-	26/1
79	2								2			2			2	2	4									2	-	28/2	26/3
80	8		3			4				K									5*			6				4	-	-	25/1
82	7	3			3						1				2	2	3									1	-	28/1	17/5
85	9		3										2		4	3	3		2			3	K				19/8	15/8	-
87	3		4			5													2½			3				2½	9/9	-	14/1
88	6												2		2	2	3		1							1	-	5/8	5/6
92	1																		5							4	-	-	18/2
93	9												4	2	3	2½	3		2					3		1	-	1/3	21/6
95	9												6	2	5	5	5		2½	K							-	6/9	-
97	1																		5							4	-	-	22/2
101	6												4											3		2	-	24/9	6/9
103	1																			5						4	-	-	7/3
104	1																			5½						4	-	-	25/3
106	6												3	0	4	3	4		2					3		1	-	7/9	2/9
109	1																				4		4			3½	-	-	2/4
110	6												3	0	4	3	4		2					3		1	-	29/9	19/8
111	11		0			0							1		1	1	2		0			1	K				9/8	12/8	-

K = culled
* = replacement 2 year old.

Table 5.2 (continued)

ID	1976 Age	Month in which serum sample was collected: S	O	N	D	'76 J	F	M	A	M	J	J	A	S	O	N	D	'77 J	F	M	A	M	J	J	A	S	Calving Date 75	76	77
112	4		5			7							3		4	3	3		2							1	17/9	7/8	19/8
113	8	2			2						1	K														K	-	20/5	-
114	5	3			2						1											2½	2½			4	-	20/1	1/1
115	11	4	K																		3*		4½			0	-	-	5/4
116	4																		0								-	-	28/2
117	8																		2			3	3		K		-	-	2/3
118	7														3	2	3					3	2			1	-	16/4	30/3
120	9												0	0	0	0	0		0					0		0	-	9/9	3/8
124	12													4	2	K											10/8	4/9	-
126	7	4			5						4				5	K											-	7/2	-
127	7		2			4							3	2	3	2	3		2			3				2	26/7	30/8	2/8
129	8												1		2	2	2		1					2		1	-	8/8	5/8
130	8														4	3	4					2½	3	3		1½	-	18/1	20/4
134	7							2				3			3	3	4			1			2½	2		2	-	14/3	1/3
135	7																		1				2				-	-	13/1
139	7							0				0			1	1	3			½			1	½		0	-	20/4	3/3
141	11				3						2				3	K											-	2/4	-
144	7		3			5			3				2		4	3	4		2							2	7/3	23/8	4/7
145	4		1			K																					11/9	-	-
146	7								2			2			3	3	3		2			3	2			2	-	23/3	26/2
147	8																				0	0				0	-	21/5	19/4
148	6											2	K														-	14/3	-
152	4		5			7							5		6	5	5		4					5	K		4/9	24/8	-
156	3																					3	4	3		2	-	4/3	13/4
157	4																			3			4			4	-	27/1	26/3
158	6		0			1							1		2	1	1		0				0	1		0	6/8	11/6	24/4
159	4		3			4				K																	23/7	-	-
160	6																			2		4		K			-	-	5/3
161	5	2			2							K															-	-	-
165	7												4	2	3	2½	4		2							1	-	4/9	5/8
166	4												3											4½		3	-	3/8	25/8
171	5												3		4	3	4		3				4			2	-	10/8	4/8
172	6								3			5			4	3	4					2	4	2½		2	-	14/3	25/4
173	7								2			5			4	3	4		3				3	3		2	-	11/3	28/2
174	7	2			3						0				1	1	2					1	2	2		1	-	28/2	15/4

K = culled
* = replacement 2 year old.

Table 5.2 (continued).

		Month in which serum sample was collected																						Calving Date		
	1976					'76											'77									
ID	Age	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	75	76	77
175	6																		2			3	3				-	-	26/12
177	6		3			5				K																	6/8	-	-
178	7																				2½	3	3			2	-	1/1	31/3
180	6											2			2	2	3		2					2½		1	-	14/8	24/8
181	6																			3		3	3½				-	28/5	21/3
187	5																					3	3			3	-	-	15/3
191	5											3			4	3	4		3		•			3		2	-	22/8	15/8
192	6		3			5				K																	26/7	-	-
195	7											1			2	2	3		1½					2			-	26/8	1/8
199	5														3	2	3							2		1½	14/3	-	15/8
202	5	2			1						0				2	K											-	30/4	-
206	4	2			2						2				2	2	4			2		2½	2½			1	-	4/3	2/3
207	5																				½	2	1			1	-	13/1	24/4
208	4	4			3	K																					-	-	-
214	11	2	K																								-	-	-
215	5											3			4	3	4			3		4	2½			1	-	1/3	2/3
216	5	2			2				2		0	3			3	2	3							2½		1	-	17/4	4/8
218	4																				3½	4	4			3	-	20/4	31/3
221	4											2			3	2	4		1½					2½		1½	-	11/8	21/8
222	4											2			2	2	3		2							1	-	21/8	23/8
223	4											3	2		3	2	3		2						K		-	21/9	-
224	3		0			2						2			1	1	3		1			1½				0	12/8	28/9	2/8
225	3		5			6						4			5	5	6		4					5		4	14/8	13/8	11/8

K = culled

Table 5.3

Coded *pomona* titres by month of sampling in cows from the No. 1 dairy farm.

		Month in which serum sample was collected																							Calving Date		
	1975	'76													'77												
ID	Age	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	75	76	77
5	7		4			6									4	3	3		2							2	7/8	8/8	10/8
6	11				3							3			3	2	2				1½	3	3			3½	-	4/3	26/4
32	10		2									2			2	K[α]											-	13/3	24/1
38	5	4			3				2		3	4			4	3	1½					3	2½			4	-	31/3	26/2
46	12	3			4	K																					-	-	-
47	7	3			1										2	K											-	20/5	-
52	6		3			3							3		2	2	2		1							2	31/8	10/8	1/8
54	6	5			4	K																					-	-	-
57	8	1			0							K															-	-	-
61	7	1			0				0			0			1	0	0		½	1	K						-	25/3	-
76	4													1	2	0	0		0	K							-	13/8	-
79	7								1			2			2	2	1									2	-	28/2	26/3
80	7		0			1				K																	-	-	25/1
82	6	3			3						2				3	2	1						0			3	-	28/1	17/5
85	8		2										2		2	0	0		0			0	K				19/8	15/8	-
88	5												3		3	0	2		2							2	-	5/8	5/6
93	8												0	2	1	0	0		0					0		0	-	1/9	21/8
95	8												4	4	4	4	4		3	K							-	6/9	-
104	8											2			0	K											-	-	-
113	7	3			2						0		K														-	20/5	-
114	4	5			4						2												3		K		-	20/1	1/1
115	10	3	K																								-	-	-
120	8												3	3	2	2½	2		1					0		3	-	9/9	9/8
124	11													2	1	K											10/8	4/9	-
126	6	1			1						0				0	K											-	7/2	-
127	6		2			5							3	2	2	2	2		1				2½			2	26/7	30/8	2/8
129	7												3		4	0	0		1½					0		3	-	8/8	5/8
130	7														4	1	2				0	3	2			0	-	18/1	20/4

α = Culled

Table 5.2 (continued)

ID	1975 Age	Month in which serum sample was collected				'76											'77								Calving Date		
		S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	75	76	77
134	6								1						2	0	2			0		2	1			2½	-	14/3	7/3
139	6								0			2			0	0	0		0			2	1			0	-	20/4	3/3
141	10				4						2				4	K[α]											-	2/4	-
146	6								1			3			3	2	1		0			2	3			3½	-	23/3	26/2
148	5											3	K														-	14/3	-
160	5																			½		2½	K				-	-	5/3
165	6												1	1	1	0	0		0							0	-	4/9	5/8
171	4												3		3	3	2		2			3½				3	-	10/8	4/8
174	6	1			1						0				2	0	1				0	1	0			1	-	28/2	1 5/4
199	4														5	3	4							3		4	14/3	-	15/8
202	4	2			0						0				1	K											-	30/4	-
214	10	1	K																								-	-	-

α = Culled

of *pomona* titres in cows aged five years or more is not significantly different from one expected by chance alone ($P > 0.20$).

Table 5.4

Numbers of cattle sampled in each age group and proportions sero-positive and sero-negative based on their notional age at July 1976.

Age(years)	Sample Size	*hardjo* Sero-positive	*hardjo* Sero-negative	*pomona* Sero-positive	*pomona* Sero-negative
1	9	100%	0%	0%	100%
2	17	100%	0%	0%	100%
3	10	100%	0%	0%	100%
4	18	89%	11%	0%	100%
5	15	100%	0%	42%	58%
6	18	100%	0%	22%	78%
7	19	100%	0%	52%	48%
8	12	92%	8%	67%	33%
9	7	86%	14%	86%	12%
≥10	11	100%	0%	64%	36%
Total	133	97%	3%	30%	70%

During the course of this study 21 of the 40 cows with *pomona* titres were culled from the herd for reasons of age or poor production.

Changes in leptospiral titres of cows which were sampled repetitively.

The precision of the MAT procedure used in this laboratory was estimated on the basis of titres recorded for 600 sera tested on two separate occasions. In addition the precision of reading individual test results was tested on 73 sera tested twice, using a double blind technique. The results of these repeated tests (Table 5.5) indicate that the precision between tests is lower than the precision of reading a given test. However, in both cases more than 90% of sera had titres at the second test which were within one dilution of that obtained at the first. The result implies that the 90% confidence limit of titre estimation

is within ± one dilution. Also there were almost equivalent numbers of higher or lower titres recorded at the second tests so that the mean coded titres at each test were virtually identical.

Table 5.5

Precision of MAT technique.

Change in coded titre.	Numbers and proportions of sera Tested on two separate occasions (n = 600).		Read twice at the same test (n = 73).	
0	$\frac{345}{600}$	57.5%	$\frac{54}{73}$	73.9%
± ½	$\frac{87}{600}$	14.5%	$\frac{10}{73}$	13.7%
± 1	$\frac{120}{600}$	20.0%	$\frac{8}{73}$	11.0%
± 1½	$\frac{13}{600}$	2.2%	—	0%
± 2	$\frac{30}{600}$	5.0%	$\frac{1}{73}$	1.4%
± 2½ or more	$\frac{5}{600}$	0.8%	—	0%

Hardjo titres of sera collected from 131 cows at various times over the two year period September 1975 to September 1977 are shown in Table 5.2. Inspection of this table, and also Table 5.3, which recorded sequential *pomona* titres observed in 40 animals over the same period, demonstrates that titres in individuals were relatively constant. Most of the fluctuations observed in these titres were generally within the range of plus or minus one titre dilution as was predicted by the estimate of the test precision; the very occasional fluctuations of plus or minus two dilutions which were observed were no more frequent than was predicted by the precision estimates.

There was, however, a slight declining trend in titres over the period of observation. There were 20 cows, first sampled in September and October 1975, which were finally sampled in September 1977. Their mean coded titres had declined from 2.85 ± 0.33 to 1.85 ± 0.28; the regression equation of 1977 titres (y) on 1975 titres (x) was $y = 0.07 - 0.62x$. Similarly, 37 cows sampled in August 1976 were again sampled in September 1977 and mean coded

titres of these animals had declined from 2.97 ± 0.20 to 1.86 ± 0.19; the regression equation of 1977 titres (y) on 1976 titres (x) was y = 0.08 - 0.60x. In both cases the regression coefficients were highly significant ($P < 0.001$). The mean ages of animals in these groups at the commencement of the study periods were 5.56 ± 0.44 years and 5.08 ± 0.38 years respectively, and there were relatively many more two and three-year-old animals in the second group.

Twenty-two cows which calved in spring 1976 and autumn 1977 were sampled four to six weeks before calving, within 48 hours of calving and four to eight weeks after calving (Table 5.6). In only four cows was there no decline in titre observed at calving and three of these cows were sero-negative throughout. Mean coded *hardjo* titres were significantly ($P < 0.05$) lower than before or after by a one-way analysis of variance.

Association between hardjo titres and age of cow.

MAT titres were obtained for 127 cows between April and September 1977. A correlation and regression analysis was conducted between the coded titres of serum samples taken closest to July 1977 and the ages of the cows at July 1977.

The corrected correlation coefficient obtained was -0.49 and was highly significant ($P < 0.001$). This degree of correlation indicates that older animals tend to have lower titres and that approximately 24% of the variation in cows' titres may be attributable to variation in age. The fitted regression line (coded *hardjo* titre = 4.39 - 0.23 x Age) indicates that the decline in titre with age is gradual with an average decline of only 0.23 coded units of titre per year of age.

Discussion.

It was apparent, at an early stage in these investigations of the Massey No. 1 dairy herd, that the management of the spring and autumn calving cattle was not identical. Although some cows moved between these groups in alternate years, and although there were frequent periods of contact between the two groups, they were largely grazed as separate groups and calved under very different climatic conditions. It was therefore considered necessary to

Table 5.6

Changes in coded *hardjo* titres occurring at calving.

Cow Identification	Coded *hardjo* Titre: 3 - 6 Weeks Before Calving	Within 48 Hours Of Calving	4 - 8 Weeks After Calving
1	5	3	4
27	5	3	3½
30	5	4	4
43	0	0	0
68	4	2	3½
72	4	2	3
93	4	2	3
95	6	2	5
106	3	0	4
113	5	5½	4½
116	0	0	0
120	0	0	0
121	5	3½	5
124	5	4½	5
127	3	2	3
134	4	1	2½
139	3	½	1
146	3	2	3
165	4	2	3
173	4	3	3
215	4	3	4
223	3	2	3
Totals 22 animals	79	47	67
Means	3.59 Aa*	2.14 Ab	3.05 Aa

* Notation: Duncan's lettering; those means with a capital letter in common do not differ at the 1% level of probability and those with a lower case letter in common do not differ at the 5% level.

select sufficient samples of animals from both groups at various times during the study period to allow a statistical comparison between the prevalence and mean of *hardjo* titres. The prevalence of titres ≥ 1:17 to *hardjo* was consistently in excess of 95% in both the spring and autumn calving groups and the mean titres in each group were not significantly different. Therefore, all other investigations of adult cattle leptospiral titres were conducted on pooled data from spring and autumn calving cattle.

The only significant leptospiral titres detected in this study were against serovars *hardjo* and *pomona*. The small numbers of very low titres (all ≦ 1:24) to serovars *ballum, copenhageni* and *tarassovi* appear to represent cross-reactions since all were detected in sera which also had titres of up to 1:400 against *hardjo* or *pomona*. No sero-conversion to these former three serovars were detected in any cattle during the course of this study except for the low titre cross-reaction titres reported in Chapter Four in yearlings recently infected with *hardjo*. Also the low titres detected to these serovars in adult cattle were never detected more than once in the same animal.

There were distinctly different age-related distributions of titres to *pomona* and *hardjo* in adult cow sera. No true sero-conversion to either serovar was observed in adult cows throughout the two year study period, though fluctuations of low titre sera above and below the threshold of serological detection were observed in some cows.

Titres to *pomona* were detected in only 30.1% of cows tested compared with *hardjo* in 97.0%. No *pomona* titres were detected in cows less than five years old at July 1976 but they were detected in 22% to 86% of all older age groups (Table 5.4). This most significantly skewed distribution implies that no new cases of *pomona* infection have occurred in the herd since the 1971 - 1972 season. However, it is possible that new cases in the adult herd continued until 1974 when the two-year-old cattle introduced failed to become infected. Moreover, a major outbreak of *pomona* occurred in the herd in 1971, with accompanying bovine abortion, and a case

of human *pomona* infection in a dairyshed worker.

It is therefore believed that the *pomona* titres detected in cows aged five years and older, represent convalescent titres remaining from a major *pomona* outbreak which occurred five years earlier, and which was self-limiting. This belief is reinforced by the absence of any new case of *pomona* infection in any animal born between September 1971 and September 1977, and by the fact that the variation by age in the prevalence of *pomona* titres amongst animals aged five years or older is not statistically significant. The declining prevalence of *pomona* titres in adult animals observed during the study period is almost entirely attributable to the culling from the herd of older cows with *pomona* titres.

This conclusion is in direct contrast to the opinion expressed by Hanson (1976) that persistant titres are attributable to chronic infection. Nor is there any evidence from this study to support Hanson's (1977) suggestion that these persistant titres are caused by infection with related antigens. The skewed age distribution of *pomona* titres in the No. 1 dairy herd and the absence of any evidence of new cases of *pomona* infection, or seroconversion to *pomona*, implies that these titres are simply persistant convalescent titres. It is apparent that Hanson's need to find a consistently available antigen to explain these persistant titres stems from his misconception of the role of IgG in the MAT response of cattle to leptospiral infection (Hanson, 1973; 1976; 1977). Hanson has argued that the MAT response is primarily an IgM response and that the continuing presence of antigen is therefore necessary for the maintenance of MAT titres. The evidence presented in Chapter Six clearly demonstrates a major role for IgG antibodies in the persistant MAT reaction.

The prevalence of *hardjo* titres in the No. 1 dairy herd ranged between 86% and 100% in all age groups. This consistently high prevalence in all adult age groups in the absence of any evidence that these animals experience new infections, clearly indicates that life-long persistance of *hardjo* titres is the rule. Mathematically expressed, prevalence (P) is a function (f) of the

product of incidence (I) and the duration (D) of a condition; P = f(I x D) (MacMahon and Pugh, 1970). Amongst adult animals P (prevalence of *hardjo* titres) is very high in all ages and I (incidence of new cases) is very low beyond 18 months of age, therefore D (duration of titres) must take high values approaching the life-span of each age group in which titres were detected.

This predicted persistance of most convalescent leptospiral titres for more than nine years is in agreement with the observations of Roberts (1958) and Hanson *et al* (1964). The persistance of these titres, when considered in the light of the 15 to 17 day half-life of passively-acquired bovine immunoglobulin reported in Chapter Three, indicates a continuing synthesis of anti-leptospiral immunoglobulin throughout the life of most cows. Titres to *pomona* persist though the antigen is apparently absent from the population, and similarly, the evidence presented in Chapter Seven indicates that there is relatively little exposure of adult cows to *hardjo* antigen. It is also demonstrated in Chapter Nine that animals with *hardjo* titres show a very slight or no anamnestic response to massive challenge with this serovar.

These facts raise some intriguing questions about the nature of the immune response to leptospiral infection. Ryan (1978) has demonstrated that B lymphocytes, specifically sensitised to *pomona* antigen, are found in sows up to five years after they experienced *pomona* infection, and long after leptospiruria ceased. The mechanism by which these B cells continue synthesising low levels of anti-*pomona* antibodies is unknown but it is likely that similar mechanisms apply in the bovine.

The long-term fluctuations observed in individual titres are extremely hard to interpret. It is apparent that the precision of the MAT procedure used in this laboratory is not sufficiently great to exclude the possibility that many of these fluctuations are attributable to the test, rather than to biological phenomona. However, detailed inspection of Tables 5.2 and 5.3 does suggest a tendency for long-term fluctuations to occur in some individuals with relatively higher titres being more prevalent in the spring and relatively lower titres in the summer.

It appears that at least some of the observed long-term variation in titres is attributable to the loss of maternal immunoglobulin into colostrum just before and at calving. The data presented in Table 5.6 indicates a significant decline in maternal titres accompanying calving followed by a rise in titre, returning to near to pre-calving levels. This result is in close agreement with the report of Brandon, Watson and Lascelles (1971) that the concentration of serum IgG dropped by up to 50% in cows two to three weeks before calving. They also observed that levels returned to those observed before the decline, by four weeks after parturition.

The other confirmed change in adult cow titres observed in this study was the long-term trend for titres to gradually decline. This observation appears to explain the highly significant, negative correlation between titre and age observed in this study. The correlation coefficient of -0.49 between cow's age and titre obtained in this series agrees well with the value of -0.54 obtained for similar data from different animals in Chapter Three.

These findings confirm and extend the observations reported in Chapter Four which indicated that the rate of decline in *hardjo* titre became less as time after infection increased. The fitted regression equations calculated for 1975 and 1977 titres, and 1976 and 1977 titres, indicate that mean coded *hardjo* titres declined by 31% and 32% respectively. The 32% decline observed between 1976 and 1977 represents a more rapid rate of decline, but this is probably attributable to the presence of relatively more young animals in this group. These animals were probably still experiencing logarithmic rates of decay in true titres. Nevertheless, these rates of decay in coded titre are considerably lower than the 62% and 89% reported respectively for the first one year and first two years after sero-conversion (Chapter Four). The regression of coded *hardjo* titre on age obtained in this study indicates an average decay rate of approximately 5% per year in older animals.

These combined findings clearly demonstrate that the initial

logarithmic decline in convalescent titres is tending to become linear as time after infection elapses, and that on average, titres could persist for at least 19 years. Thus convalescent *hardjo* titres are life-long in almost all cows and as suggested by Kiktenko *et al* (1977) all titres, even those which are very low, are relevant in defining the history of leptospiral infection in a herd. A high prevalence of titres in all adult age groups implies that the endemic level of infection is close to 100%, though further studies are necessary to define the ages at which infection actually occurs.

An interesting corollary to these observations is the fact that all animals in the herd have become infected at some time. However, the concept of herd immunity implies that not all susceptible members of a population will become infected in an epidemic (Fox *et al*, 1971). This contradiction may well be unique in the study of the epidemiology of infectious diseases. It implies that the susceptibility of the herd is equivalent to, rather than less than, the summed susceptibilities of individuals within the herd. This in turn implies that infectives either remain susceptible or that there is a very long period before infectives cease shedding and become immune, thereby ensuring the infection of all susceptibles.

The detection of a very low number (3.1%) of sero-negative animals in this herd appears to contradict the arguement presented above. However, other evidence presented in Chapter Four and Chapter Nine indicates that these animals had probably lost titres. Cows have been observed to lose titres in the course of this study (Tables 4.5, 4.9, 5.2 and 5.3) and the four consistently negative animals were aged four, eight and nine years. A serologically negative cow resisted experimental challenge (Chapter Nine) and this observation may be explained by persistence of antibody not detected by the MAT as has been suggested to occur in neonates (Hathaway, 1978) and vaccinates (Negi, Meyers and Segre, 1971; Hanson, 1973; 1977).

The precision estimate of the MAT technique used at this laboratory indicates that 90% of titres were repeatable to within ± one doubling dilution. This degree of precision is the same as

that obtained by Ryan (1978) and Graves and Faine (1970). A study on the precision of the MAT conducted by Graves and Faine (1970) indicated that up to a 40% variation in antigen concentration and incubation times between 20 and 140 minutes produced no variation in test precision. Antigen concentration was always held within these limits in the present study and a standard 90 minute incubation time was always used. The data on MAT precision presented in Table 5.5 indicates that there is a greater variation in test results between tests than between readings within the same test. This suggests that while reading errors account for some of the variation observed, errors in serum identification, serum dilution and in antigen concentration account for most of the observed variation.

Summary.

1. More than 95% of adult cows in the No. 1 dairy herd had *hardjo* titres $\geq$ 1:17 and 30.1% had *pomona* titres.
2. No significant titres were detected to serovars *ballum, copenhageni* and *tarassovi*.
3. No adult animal was observed to sero-convert to any leptospiral serovar or to develop an anamnestic response.
4. *Pomona* titres were observed only in cows aged five years or more and appeared to be convalescent titres resulting from a major outbreak of infection which had occurred in the herd five years earlier.
5. Convalescent leptospiral titres appear to persist for life in most cows and it appears that the continuing presence of antigen is not necessary to maintain these titres.
6. There is some indication that long-term low-level fluctuations in convalescent titres may occur though these could be explained by the precision of the MAT.
7. Titres fall at calving and rise again four to eight weeks later. This appears to be caused by the loss of maternal immunoglobulin into the colostrum.
8. The rate of decline of titres slows as time after infection elapses from a geometric to a linear rate of decay and the regression of titre on age predicts that convalescent titres should persist for 19 years.
9. The extremely high prevalence of convalescent titres in

adult cows indicates that the endemic level of infection in this herd is close to 100%. The implications of this observation with regard to the concept of herd immunity are discussed.

10. The precision estimate of the MAT technique used in this study indicates that 90% of titres were repeatable within ± one doubling dilution.

CHAPTER SIX

IMMUNOGLOBULINS AND LEPTOSPIRAL INFECTION

Animals normally respond to experimental or natural infection with leptospiral serovars by producing antibodies. These antibodies have been detected by a variety of serological methods but most emphasis has been placed on the use of agglutination tests, particularly by the microscopic agglutination test (MAT) (Turner, 1968; Hanson, 1973; 1977; Alexander, 1976). A number of studies have been conducted to establish the class of host immunoglobulin involved in agglutination reactions.

Hocker and Bauer (1965) reported that only IgM agglutinating antibody was produced by rabbits challenged repetitively over an 18 month period with serovar *biflexa*. However, Pike and Schultz (1965) and Pike *et al* (1965) found that a proportion of the agglutinating activity in serum from rabbits experimentally challenged with serovars *pomona, grippotyphosa* and *sejroe* was attributable to IgG antibody, and that this proportion increased as time after infection elapsed. At 44 days post infection (p.i.) 83% of anti-*sejroe* activity was IgG antibody. The transition from an initial pure IgM to a later mixed IgM/IgG or solely IgG response has since been confirmed by observations made on rabbits experimentally infected with a variety of serovars including *biflexa, grippotyphosa* and *copenhageni* (Samedov and Sharabchiev, 1969; Graves and Faine, 1970; Kadlcik *et al*, 1973). Graves and Faine (1970) observed, in a single rabbit repeatedly inoculated with *biflexa*, that at 216 days p.i. 33% of agglutinating antibody was of the IgM class though Samedov and Sharabchiev (1969) reported that the transition to a total IgG response could occur as early as 42 days p.i. Similarly, the agglutination reaction of guinea pigs experimentally infected with serovar *kennewicki* was initially entirely attributable to IgM but by 50 days p.i. IgG was the predominant immunoglobulin involved (Crawford, 1972a; 1972b). Morris and Hussaini (1974) observed in serum from three cows with natural infections of *L. canicola* and *L. icterohaemorrhagiae* of unknown duration that agglutinating activity was present in both IgM and IgG classes. They further

demonstrated that this activity was in the IgG_1 but not in the IgG_2 subclass.

A number of investigators have studied immunoglobulin classes involved in leptospiral agglutinating responses of humans following both vaccinations and natural infections. Initial reports stated that only 19S antibody was involved (Hartman *et al*, 1964; Lataste-Dorolle *et al*, 1964) although in 10 of 36 cases with agglutinating titres of less than 1:1000, 19S antibody with anti-leptospiral agglutinating activity was not detected (Lataste-Dorolle *et al*, 1964). These authors did not state the stage of infection at which serum was taken. Tong *et al* (1971) reported that IgM was the predominant immunoglobulin involved in the MAT in three human cases during the first three weeks of infection.

A study by Gsell *et al*, (1971) of a human *in utero* infection showed IgM to be the predominant leptospiral-agglutinating antibody until the 26th day after birth when IgG became predominant, remaining so until the study was terminated at the 110th day after birth. In a study on sera taken from 669 human patients at various stages of infection and convalescence both IgM and IgG antibodies were shown to participate in leptospiral agglutination (Chernukha *et al*, 1976). They reported that the initial reaction up to the time of peak titre development, at about 20 days p.i., was predominantly due to IgM and that an increasing proportion of the reaction was attributable to IgG as time from infection increased. Conversely, in humans vaccinated with a heat-treated polyvalent vaccine, only low levels of IgG antibodies were formed (Shishkina *et al*, 1976). These authors postulated that the appearance of these largely IgM titres is attributable to the heat-treatment of the vaccine leaving only thermostable carbohydrate antigens. It has been previously reported that while carbohydrates stimulate the production of IgM, proteins are necessary to stimulate IgG production (Pike, 1967).

The tendency of sera from infected animals, particularly in the acute phase of infection, to cross-react with a variety of non-infecting and even unrelated serotypes has been reported by many authors (Turner, 1968; Feigin and Anderson, 1975). Most evidence

now indicates that such cross-reactivity is largely or entirely confined to the IgM component of the host immunoglobulin (Pike *et al*, 1966; Kadlcik *et al*, 1973; Chang and Faine, 1974; Chernukha *et al*, 1976).

The major immunoglobulin classes of the cow are IgG, IgA and IgM (Butler, 1969); IgG is further subdivided into IgG_1 and IgG_2 on the basis of antigenic and electrophoretic differences (Nansen, 1970). There is also evidence for the occurrence of serum IgE (Williams *et al*, 1975). Bovine IgM comprises less than 10% of serum immunoglobulin (Butler, 1969; Williams *et al*, 1975). It is eluted in the first peak from Sephadex G-200 (Butler, 1969; Duncan *et al*, 1972; Fey *et al*, 1976), it is a most effective agglutinating antibody (Rose and Roepke, 1964) and it predominates in the complement-fixation reaction (Butler, 1969).

Bovine IgG_1 and IgG_2 comprise 85% - 90% of serum immunoglobulins (Klaus *et al*, 1969; Nansen, 1970; Williams *et al*, 1975). These sub-classes are eluted from Sephadex G-200 in the second peak (Nansen, 1970; Duncan *et al*, 1972) and can be isolated by anion-exchange chromotography (Nansen, 1970; Fey *et al*, 1976) or zone electrophoresis (Duncan *et al*, 1972; Fey *et al*, 1976). IgG also has agglutinating activity and a progression from IgM to IgG agglutinating activity can be observed for up to 18 months after infection with various micro-organisms (Butler, 1969). IgG_1 has complement fixing activity (Butler, 1969) which may be blocked by IgG_2 which does not appear to be complement fixing (Plackett and Alton, 1975). In addition IgG_1 is selectively concentrated in colostrum and is the predominant immunoglobulin in this secretion (Brandon *et al*, 1971; Porter, 1972).

This study was undertaken in order to define the sequence of appearance of the various immunoglobulins during the course of natural leptospiral infection of the bovine. A series of serum samples obtained from cattle, at various times after they became naturally infected with *hardjo*, were fractionated. These sera were subjected to gel filtration or to anion exchange chromotography in order to determine which immunoglobulins were associated with leptospiral agglutinating activity at the various stages of

infection.

Materials and Methods.

Gel Filtration.

Bovine sera were fractionated on Sephadex G-200 (Pharmacia) columns. The gel beads were swollen at room temperature for 48 hours in the solution used for elution, namely, 0.85% sodium chloride (Analar) containing 0.02% sodium azide (Analar) and buffered to pH 7.2 with 0.05M phosphate buffer (Appendix III). During the swelling period fines were removed two or three times according to the manufacturers'recommendations. The swollen gel was degassed under a vacuum of 700 mm Hg until it ceased boiling after five to ten minutes. The gel was poured as a slurry, consisting of approximately two parts of gel to one part of buffer, into a column measuring 70 x 2.5cm (Pharmacia) and packed in one batch by using an attached reservoir (Pharmacia) with a constant pressure head of five to ten centimetres of buffer,

The column was operated at room temperature, under a constant pressure head ranging from five to fifteen centimetres of buffer, at a flow-rate of approximately 20ml/hr. One ml of serum was carefully applied to the top of the column at the start of each fractionation run. Approximately 24 fractions, each of 6ml, were collected by using an automated fraction collector (ISCO) in drop-counting mode. The optical density of protein in the elute at 280 nm was continuously monitored and recorded graphically using an automated system (ISCO).

Fractions were held at $4^{\circ}C$ until subjected to the MAT, usually within 24 hours and always within 72 hours of fractionation. Pooled fractions from each peak were concentrated to one ml by dialysis against polyethylene glycol (M.W.20,000).

Anion Exchange Chromotography.

Anion exchange chromotography was performed using preswollen diethylaminoethyl - cellulose (DEAE) (DE52 - Whatman). The cellulose was equilibrated to a pH of 8 using 1.0M phosphate buffer (Appendix III) and degassed under a vacuum of 700 mm Hg. Fines were removed two or three times according to manufacturers' specifications.

The column which measured 40 x 2.5 cm (Pharmacia) was packed under a pressure of 100 cm of starting buffer, 0.01M phosphate buffer pH 8, and run continuously until the elute had reached a molarity of 0.01M as assessed by the measurement of conductance using a conductivity meter (Yellow Spring Instrument Company). Elution was carried out under a continuous gradient at a constant pH of 8 using a multiple chamber mixing device (Varigrad) with 55 mls of 0.01M buffer in chambers one to seven inclusive and 55 mls of 0.60M buffer in chambers eight and nine. Serum was dialysed against starting buffer for 24 hours at 4°C before 2 ml was applied to the column. Fractions, each of 10 ml, were collected using the same collection system described above. Fractions from each peak were tested for agglutinating activity and then pooled and concentrated as described above.

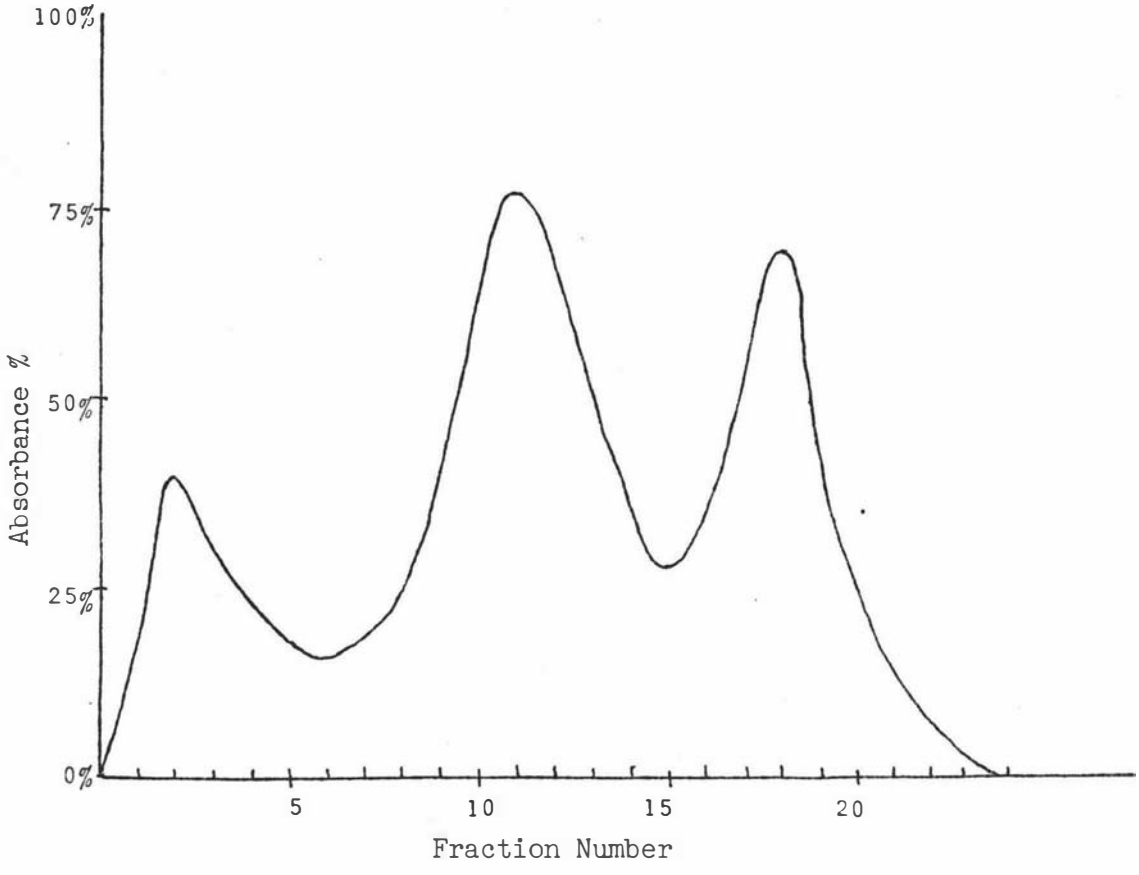
After each run the anion exchange resin was re-equilibrated by using one litre of 1.0M sodium chloride followed by sufficient 0.01M phosphate buffer to return the elute to starting buffer molarity as assessed by conductance readings.

Analysis of Serological Results.

Eight serial doubling dilutions of each fraction were prepared in isotonic saline in microtitre plates (Microtitre) and equal volumes of live antigen were added to each dilution. Since one ml of serum had been applied to the column and 6 ml fractions were collected the dilutions of each fraction ranged from 1:12 to 1:1572. Titres were read as described in Chapter Two and were converted into MAT units per fraction by taking the reciprocals of the fraction titres and rounding them to the nearest 10 units (e.g. a titre of 1:96 converted to 100 MAT units). Whole serum was also tested with each set of fractions and the reciprocal of the whole serum titre was taken as a measurement of the number of MAT units applied to the column. MAT units in the fractions of each peak were totalled to determine the number of units recovered in each peak and the amounts of the activity in each peak expressed as percentages of total activity recovered. The total number of units recovered were also expressed as a percentage of the number of units applied to the column to provide an estimate of the efficiency of recovery following fractionation.

Figure XV

Elution Profile of Bovine Serum Fractionation by Gel Filtration.



Immunoelectrophoresis.

Plates for immunoelectrophoresis (IEP) were prepared by pouring 1% Ionagar (Difco) dissolved in 0.5% sodium barbitone buffer, pH 8.6 (Appendix III), onto glass plates which were left overnight at 4°C before wells and troughs were cut. Plates measured 12 x 9cm and five troughs, measuring 6 x 0.5 cm running across the plate, and six wells, 0.5 cm in diameter, arrayed down the longitudinal axis, were cut at 1.0 cm centres and agar plugs were removed from the wells.

Aliquots of pooled and concentrated fractions, representative of the protein peaks obtained by gel filtration and cellulose chromotography, were placed in the wells, and the plates were run at 100 volts and 15 milliamps (10 volts/cm) for three to four hours. Agar was then removed from the troughs and anti-serum was added to the troughs and the plates left at room temperature for 24 hours for precipitin lines to develop. The anti-sera used were anti-bovine IgM (Miles Laboratories), anti-bovine IgG (kindly supplied by Mr D.V. Timbs, Central Brucellosis Laboratory, Wallaceville), and anti-bovine whole serum prepared in this laboratory (Appendix IV).

Serum Samples.

Representative samples were taken of the sera collected from the various groups of animals described in the three preceding chapters. In the cases of both neonates and recently-infected animals a series of samples was taken from selected individuals. For the adult animals samples were selected from animals with a range of ages and titres.

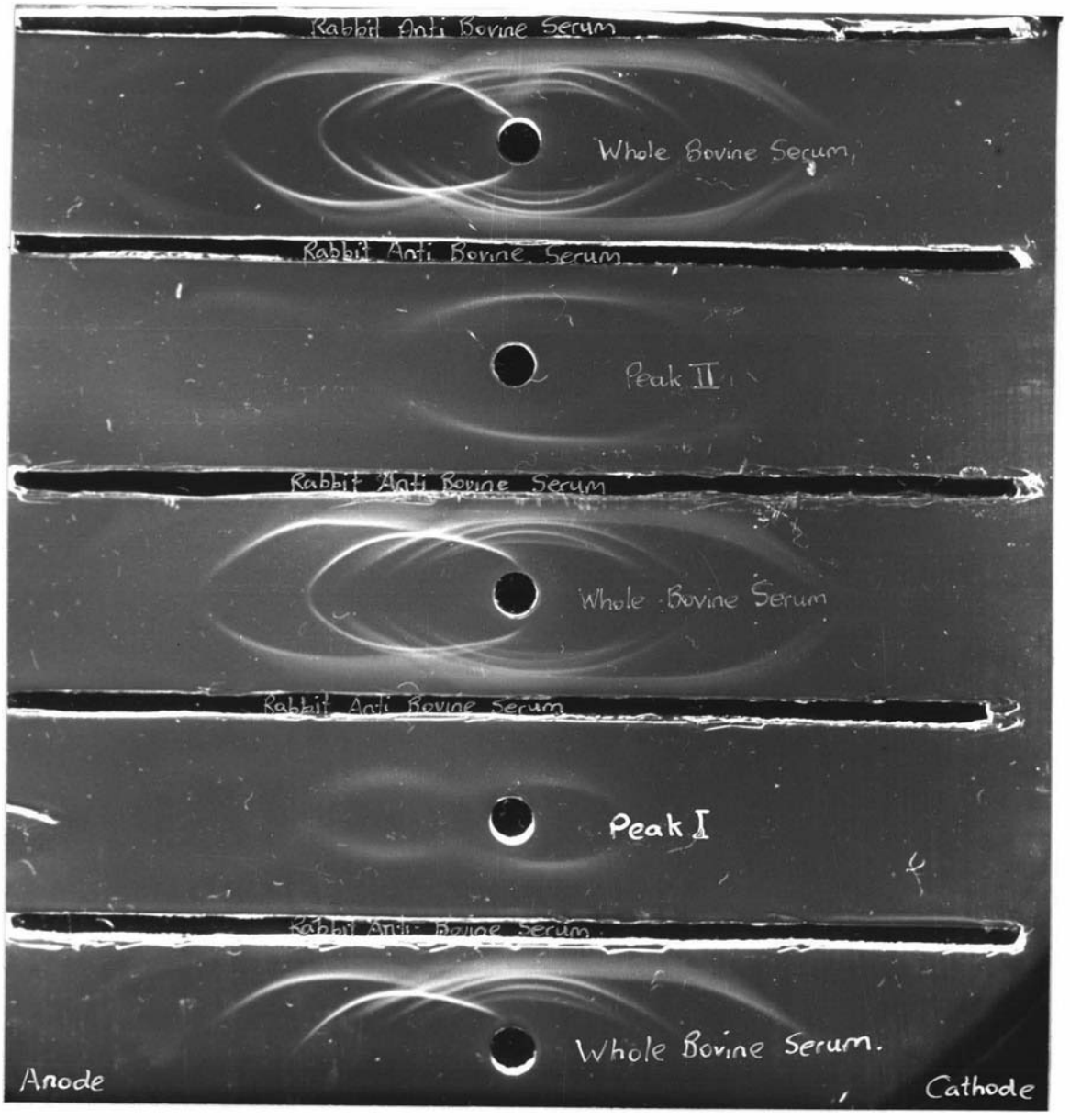
Results.

Gel Filtration.

A consistent three-peaked elution profile was obtained from each serum fractionation. Fractions 1 to 6 inclusive comprised peak I, fractions 7 to 15 peak II and fractions 16 to 24 the peak III (Figure XV). Pooled fractions were concentrated for some of the serum fractionations and were submitted to IEP. The typical precipitin reaction with anti-bovine whole serum is shown in Figure XVI. The precipitin lines indicate that IgM was found in peak I, and IgG in peak II. To confirm this observation,

Figure XVI

Immunoelectrophoretic Reaction of Concentrated Gel Filtration Fractions of Bovine Serum with Rabbit Anti-Bovine Serum.



concentrated samples of peaks I, II and III and whole serum were again run in IEP. Anti-sera to bovine IgM and IgG were used to produce the precipitin lines shown in Figure XVII. Anti-bovine IgM only produced precipitin lines against peak I and whole serum. However, while anti-bovine IgG produced strong precipitin lines against peak II and whole serum, there were trace amounts of IgG present in peaks I and III.

MAT reactions of gel filtration fractions.

The results obtained by fractionating sera taken from calves aged between one and 52 days, are given in Table 6.1. Only sera from calves which had suckled were frationated. All five samples taken from one-day-old calves had MAT activity in some peak I fractions. The amount of peak I activity for these calves ranged from 17% to 55% of the total MAT activity recovered. Only two other serum samples from neonatal calves had peak I activity. The calves from which these sera were taken (W15/23 and G62/2) were aged 15 and 21 days and the proportions of peak I activity detected in these samples were 12% and 2% respectively. The results obtained by the fractionation of paired serum samples taken from calves W4, W13, W15, W16 and W17 shortly after birth and again 10 to 20 days later indicate that the activity in peak I fractions was lost more rapidly than in peak II fractions (Table 6.1).

The results obtained by frationating sera taken at various times from five animals which experienced natural *hardjo* infection and sero-converted on 2/9/76 are given in Tables 6.2 to 6.7. The first day on which titres were detected was taken as the seventh day of infection and stages of infection at subsequent tests are based on this approximation. On day seven (Table 6.2), 88% to 100% (mean 97%) of the total MAT activity of these sera was found in peak I fractions, on day 21 (Table 6.3) 73% to 93% (mean 80%), on day 42 (Table 6.4) 29% to 66% (mean 40%), on day 105 (Table 6.5) 14% to 30% (mean 21%), on day 204 (Table 6.6) 15% to 39% (mean 21%) and on day 385 (Table 6.7) 0% to 23% (mean 8%) remained. In every case the balance of MAT activity was in peak II; some tailing of antibody activity occurred in peak III (Figure XVIII).

Figure XVII

Immunoeletrophoretic Reaction of Concentrated Gel Filtration Fractions of Bovine Serum with Anti-Bovine IgM and IgG.

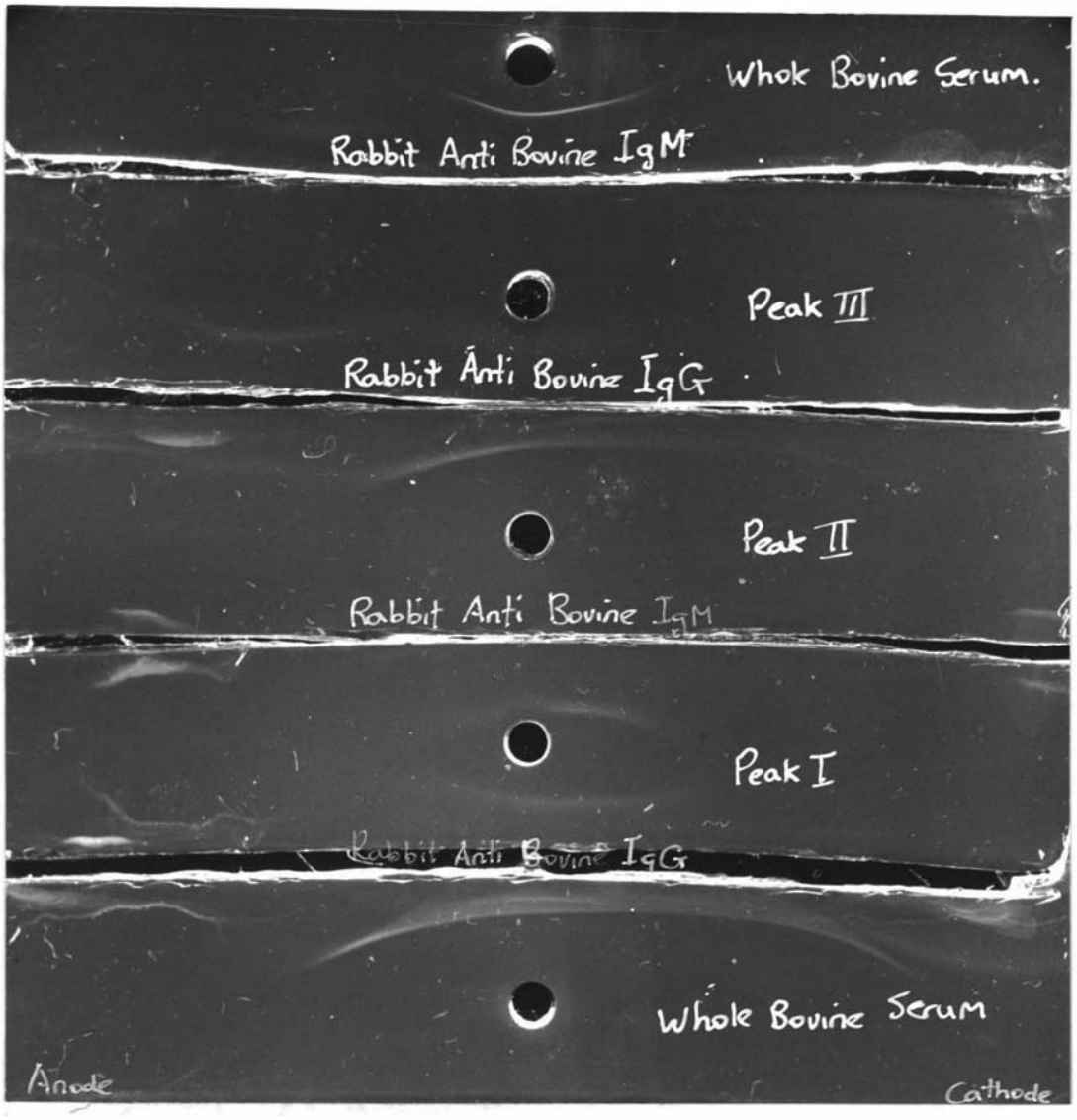


Table 6.1

Distribution of leptospiral agglutinating units in gel filtrations of sera, from calves which had suckled, taken at various times after birth.

Fraction Number	Sample Identification W4/21a	W4/21b	W13/22	W13/23	W15/22	W15/23	W16/22	W16/23	W17/22	W17/23	W12/22	G62/2	W9/22	G43/16	G52/2	W3/21	W14/22	Calf ex C139/21
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	10	-	-	-	10	-	20	-	-	-	-	-	-	-	-	-	-	-
3	20	-	10	-	20	10	70	-	10	-	-	-	-	-	-	-	-	-
4	-	-	20	-	50	10	40	-	10	-	-	10	-	-	-	-	-	-
5	-	-	10	-	20	-	20	-	-	-	-	-	-	-	-	-	-	-
6	-	-	10	-	10	10	20	-	-	-	-	-	-	-	-	-	-	-
7	20	10	10	10	20	10	40	10	-	-	-	10	-	-	-	-	-	-
8	20	20	20	10	40	50	50	20	-	10	10	20	10	10	-	-	-	-
9	50	40	30	40	50	50	30	50	-	20	20	100	20	20	20	-	-	-
10	20	40	20	40	40	20	10	20	10	40	10	140	50	20	30	-	-	-
11	10	20	10	20	10	-	10	20	10	10	10	70	10	10	20	-	-	-
12	10	-	10	-	10	10	-	20	40	10	-	50	10	-	10	-	-	-
13	-	-	-	-	-	-	-	10	20	10	-	20	10	-	-	-	-	-
14	-	-	-	-	-	-	-	-	10	10	-	20	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Whole serum	290	190	190	190	290	290	380	190	140	140	140	770	140	100	100	0	0	0
Peak I	30	0	50	0	110	20	170	0	20	0	0	10	0	0	0	-	-	-
Peak II	130	130	100	120	170	150	140	150	100	110	50	430	110	60	60	-	-	-
Recovered	160	130	150	120	280	170	310	150	120	110	50	440	110	60	60	-	-	-
Recovered%	55%	68%	79%	63%	97%	59%	82%	79%	86%	78%	36%	57%	79%	60%	80%	-	-	-
Peak I %	19%	0%	33%	0%	39%	12%	55%	0%	17%	0%	0%	2%	0%	0%	0%	-	-	-
Peak II %	81%	100%	67%	100%	61%	88%	45%	100%	83%	100%	100%	98%	100%	100%	100%	-	-	-
Age (days)	1	10	2	20	1	15	1	12	1	11	5	21	23	34	52	1	1	1

Table 6.2

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on approximately the seventh day after sero-conversion.

Fraction Number	Animal Identification G39/13	G42/13	G54/13	G55/13	G63/13	Total	Mean
1	50	30	40	20	20	160	32
2	120	100	150	100	150	620	124
3	190	320	640	440	190	1780	356
4	290	870	190	420	70	1840	368
5	160	320	30	130	20	660	132
6	40	30	10	40	-	120	24
7	-	-	-	20	-	20	4
8	-	-	10	20	-	30	6
9	-	-	10	20	-	30	6
10	-	-	-	20	-	20	4
11	-	-	-	20	-	20	4
12	-	-	-	10	-	10	2
13	-	-	-	10	-	10	2
14	-	-	-	10	-	10	2
15	-	-	-	10	-	10	2
16	-	-	-	10	-	10	2
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	770	2300	3070	1540	1150	8830	1766
Peak I	850	1670	1060	1150	450	5180	1036
Peak II	-	-	20	150	-	170	34
Total recovered	850	1670	1080	1300	450	5350	1070
Recovered %	110%	73%	35%	84%	39%	61%	
Peak I %	100%	100%	98%	88%	100%	97%	
Peak II %	-	-	2%	12%	-	3%	

Table 6.3

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on the 21st day after sero-conversion.

Fraction Number	Animal Identification G39/14	G42/14	G54/14	G55/14	G63/14	Total	Mean
1	-	100	20	0	0	120	24
2	100	260	50	70	180	660	132
3	130	220	380	140	510	1380	276
4	60	130	380	100	160	830	166
5	20	60	100	50	40	270	114
6	-	50	50	20	10	130	26
7	-	40	40	-	10	90	18
8	-	10	70	-	30	110	22
9	-	20	70	-	40	130	26
10	-	20	100	-	30	150	30
11	20	40	70	10	20	160	32
12	20	40	20	10	-	90	18
13	20	30	-	-	-	50	10
14	-	20	-	10	-	30	6
15	-	20	-	-	-	20	4
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	770	1540	1540	580	2300	6730	1346
Peak I	310	820	980	380	900	3390	678
Peak II	60	240	370	30	130	830	166
Total recovered	370	1060	1350	410	1030	4220	844
Recovered %	48%	69%	88%	71%	45%	63%	
Peak I %	84%	77%	73%	93%	87%	80%	
Peak II %	16%	23%	27%	7%	13%	20%	

Table 6.4

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on the 42nd day after sero-conversion.

Fraction Number	Animal Identification G39/16	G42/16	G54/16	G55/16	G63/16	Total	Mean
1	10	-	-	-	30	40	8
2	20	-	100	20	100	240	48
3	30	30	100	50	180	390	78
4	50	50	70	20	80	270	56
5	50	30	50	10	40	180	36
6	40	-	40	-	20	100	20
7	30	-	50	-	40	120	24
8	40	20	100	-	50	210	42
9	60	50	190	-	70	370	74
10	100	80	190	20	40	430	86
11	130	50	100	40	30	350	70
12	60	20	50	50	-	180	36
13	20	-	20	50	-	90	18
14	-	-	-	20	-	20	4
15	-	-	-	40	-	40	8
16	-	-	-	20	-	20	4
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	770	380	1150	380	770	3450	690
Peak I	200	110	360	100	450	1220	244
Peak II	440	220	700	240	230	1830	366
Total recovered	640	330	1060	340	680	3050	610
Recovered %	83%	87%	92%	89%	88%	88%	
Peak I %	31%	33%	34%	29%	66%	40%	
Peak II %	69%	67%	66%	71%	34%	60%	

Table 6.5

Distribution of leptoapiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on the 105th day after sero-conversion.

Fraction Number	Animal Identification G39/18	G42/18	G54/18	G55/18	G63/18	Total	Mean
1	20	-	20	-	-	40	8
2	20	10	20	10	10	70	14
3	20	20	30	20	20	110	22
4	10	20	50	30	20	130	26
5	10	20	50	40	20	140	28
6	10	10	40	30	20	110	22
7	10	10	20	20	10	70	14
8	20	20	20	30	40	130	26
9	90	50	40	70	50	300	60
10	130	40	50	130	100	450	90
11	140	30	60	100	70	400	80
12	100	20	70	140	50	380	76
13	40	10	80	80	40	250	50
14	10	10	80	30	10	140	28
15	-	-	60	10	-	70	14
16	-	-	50	-	-	50	10
17	-	-	10	-	-	10	2
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	580	380	770	770	580	3080	616
Peak I	90	80	210	130	90	600	120
Peak II	540	190	540	610	370	2250	450
Total recovered	630	270	750	740	460	2850	570
Recovered %	109%	71%	97%	96%	79%	93%	
Peak I %	14%	30%	28%	18%	20%	21%	
Peak II %	86%	70%	72%	82%	80%	79%	

Table 6.6

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on the 204th day after sero-conversion.

Fraction Number	Animal Identification G39/22	G42/22	G54/22	G55/22	G63/22	Total	Mean
1	-	-	-	-	-	-	-
2	20	10	-	10	10	50	10
3	50	50	20	10	20	150	30
4	50	20	20	20	20	130	26
5	40	10	10	10	20	90	18
6	10	-	-	10	10	30	6
7	50	20	10	20	20	120	24
8	150	40	20	50	40	300	60
9	380	50	50	80	40	600	120
10	190	20	50	50	30	340	68
11	100	10	30	40	40	220	44
12	50	-	10	10	20	90	18
13	20	-	-	-	10	30	6
14	10	-	-	-	10	20	4
15	-	-	-	-	-	-	-
16	-	-	-	-	10	10	2
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	1150	380	380	270	380	2560	512
Peak I	170	90	50	60	80	450	90
Peak II	950	140	170	250	220	1730	346
Total recovered	1120	230	220	310	300	2180	436
Recovered %	97%	61%	58%	115%	79%	85%	
Peak I %	15%	39%	23%	19%	27%	21%	
Peak II %	85%	61%	77%	81%	73%	79%	

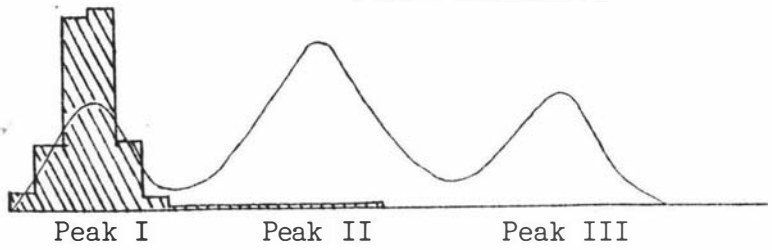
Table 6.7

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from naturally-infected cattle on the 385th day after sero-conversion

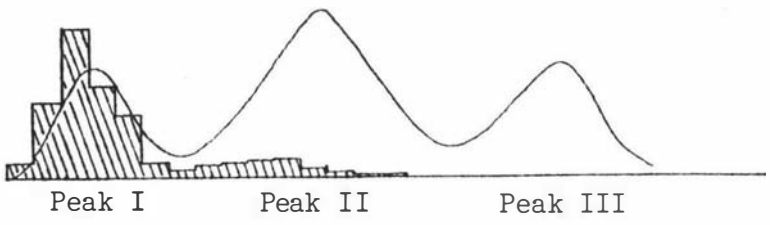
Fraction Number	Animal Identification G39/37	G42/37	G54/37	G55/37	G63/37	Total	Mean
1	-	-	-	-	-	-	-
2	10	-	-	10	-	20	4
3	10	-	-	10	-	20	4
4	10	-	-	10	-	20	4
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-
7	-	-	-	10	10	20	4
8	10	-	20	10	20	60	12
9	10	10	20	40	20	100	20
10	20	20	30	20	20	110	22
11	50	20	10	20	10	110	22
12	50	20	10	-	10	90	16
13	70	10	-	-	-	80	14
14	40	10	-	-	-	50	10
15	20	-	-	-	-	20	4
16	10	-	-	-	-	10	2
17	10	-	-	-	-	10	2
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	380	140	190	190	140	1040	
Peak I	30	0	0	30	0	60	
Peak II	290	90	90	100	90	660	
Total recovered	320	90	90	130	90	720	
Recovered %	84%	64%	47%	68%	64%	69%	
Peak I %	9%	0%	0%	23%	0%	8%	
Peak II %	91%	100%	100%	77%	100%	92%	

Figure XVIII

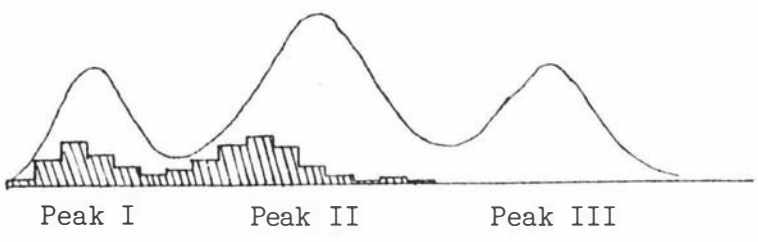
Mean Distribution of Leptospiral Agglutinating Activity in Gel Filtration Fractions of Bovine Sera, at Various Times After Infection.



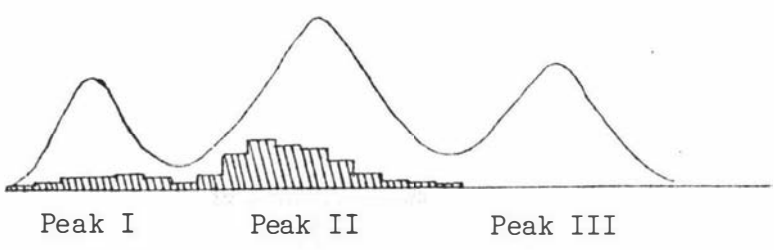
7 days p.i.



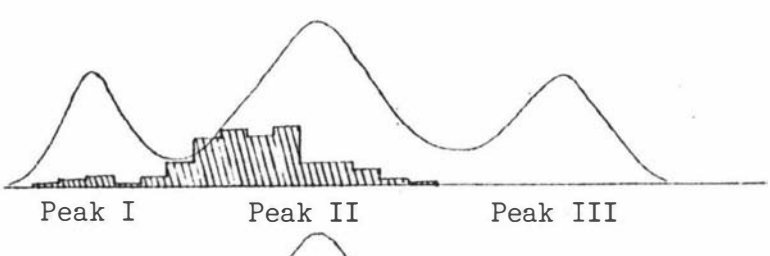
14 days p.i.



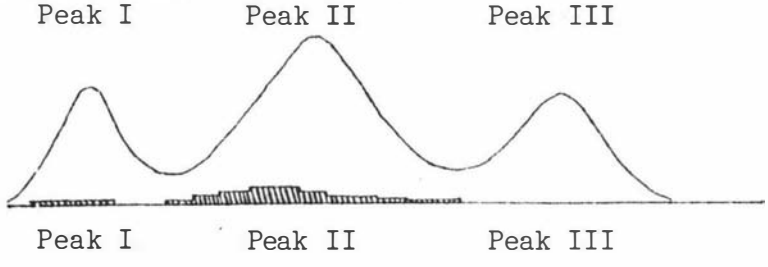
35 days p.i.



98 days p.i.



204 days p.i.



385 days p.i.

A number of sera taken at various times from five heifers prior to, and shortly after, their introduction into the milking herd were also fractionated. It was estimated that these animals had become infected during a period of up to 60 days before they were first sampled (see discussion on blue tag calves, Chapter Four) and all were consistently sero-positive when they were tested approximately every three months during the next year. The distribution of MAT activity in serum fractions from these animals and the estimated ages of their titres are given in Table 6.8. One animal with an infection of an estimated 40 days duration had 23% of its MAT activity in peak I, four animals with titres estimated to be 140 days old had 7% to 22% of MAT activity in peak I and six serum samples taken from two animals at various times between 260 and 420 days after infection had from 0% to 10% of their MAT activity in peak I. Apart from occasional tailing of high titres into peak III, all remaining MAT activity was found in peak II for all of these animals.

The final series of gel filtrations were conducted on sera from seven adult cows between two and six years old and possessing MAT titres ranging between one and six. The results of these fractionations are given in Table 6.9. These animals had 0% to 33% of their MAT activity in peak I and the balance in peak II. The three animals with relatively large proportions of peak I activity (C218/37, C121/37 and C156/37 with 29%, 33% and 25% peak I activity respectively) had low total MAT activity.

Anion Exchange Chromotography.

A consistent elution profile composed of six peaks was obtained; this is shown in Figure XIX with the molarity of the fractions as assessed by conductivity meter readings superimposed.

In order to assess the distribution of immunoglobulins in the eluted fractions, pools of peaks I to VI inclusive from a representative fractionation were concentrated down to 2 ml, equivalent to the starting volume of whole serum, and subjected to IEP. The precipitin lines produced against these concentrated pools by rabbit anti-bovine whole serum indicate that while peak I contained IgG_1, peaks II to V contained varying amounts of IgG_1 and peaks IV to VI varying amounts of IgM (Figure XX).

Table 6.8

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from heifers at various times up to 420 days after sero-conversion.

Fraction Number	Animal Identification										
	B1/1	B1/5	B4/5	B22/5	B50/5	B50/7	B50/10	B50/13	B56/7	B56/10	B56/13
1	10	-	-	-	-	-	-	-	-	-	-
2	20	10	-	-	-	10	-	-	10	-	-
3	20	20	20	20	20	10	20	-	-	-	10
4	30	100	70	40	70	-	20	-	20	10	10
5	20	140	100	20	20	-	-	-	30	20	10
6	10	100	100	10	10	-	-	-	20	-	10
7	20	140	140	20	20	-	-	-	40	-	20
8	20	190	100	20	20	20	20	10	100	10	20
9	30	380	290	50	40	30	70	20	180	20	20
10	50	380	580	100	140	60	80	30	190	40	30
11	140	1540	380	100	190	80	100	50	160	90	50
12	70	1540	580	20	140	90	70	40	60	50	50
13	10	770	140	10	50	60	50	30	10	30	50
14	10	190	20	-	20	50	20	20	-	40	50
15	10	100	-	-	-	20	-	-	-	10	30
16	-	20	-	-	-	-	-	-	-	-	20
17	-	10	-	-	-	-	-	-	-	-	10
18	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-	-
Unfractionated whole serum	770	6140	3072	580	770	380	580	290	1150	380	770
Peak I	110	370	290	90	120	20	40	-	80	30	40
Peak II	360	5260	2230	320	620	410	410	200	740	290	350
Total Recovered	470	5630	2520	410	740	430	450	200	820	320	390
Recovered %	61%	92%	82%	71%	96%	113%	76%	69%	71%	84%	51%
Peak I %	23%	7%	12%	22%	16%	5%	9%	0%	10%	9%	10%
Peak II %	77%	93%	88%	78%	84%	92%	91%	100%	90%	91%	90%
Days after s-c[α]	40	140	140	140	140	260	340	420	260	340	420

α estimated days after sero-conversion

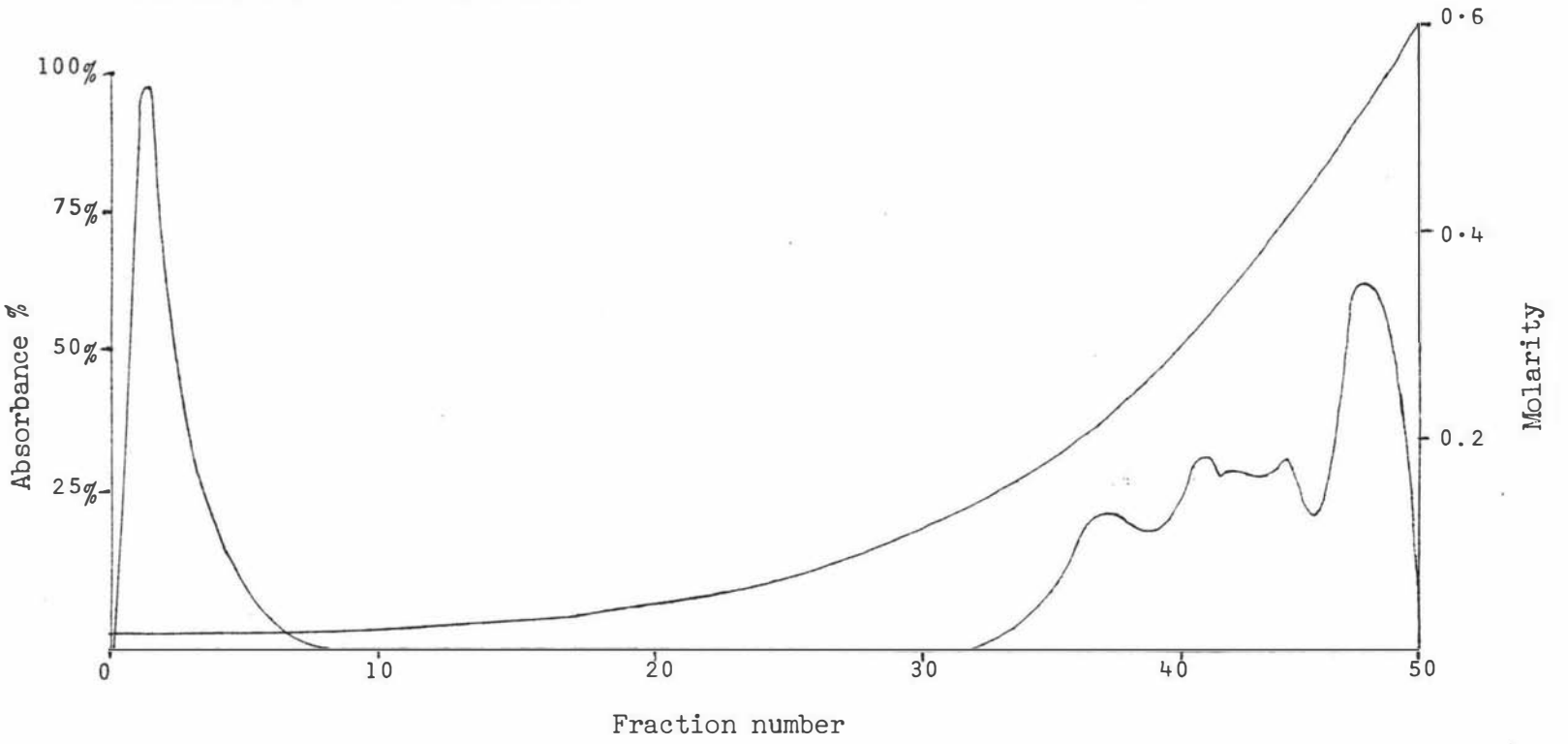
Table 6.9

Distribution of leptospiral agglutinating units in gel filtration fractions of sera taken from adult cows of various ages.

Fraction Number	Animal Identification C119/37	C25/37	C218/37	C121/37	C156/37	C51/24	C172/24
1	-	-	-	-	-	-	-
2	-	-	-	10	-	-	-
3	-	-	10	10	10	-	-
4	-	10	10	10	-	-	-
5	-	-	-	10	-	-	-
6	-	-	-	10	-	-	-
7	10	10	-	-	-	-	-
8	10	20	-	10	-	20	-
9	20	50	10	20	10	40	-
10	50	50	10	40	10	50	10
11	70	30	20	20	10	20	10
12	90	20	10	10	-	10	-
13	90	-	-	-	-	-	-
14	10	-	-	-	-	-	-
15	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-
Unfractionated whole serum	770	290	70	100	40	100	20
Peak I	0	10	20	50	10	0	0
Peak II	350	180	50	100	30	140	20
Total Recovered	350	190	70	150	40	140	20
Recovered %	45%	66%	100%	150%	100%	140%	100%
Peak I %	0%	5%	29%	33%	25%	0%	0%
Peak II %	100%	95%	71%	67%	75%	100%	100%
Age (years)	3	4	5	3	6	3	6

Figure XIX

Elution Profile of Bovine Serum Fractionated by Cellulose Chromotography.



MAT Reactions of Anion Exchange Chromotography Fractions.

The MAT activity of fractions obtained by anion exchange chromotography of sera from ten adult cows and two new-born calves are presented in Table 6.10. Five animals (C39/3, C53/3, C125/3, C38/8, C73/8) had low levels of activity, ranging from 4% to 14% of the total activity recovered, in peak I. All other MAT activity was found in fractions 33 to 49, comprising peaks II to VI with the highest levels of activity found in fractions 33 to 40. Histograms indicating the distribution of MAT activity in different fractions are presented in Figure XXI. Two animals (C158/3 and C139/8) which had no MAT activity in whole serum at a titre of 1:20 had no activity in any fractions.

Discussion.

The immunoelectrophoretic studies indicated that while gel filtration separated IgM into peak I fractions and most of the IgG into peak II fractions, small amounts of IgG were present in peak I and peak III. In all cases leptospiral agglutinins occurred exclusively in peaks I and II apart from the occasional tailing of some activity of high titre sera from peak II into peak III. These observations were interpreted to mean that agglutinating antibody detected in peak I was of the IgM class, and that in peak II of the IgG class. It is apparent that small amounts of IgG agglutinins will have been attributed to the IgM class by this approach. However, inspection of Tables 6.2 to 6.9 and Figure XVIII indicates that this approximation will have had only a minor effect on the calculated distributions of agglutinins reported in this study.

The results obtained from gel filtration of sera taken from new-born calves after suckling (Table 6.1) indicate that 19% to 55% of leptospiral agglutinins in the sera of one-day-old calves were of the IgM class but that these agglutinins were lost more rapidly than IgG agglutinins. It appears that IgM contributes a significant proportion of the MAT activity in neonatal calf sera for just a few days after birth. The presence of only 2% IgM agglutinins in the high titre serum of calf G62/2 at 21 days of age indicates that this is likely to be near to the upper limit for the persistence of passively-acquired IgM antibody.

Figure XX

Immunoelectrophoretic Reaction of Concentrated Cellulose Chromotography Fractions of Bovine Serum with Rabbit Anti-Bovine Serum.

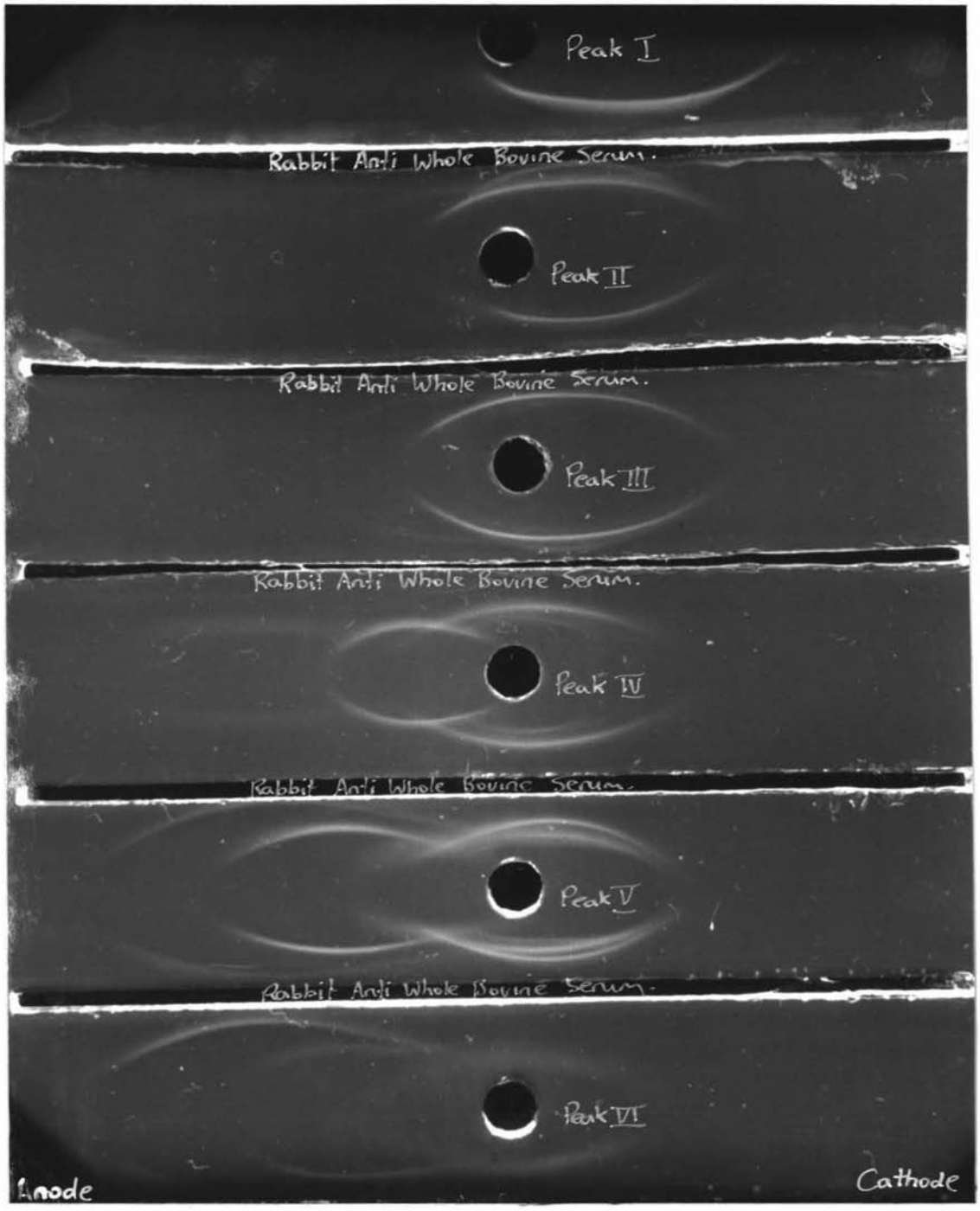


Table 6.10

Distribution of leptospiral agglutinatng units in anion exchange chromotography fractions of sera from cows and calves of various ages.

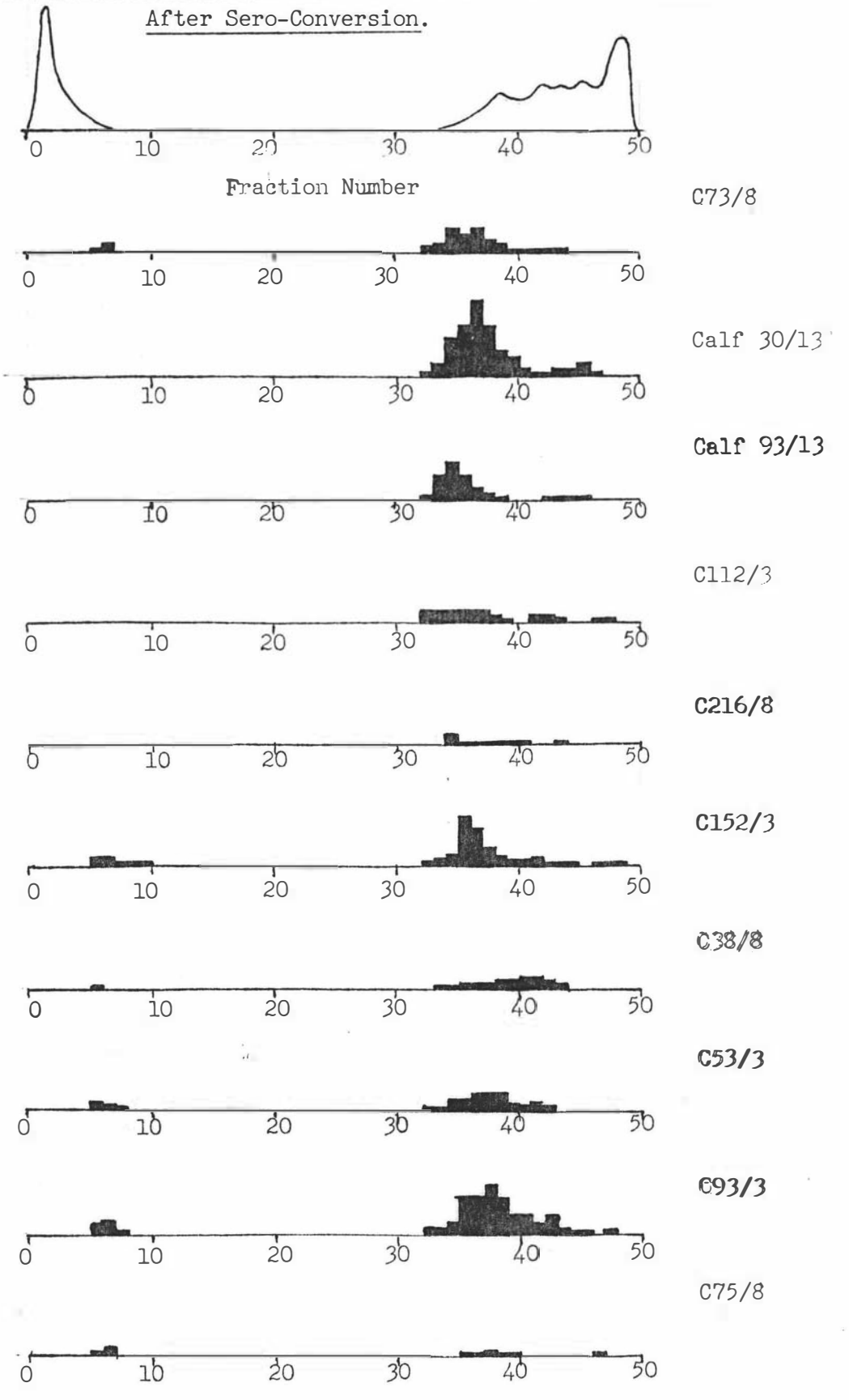
		Animal Identification					
	Fraction Number	Calf 30/13	Calf 93/13	C112/3	C216/8	C152/3	C38/8
Peak I	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	6	-	-	-	-	20	10
	7	-	-	-	-	20	-
	8	-	-	-	-	10	-
	9	-	-	-	-	10	-
	10	-	-	-	-	10	-
Peaks II to VI	33	10	10	30	-	10	-
	34	40	80	30	-	20	10
	35	120	120	30	30	40	10
	36	160	80	30	10	160	20
	37	240	40	30	10	120	20
	38	160	20	20	10	60	20
	39	80	10	10	10	30	30
	40	60	-	-	10	20	30
	41	20	-	-	10	20	40
	42	10	-	20	-	30	40
	43	10	10	20	-	10	30
	44	20	10	10	10	10	20
	45	20	10	-	-	10	-
	46	40	10	-	-	-	-
	47	10	-	10	-	10	-
	48	-	-	10	-	10	-
	49	-	-	-	-	10	-
	50	-	-	-	-	-	-
	Whole serum	1280	480	320	120	1280	320
	Peak I	0	0	0	0	70	10
	Peaks II to VI	1000	400	240	100	570	270
	Total recovered	1000	400	240	100	640	280
	Recovered %	78%	83%	75%	83%	50%	88%
	Peak I %	0%	0%	0%	0%	12%	4%
	Age	1 day	1 day	3 yrs	4 yrs	4 yrs	5 yrs

Table 6.10 (contd.)

	Fraction Number	Animal Identification C53/3	C39/3	C75/8	C73/8	C158/3	C139/8
Peak I	1	-	-	-	-	-	-
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
	5	-	-	-	-	-	-
	6	30	30	-	10	-	-
	7	20	40	-	30	-	-
	8	10	10	-	-	-	-
	9	-	-	-	-	-	-
	10	-	-	-	-	-	-
Peaks II to VI	33	10	10	-	20	-	-
	34	10	20	-	30	-	-
	35	40	40	-	80	-	-
	36	40	120	10	60	-	-
	37	60	120	10	80	-	-
	38	60	160	20	40	-	-
	39	60	120	10	30	-	-
	40	30	60	10	10	-	-
	41	20	60	-	10	-	-
	42	30	30	-	10	-	-
	43	20	60	-	10	-	-
	44	-	20	-	10	-	-
	45	-	10	-	-	-	-
	46	-	10	-	-	-	-
	47	-	-	10	-	-	-
	48	-	10	-	-	-	-
	49	-	-	-	-	-	-
	50	-	-	-	-	-	-
Whole serum		640	960	120	320	0	0
Peak I		60	80	0	40	0	0
Peaks II to VI		380	850	70	370	0	0
Total recovered		440	930	70	410	0	0
Recovered %		69%	97%	58%	128%	0%	0%
Peak I %		14%	9%	0%	10%	0%	0%
Age		6 yrs	7 yrs	7 yrs	10 yrs	5 yrs	6 yrs

Figure XXI

Distribution of Agglutinating Activity in Cellulose Chromotography Fractions of Some Bovine Sera at Various Times After Sero-Conversion.



The half-life of IgM acquired from colostrum is apparently shorter than the 15 to 17 days half-life calculated for colostrum-derived MAT titres in Chapter Three. However, insufficient data was obtained in this study to calculate the actual value of the half-life for IgM.

IgG is the predominant immunoglobulin passively transferred to calves in colostrum (Murphy *et al*, 1964; Pierce and Feinstein, 1965; Brandon *et al*, 1971; Husband *et al*, 1972). IgM has also been reported to be passively transferred to calves though in smaller amounts than IgG (Husband *et al*, 1972; Mohamad, 1975). A half-life of four days has been reported for maternal IgM in the calf (Husband *et al*, 1972; Porter, 1972), compared with a half-life of 16 to 32 days for IgG (Husband *et al*, 1972; Williams *et al*, 1975). These data are consistent with the observation made in this study.

The series of fractionations conducted on the sera from five calves which sero-converted in September 1976 (Tables 6.2 to 6.7) demonstrate a consistent pattern of transition of agglutinins from the IgM to the IgG class of immunoglobulins with increasing time after infection. The initial agglutinin response is probably exclusively IgM if an allowance is made for the fact that the sera described in Table 6.2 came from animals which sero-converted during a seven-day period between the last negative and first positive tests. This early phase was followed immediately by the appearance of IgG agglutinins. As the level of IgM agglutinins declined, the level of IgG agglutinins rose and they became the predominant agglutinins between 21 and 42 days after sero-conversion (Tables 6.3 and 6.4).

These observations are consistent with those made in experimentally-infected guinea-pigs by Crawford (1972a; 1972b) and in cattle by Hodges and Ris (1974b). These authors reported an initial IgM response which had become predominately an IgG response by 24 to 50 days p.i. Graves and Faine (1970), reporting observations on a single experimentally-infected rabbit stated that 50% of anti-*biflexa* agglutinins resided in the IgG class as early as the tenth day p.i. However, as this

rabbit had a titre to *biflexa* before challenge, it is possible that it was responding anamnestically. If this was so a more rapidly developing and predominately IgG response would be expected (Uhr, 1964).

At later stages in the serological response to *hardjo*, the proportion of agglutinins residing in the IgM class of immunoglobulins appears to stabilise. At 105 and 204 days after sero-conversion an average 21% of serum agglutinating activity in the five calves was associated with the IgM class (Tables 6.5 and 6.6). The proportion of IgM activity declined to 8% by 385 days after sero-conversion and by this time three of the five calves had no detectable IgM agglutinins (Table 6.7). This degree of transition from a predominately IgM response in the early stages of infection is considerably more marked than that observed by Graves and Faine (1970). The rabbit which they were sampling sequentially after multiple doses of antigen still had 33% of IgM agglutinin activity 216 days p.i. There are no other reports of animals being tested over such an extended period.

The results obtained by fractionating sera from heifers which had been sero-positive for periods ranging between 40 and 420 days conform to the general pattern outlined above (Table 6.8). In these animals, the titres were all predominately IgG responses. Also the proportion of total agglutinating activity contained in the IgM component became smaller as time after sero-conversion increased. On average IgM provided 23% of the agglutinating activity at 40 days p.i., about 14% at 140 days p.i., about 8% at 260 and 340 days p.i. and 5% at 420 days p.i.

The seven adult animals tested with titres of presumably long-standing duration, showed a greater variation in the proportion of IgM activity with a mean of 13% and range of 0% to 33% (Table 6.9). However, the total amounts of leptospiral agglutinins in the sera from most of these animals was low. In such sera low levels of IgM, near to the threshold of detection, can produce big changes in the values of proportions. Thus proportions have greater inherent errors in low-titre serum and should be regarded as less reliable. It is apparent that the

absolute levels of IgM agglutinins may stabilise at low values in older animals but that many will have no remaining IgM agglutinins as soon as one year after sero-conversion.

It has been argued that the leptospiral MAT primarily measures the IgM content of convalescent sera (Hanson, Tripathy and Killinger, 1972; Hanson, 1973; 1976; 1977). This claim is not supported by either the more recent reports in the literature (reviewed in the introduction to this chapter) nor by the present study which clearly indicates that often MAT titres are solely attributable to IgG and that primacy of IgM in the leptospiral MAT occurs only during the initial stages of natural *hardjo* infection; up to six weeks after sero-conversion.

There is some evidence that antigen must persist in the host to allow the continued synthesis of IgM antibody (Uhr, 1964; Pike, 1967). Some of the cattle in the present study shed *hardjo* for periods of up to fourteen months and may well have shed organisms for much longer periods (see Chapter Seven). It is also possible that leptospiral antigen may persist in the host beyond the time when shedding ceases. In these animals it is clear that the major transition from an IgM to an IgG response occurred well before shedding terminated. It is possible that the total disappearance of IgM agglutinins from some convalescent sera occurred at about the time that shedding may have terminated (e.g. G42/37, G54/37 and G63/37) but other, older, cattle still had IgM agglutinins (Table 6.9).

The results of this study have added significance as they are parameters of the situation pertaining in natural field infections rather than studies on experimentally-infected animals. The findings from the five calves which are reported in Tables 6.2 to 6.7 were made during a period of more or less continuous exposure to other actively-shedding cattle (see Chapter Seven). At present there is no serological test which can consistently determine which cattle in a group have recently become infected. The complement fixation test (CFT) appears to identify those animals which have comparitively recent infections (Robertson and Boulanger, 1963; Palit and Sharma, 1971; Hodges and Ris, 1974)

but is a more difficult test to apply than the MAT. The MAT itself is not a satisfactory test to detect acute cases (see Chapter Four). However, the results of this study indicate that a simple modification to the MAT should provide a method to detect recent infections.

The application of a procedure which selectively inactivates the IgM antibody in whole serum, would enable the ratio of IgM and IgG agglutinins to be determined by comparing MAT titres before and after IgM inactivation. Such procedures currently available are heat-inactivation, 2 - ME inactivation or Rivanol precipitation (Rose and Roepke, 1964; Brindley-Morgan, 1967; Anon, 1977c). Based on the results presented in Tables 6.2 to 6.9, high ratios of IgM:IgG activity ranging from 100:0 down to 80:20 would indicate titres of up to three weeks duration and lower ratios would indicate progressively longer-standing titres down to 40:60 by six weeks and 20:80 by approximately three months and subsequently. Such a test could be performed in any laboratory able to conduct the MAT and would have considerable epidemiological significance in identifying recent cases. This information would facilitate more detailed investigations of the dynamics of leptospiral infection in a group of cattle. The detection of new cases by the detection of IgM leptospiral agglutinins has been suggested previously, though only in general terms (Samedov and Sharabchiev, 1969).

The results obtained from immunoelectrophoretic examination of proteins in fractions obtained by cellulose chromotography of cattle sera indicate that immunoglobulins were distributed as previously reported (Nansen, 1970; Duncan *et al*, 1972). The only fractions containing a single type of immunoglobulin were those of peak I which contained IgG_2. The other peaks contained mixtures of immunoglobulins with most of the IgG_1 contained in peaks II and III and most of the IgM in peaks IV and V (Figure XX).

The distribution of MAT units amongst the peaks obtained by cellulose chromotography (Table 6.10) indicates that the MAT activity of one-day-old calves resides predominately in the IgG_1

component with the balance in the IgM component; there was no IgG_2 activity. This is consistent with the findings from the gel filtration studies reported above and the reports of other workers that little IgG_2 is passively transferred to new-born calves (Husband *et al*, 1972: Mohamad, 1975). The adult cattle studied all had predominantly IgG_1 responses and though it was not possible to distinguish clearly between IgG_1 and IgM activity in peaks III to VI because of the mixtures of immunoglobulins present, it appears that most had low levels of IgM agglutinins (Table 6.10). Five animals also had some IgG_2 activity. There is some evidence that IgG_2 agglutinins occurred in older animals (the mean age of the five cattle with IgG_2 agglutinins was 6.4 ± 1.0 years compared with 4.7 ± 1.2 years for those lacking IgG_2 antibody) but insufficient animals were studied to be certain of this trend. This result is consistent with reports that IgG_2 appears at a later stage in the immune response than IgG_1, and that IgG_2 is a more important immunoglobulin in older animals (Beh, 1974; Williams *et al*, 1975). This is the first report of leptospiral agglutinins occurring in the IgG_2 subclass of immunoglobulins. Morris and Hussaini (1974) investigating leptospiral agglutinins in three cows, at unknown stages of infection, reported that no agglutinins occurred in this subclass. The presence of IgG_2 agglutinins in the sera of older cows may be attributable to the progressive increase in antibody quality which has been reported to occur during the development of an immune response (Uhr, 1964).

The ratio of serum IgG_1:IgG_2 antibody varies in ruminants (Nansen, 1970; Williams *et al*, 1975). Relatively high levels of serum IgG_2 occur at the time of parturition when IgG_1 is selectively lost into the colostrum (Brandon *et al*, 1971) while relatively low levels occur in animals experiencing secondary antigenic stimulation (Margni, Castrelos and Paz, 1973). Plackett and Alton (1975) have demonstrated an inhibitory interaction between IgG_1 and IgG_2 antibodies resulting in negative *Brucella* CFT reactions in infected cattle. In the present study there were no cases where separation of IgG_1 and IgG_2 into different fractions resulted in an apparent increase in agglutinating activity compared with

that of whole serum. Neither were agglutinins detected in cellulose chromotography fractions of sera from two cows which had negative whole serum tests (Table 6.10).

Summary.

1. New-born calves passively acquire both IgM and IgG anti-leptospiral agglutinins. The IgG agglutinins are exclusively of the IgG_1 subclass.
2. The IgM agglutinins acquired by new-born calves are catabolised within three weeks of birth and the persisting agglutinin titres observed in these calves belong to the IgG_1 subclass.
3. Cattle responding to natural *hardjo* infection initially produce only IgM agglutinins but within a week IgG_1 agglutinins appear. These represent 20% of the total agglutinating response by about three weeks after sero-conversion, 60% by six weeks and 80% by three minths.
4. Later in the serological response 90% to 100% of the agglutinins are in the IgG class though some low-titred animals have higher proportions of IgM.
5. It is suggested that by modifying the MAT, by the use of an appropriate IgM inactivation technique such as heat, 2-ME inactivation or Rivanol precipitation, the ratio of IgM:IgG agglutinins in a serum sample can be calculated. This ratio will provide a good estimate of the stage of infection during the first three months after sero-conversion and would have considerable value as an epi-demiological tool.
6. Cows with long-standing MAT titres possess some agglutinins in the IgG_2 class. It is suggested that this may, in part, reflect the refinement in antibody quality which occurs as the immune response progresses.
7. This is the first report that antileptospiral agglutinins may occur in the IgG_2 subclass of immunoglobulins.
8. There is no evidence for an inhibitory interaction between the IgG_1 and IgG_2 subclasses occurring in the leptospiral agglutination reaction.

CHAPTER SEVEN

OBSERVATIONS ON NATURALLY-OCCURRING *hardjo* EPIDEMICS IN A HERD OF CATTLE.

Introduction.

The results of an observational study of the epidemiology of an infectious disease are determined by the opportunities which are presented when outbreaks occur. The problems in detecting outbreaks in this type of study also render it extremely difficult to precisely define infection times for individuals, or even groups (see Chapter Four Discussion). Nevertheless, the value of the information obtained by studying naturally-occurring epidemics is sufficiently great to outweigh the limitations which accompany this approach. In the present study a number of outbreaks of *hardjo* infection were investigated, and the results of these investigations are presented in this Chapter. This appears to be the first report of an attempt to observe in detail the epidemiology of bovine *hardjo* infection in the field.

One outbreak of bovine *pomona* infection was also studied. Observations made during this outbreak and other information obtained concerning the epidemiology of bovine *pomona* infection are presented in Chapter Eight.

Aspects of the epidemiology of bovine *hardjo* infection have been the subjects of studies by various authors. Infection of young cattle with *hardjo*, before nine months of age, has seldom been reported and it appears that in herds with endemic *hardjo* that the first cases are observed after calves have lost colostral titres (Hanson *et al*, 1964; Cacchione *et al*, 1968). Ellis and Michna (1976c), discussing three outbreaks of *hardjo* abortion in Scotland, reported that on two of the farms involved, there was a high prevalence of *hardjo* infection in adults, but not in young stock grazed in isolation. Earlier they had reported a rapid rise in the prevalence of *hardjo* infection in cows between the second and third years of life (from 37% to 72%), coinciding with their first gestation and introduction into the adult herd (Ellis and Michna, 1976a). After this time relatively constant proportions

of adult cattle were serologically positive to *hardjo* (Ellis and Michna, 1976a). They considered that epidemics of *hardjo* abortion followed either the introduction of the infection into a susceptible population, or the introduction of susceptible cattle into an infected population (Ellis and Michna, 1976b). Hoare and Claxton (1972) had earlier reported that epidemics of *hardjo* mastitis followed the initial introduction of infection into susceptible herds, but that subsequently asymptomatic infection became increasingly common, owing to changes either in host resistance, or in the virulence of the organism.

Bovine *hardjo* infection has been widely reported to follow recent periods of heavy rainfall, or to occur in wet seasons or environments (Hanson *et al*, 1964; Martin *et al*, 1967; Sakula and Moore, 1969; Sullivan and Callan, 1970; Hoare and Claxton, 1972; Johnson *et al*, 1974; Ellis and Michna, 1976b; Gordon, 1977). However, a number of workers have reported outbreaks which occurred during dry periods or without any apparent seasonal association (Hanson *et al*, 1964; Schnurrenburger *et al*, 1970; Hoare and Claxton, 1972; Blackmore *et al*, 1976; Ellis *et al*, 1976. Close confinement on muddy wet pastures has been suggested as the factor responsible for outbreaks under wet conditions (Hoare and Claxton, 1972; Ellis and Michna, 1976b), and the behavioural trait of urine licking amongst bovines has been cited to explain transmission under dry conditions (Blackmore *et al*, 1976; Ellis and Michna, 1976b; Ellis *et al*, 1976.

Other suggested modes of transmission include direct contact with shedding cattle at pasture (Sullivan, 1970a; 1972), spread through milking machines (Ellis *et al*, 1976. Introduction of carrier cattle into a susceptible population (Sullivan and Callan, 1970; Hoare and Claxton, 1972) and exposure to contaminated drinking water (Ellis and Michna, 1976b). The possibility that *hardjo* may be transmitted to cows by either natural or artificial mating, suggested by Michna *et al* (1974), has been supported by the work of Kiktenko *et al* (1976).

In spite of extensive investigation in many countries (Hanson *et al*, 1964; Martin *et al*, 1967; Hoare and Claxton, 1972;

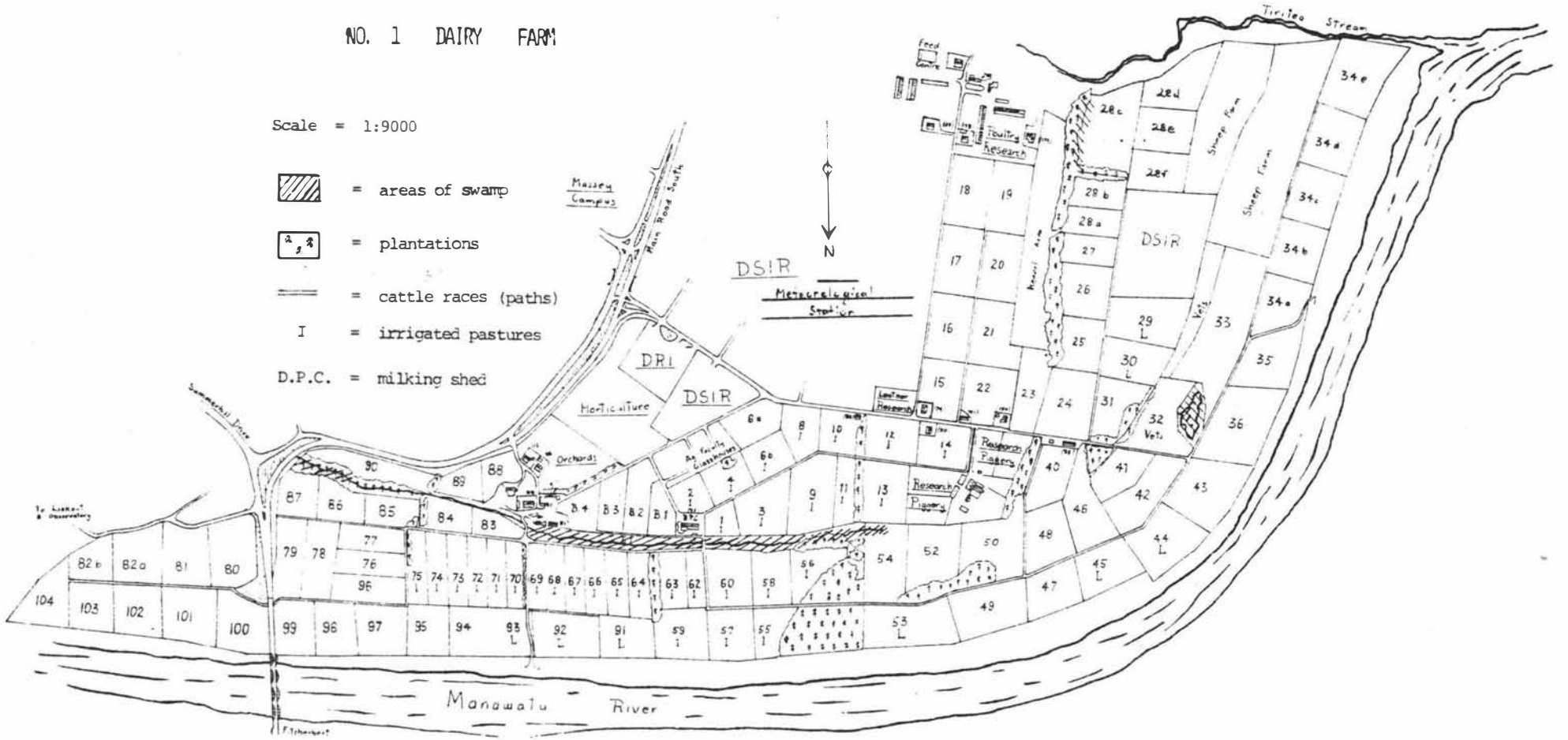
Twigg *et al*, 1969; 1972; Sullivan, 1974; Blackmore *et al*, 1976) there is no evidence, or suggestion, that any feral or wildlife species acts as a reservoir host for *hardjo* infection. There is some evidence that sheep become infected with *hardjo* (Hoare and Claxton, 1972; Ris, 1975; Marshall, 1976) but until isolates are obtained and typed the significance of *Hebdomadis* serogroup titres in sheep remains unknown. Low levels of *hardjo* titres have been reported to occur in pigs (Lyubashenko *et al*, 1966; Schnurrenburger *et al*, 1970; Farina *et al*, 1972; Anon, 1974e; Ryan, 1978). It is likely that these titres represent cross-reactions, resulting from infections either with other members of the *Hebdomadis* serogroup or, more commonly, with serovars from unrelated serogroups (Ryan, 1978). There appears to be total agreement in the literature that bovine *hardjo* infection is transmitted exclusively from cow to cow. No author has commented on the role of sheep in the epidemiology of *hardjo* infection and this aspect is still unresolved.

Experimentally-infected cattle have been reported to shed *hardjo* for various times between 17 and 153 days p.i., generally for short periods ranging from just a few days up to two months (Sullivan, 1970a; 1970b; 1972; Farina *et al*, 1972; Hodges and Ris, 1974; Ellis and Michna, 1977). However, Farina *et al* (1972) observed three of nine experimentally-infected animals consistantly shedding for about 100 days, at which time they were slaughtered, and Ellis and Michna (1977) isolated *hardjo* from the kidneys of two heifers slaughtered 153 and 174 days after infection. Sullivan (1972) observed shedding for 35 days in a naturally-infected cow.

With the aim of more precisely defining the epidemiology of *hardjo* infection in an endemically infected herd, a two and a half year investigation was undertaken of the No. 1 dairy herd at Massey University. Cultural and serological studies were conducted on different groups of cattle to determine the pattern of spread within the herd. Specific studies were undertaken on selected groups to investigate the possible role of some factors in modifying the rate of transmission of *hardjo* infection at different times of the year.

Figure XXII

Map of the Massey No. 1 Dairy Farm.



Materials and Methods.

The Herd.

The animals observed in this study were members of the Massey No. 1 Dairy Herd and the conditions under which they were managed have been described earlier (see Chapters Three to Five). Records of mating and calving, movements of stock about the farm, and periods of contact between different groups of animals, were maintained throughout the study period.

The Farm Property.

The farm is located on river flats in a bend of the Manawatu River (Figure XXII). The soil is an alluvial sandy or silty loam of recent geological origin and is free-draining. Although one or two low-lying areas become water-logged at times during the winter these areas are generally fenced off from the cattle and are only grazed during the dry seasons (Figure XXII). On one occasion during the study period, June 28th 1976, part of the farm was flooded by the Manawatu River for approximately 12 hours and large areas of standing water remained in some paddocks for several days (28a to 28f, 32, 50, 52 and 54 in Figure XXII).

The full length of the river boundary has a mixed vegetation of rank weeds, fern and willow trees as do the fenced areas bounding the large drain which runs through the farm. There are also several small pine plantations on the farm. All these areas of plantation and wasteland support small populations of possums (*Trichosurus vulpecula*), black and brown rats (*Rattus rattus* and *Rattus norvegicus*), mice (*Mus musculus*), hedgehogs (*Erinaceus europaeus*) and rabbits (*Oryctolagus cuniculus*). Mustelids and hares (*Lepus timidus*) are probably also present from time to time but only one stoat (*Mustela erminea*) was detected during the study period. In addition there is a small population of feral cats (*Felis catis*) living in the barns and out-buildings.

A large piggery is located in the centre of the dairy farm, and though run as a separate unit, there is contact between pigs and cattle through a single fence, and some drainage from the pig farm runs onto dairy pastures. There are also two large paddocks at the western end of the farm on which sheep and

occasionally beef steers are grazed. Some dogs are occasionally housed in large concrete kennels in the kennel area. With these exceptions there is no contact with any other livestock and the farm is entirely bounded by the river, highways or cropland. All the waterways flowing through or beside the farm have no stock pastured near them for at least two kilometers upstream of the farm. Effluent from both the piggery and the dairy shed is discharged directly into the Palmerston North municipal sewer.

Serological Surveillance.

As described in Chapter Four, blood samples were taken from all groups of sero-negative cattle at approximately monthly intervals, though the intervals between consecutive tests in some groups ranged from one to ten weeks. Sera were tested by the MAT as previously described (Chapter Two), against serovars *ballum, copenhageni, hardjo, pomona* and *tarassovi*.

Surveillance of Leptospiruria.

Urination was induced by manual stimulation of the perineum and 10 ml samples of mid-stream urine were collected into sterile containers. Urine samples were occasionally contaminated with small amounts of faecal materials.

All urine samples collected were routinely examined by dark-field microscopy using a simple technique. Urine was centrifuged at 3000G for five minutes to bring particulate material out of suspension and a loop full of the supernatant was transferred onto a glass slide and 20 to 50 fields examined for the presence of motile leptospires. The presence of non-motile organisms was always recorded, but low numbers of non-motile leptospires was regarded only as suspicious.

Urine dilution and culture was carried out in the laboratory within one to three hours of collection. Two ten-fold dilutions of urine were made in sterile Stuart's basal medium (BBL) and 50 µl (two drops from a pasteur pippette) of undiluted urine and of each dilution were inoculated into separate tubes containing 5 ml of medium. In the earlier part of the study urine samples were also filtered through a 13mm x 0.22µm cellulose filter (Millipore)

held in a sterile swinnex filter holder (Millipore). The first 2 ml of filtrate of undiluted urine was discarded and then successive 0.5 ml aliquots were inoculated into tubes containing 5 ml of medium.

Initially the culture medium used was commercially prepared EMJH (Difco) to which was added 0.15% agar (Difco) and 1% bovine serum albumin (BSA) (Difco). This was used on its own or after the addition of 200 µg/ml of 5FU (Sigma) prepared according to the technique of Johnson and Seiter (1977). Later there were problems in supply of the commercially prepared BSA and it was replaced by a supplement prepared in this laboratory according to the method of Johnson and Seiter (1977) (Appendix V) from a purified Fraction V bovine albumin preparation (Pentex). From that time EMJH basal medium was also prepared from basic reagents in this laboratory according to the method of Johnson and Seiter (1977) (Appendix V).

Urine samples were collected from a stratified random sample of animals representative of the different age groups of cattle managed on the farm. In addition some animals were repetitively sampled for approximately 18 months to estimate the persistance of the leptospiruric state in selected individuals.

During 1976, a series of urine samples were also tested by hamster inoculation. Pairs of weanling golden hamsters, obtained from a leptospire-free colony, were each inoculated with 0.5 ml aliquots of urine collected one to three hours previously. Twenty-one days later the hamsters were bled by cardiac puncture and euthanased. Kidneys were removed aseptically and forced through a 1cm x 18G hypodermic needle into 10 ml of sterile Stuart's basal medium to form an homogenate. Two further ten-fold dilutions of this homogenate were prepared in the same medium and 0.5 ml aliquots of each dilution inoculated into both EMJH and EMJH plus 200 µg/ml 5FU. Serum from the hamsters was tested by the MAT.

All culture tubes were incubated at 30°C for three months and

were examined as previously described (Chapter Two) at three-weekly intervals. Isolates were passaged in liquid EMJH containing 200 µg/ml 5FU prepared according to the method of Johnson and Seiter (1977) (Appendix V). When isolates were growing at a density of approximately 1 x 10^8 organisms/ml they were tested against a range of known antisera and, if applicable, cross-absorbed anti-*hardjo* and anti-*balcanica* sera as described in Chapter Two.

Meteorological Records.

Meteorological data relevant to the study period was obtained from a recording station at the Department of Scientific and Industrial Research (D.S.I.R.) laboratory, adjacent to the No. 1 Dairy Farm (FigureXXII). Daily records and weekly means of rainfall, pan evaporation, hours of sunshine and humidity, and grass minimum, soil and air temperatures were available.

Environmental Sampling.

Samples of water from puddles, drains and streams were collected from time to time in sterile 20 ml bottles and taken to the laboratory for processing. All samples were centrifuged at 3000G for 15 minutes to sediment particulate material and the supernatant inoculated into culture media and/or golden hamsters. Samples inoculated directly into media were filtered through membrane filters using the same technique described for the filtration of urine samples. Media used were EMJH plus 200 µg/ml or 400 µg/ml 5FU. Inoculated hamsters were euthanased after three weeks and processed as described before. All cultures were incubated at 30°C for three months and examined by darkfield microscopy at three-weekly intervals. Isolates were typed as previously described.

Wildlife Sampling.

An extensive survey of wildlife on the farm was undertaken during the course of this survey by Hathaway (1978). Possums, black and brown rats, mice, hedgehogs, feral cats and a stoat were captured on various parts of the farm, and in most cases cultural examinations were carried out. Sites sampled included the piggery and the calf-rearing area on the dairy farm.

Other Domestic Species.

The No. 1 Dairy Farm is solely a dairying unit. Sheep are only occasionally run at the western end of the farm (Figure XXII), and during the only period when cattle in that part of the farm experienced a *hardjo* epidemic, there were no sheep on those paddocks. The piggery was under intensive surveillance throughout the period during which these bovine studies were undertaken (Ryan, 1978).

Results.

The pattern of occurrence of new cases of hardjo infection.

1. Association with age of host.

During the two and a half year study period at the Massey No. 1 Dairy Farm, the combined crude attack rate of *hardjo* infection was 89% (78 of 88 susceptibles). All the new cases, which were detected when cattle under surveillance sero-converted, occurred in calves between 5 and 18 months of age; no new cases were observed amongst neonates or adults. In addition two other groups of cattle, consisting of a total of 41 animals aged 12 to 18 months, had apparently experienced outbreaks of *hardjo* just prior to the start of this study, as both had GMT's to *hardjo* greater than 1:400.

The ages at which all members of each group of susceptible cattle became infected were similar (Table 7.1), since each group experienced propagating epidemics rather than a succession of sporadic cases. It should be noted that based on serological evidence the endemic level of *hardjo* infection in the adult herd was close to 100%, whilst amongst calves with colostral immunity it was 0%.

The accepted meanings of "endemic" and "epidemic" are the usual frequency and the excessive frequency of a disease in a population respectively (MacMahon and Pugh, 1970). However, there are difficulties in applying these terms to a situation such as that occurring in the No. 1 dairy herd where different endemic levels apply in different age groups. In this discussion the term 'epidemic' refers to the propagating epidemics observed in 6 to 18 month old heifers and 'endemic' refers to the situation

Table 7.1

Ages of animals at the time of sero-conversion and the season in which sero-conversion occurred.

Calf Group	Age (months) at which sero-conversion occurred		Season of peak sero-conversion
	Range	Mean ± S.E.	
Autumn 1974	12-18[α]	N/A	Winter[α]
Spring 1974	9-12[α]	N/A	Winter[α]
Autumn 1975	5-17.5	14.1 ± 0.9	Winter
Spring 1975	12-13.5	12.4 ± 0.1	Winter
Autumn 1976	8-10	9.0 ± 0.2	Spring
Spring 1976	12-15	13.4 ± 0.3	Winter
Autumn 1977	7-11	9.0 ± 0.3	Spring

[α] Estimates based on titres and birth dates

N/A = not applicable

applying in the adult herd.

2. *Associations with season.*

New cases of *hardjo* infection were detected more frequently in the winter and spring (Table 7.1). Throughout the study period five calves sero-converted during the six-monthly periods between December 1st and May 31st, and 73 calves between June 1st and November 30th. There were two periods during which most infections occurred: late June to August and October/November (Table 7.2).

Table 7.2

Distribution by month of occurrence of new cases of *hardjo* infection in the No. 1 dairy herd during the period 5/9/75 to 27/1/78.

December	January	February	March	April	May
1	1	1	0	0	1
June/July	August	September	October	November	
21	12	4	19	18	

3. *Associations with exposure to other infective cattle*

1. *Epidemiological Studies.*

In order to attempt to explain the uneven occurrence of new cases in time, it is necessary to mathematically define the propagating epidemics which were observed in each group of calves. Two measurements are employed for this purpose. The term "exposure ratio" is used to express the varying ratio of infectives to susceptibles occurring in groups of calves, and the term "incidence ratio" to express those new cases occurring during a defined period, as a proportion of the total number of animals in their group (i.e. Exposure ratio = $\frac{\text{infectives}}{\text{susceptibles}}$

Incidence ratio = $\frac{\text{New cases}}{\text{susceptibles + old cases}}$

All outbreaks of *hardjo* observed in this study occurred following contact with known or suspected shedder cattle during the winter and spring. Details of new cases and periods of

contact are summarised in Tables 7.3 to 7.8. Several observations can be made from these tables. Firstly, when contact with infectives occurred while any member of a group of calves still had colostral titres, no sero-conversion occurred (Tables 7.4, 7.5 and 7.7). Secondly, though frequent contact occurred between susceptibles and infectives at all times of the year, transmission of infection did not routinely occur. Thirdly, no new cases occurred in groups of susceptible calves run in isolation through the winter until contact with infective cattle occurred in the late winter and early spring (Tables 7.5, 7.6 and 7.8). The data contained in Tables 7.3 to 7.8 is summarised in Figure XXIII. Exposure ratios varied, at different times of the year, in different groups of susceptible calves. Changes in exposure ratios occurred when infective cattle were introduced to, or removed from, each group. The infectives introduced, which were animals confirmed or suspected to be leptosiruruic, were generally cattle six to twelve months older than the susceptibles in each group. The information shown in Figure XXIII indicates that high exposure ratios failed to establish propagating epidemics in January and February 1976 and May and July 1977, whereas relatively low exposure ratios established propagating epidemics in September 1976 and September and October 1977.

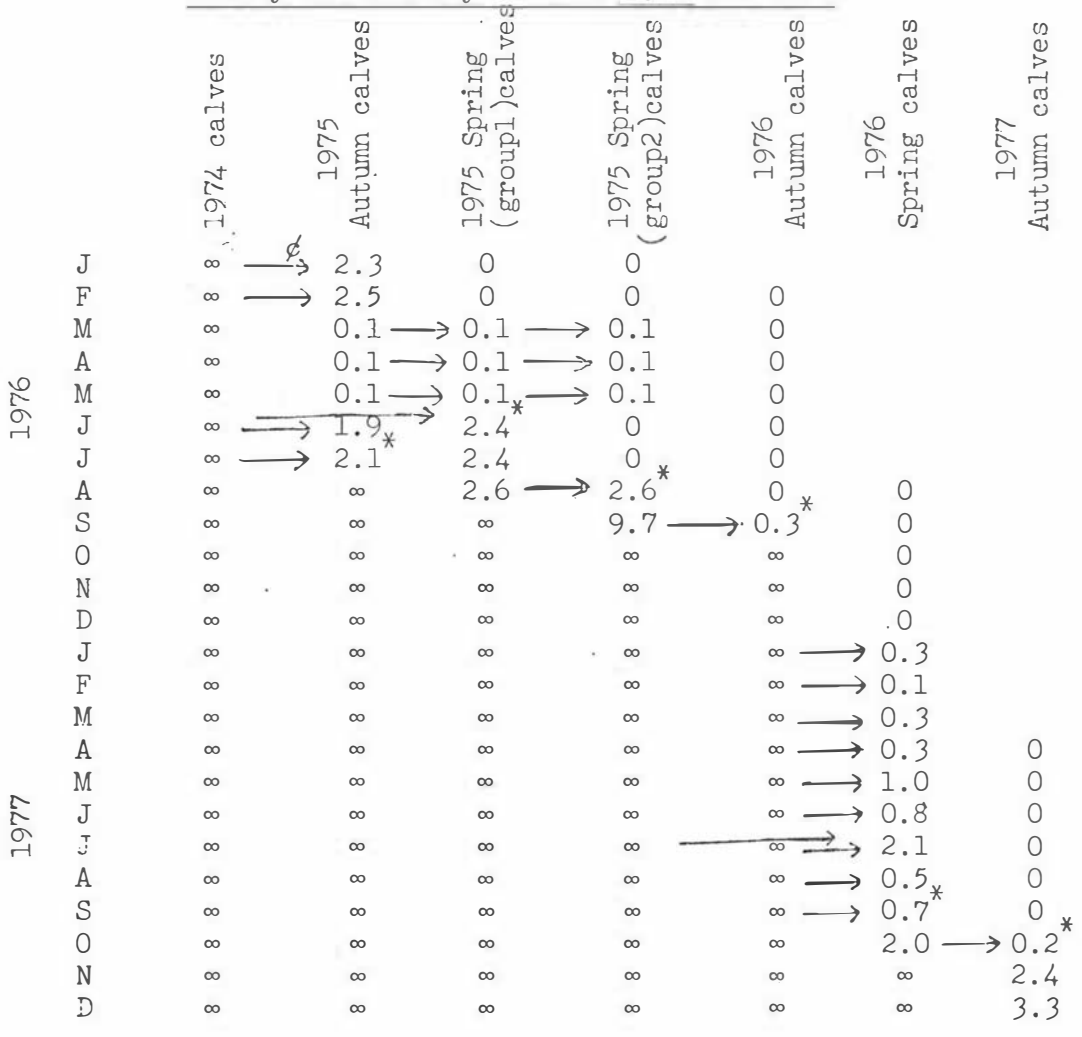
These findings are clearly apparent in the observations made on the two groups of calves which were kept under more detailed surveillance: some spring calves born in 1975 (1975 Group Two) (Table 7.5) and all spring calves born in 1976 (Table 7.7). The 1975 calves failed to become infected in the autumn of 1976 during a prolonged period of contact with suspected infectives. They later experienced an epidemic shortly after exposure to a large group of suspected infectives, which included a number of confirmed leptospiruric animals in August/September 1976. The 1976 calves were exposed to a varying number of infectives continuously from December 17th 1976 until November 2nd 1977, but no cases occurred until the main epidemic commenced in August/September 1977.

11. Bacteriological Studies.

No leptospires were detected in the urines from 26 neonates

Figure XXIII

Summary of the Occurrence of *hardjo* Epidemics at the Massey No. 1 Dairy Farm in 1976 and 1977.



* Propagating epidemic established

ϕ Arrows indicate periods of contact between various cohorts.

The numbers in this figure represent the Exposure Ratios (Infectives/Susceptibles) in successive cohorts of calves at the Massey No. 1 Dairy Farm.

Table 7.3

Autumn calves born 1975: Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
6/9/75	16	3	13	0	0	0	0
8/10/75	16	0	13	1	1	1.8	0.06
11/11/75	16	0	15	1	2	0.1	0.06
16/12/75	16	0	14	0	2	0.1	0
26/1/76	16	0	14	1	3	2.3	0.06
27/3/76	16	0	13	0	3	2.5	0
26/4/76	16	0	13	0	3	0.1	0
22/5/76	16	0	13	1	4	0.1	0.06
16/6/76	15[α]	0	12	1	4	1.9	0.06
11/8/76	15	0	11	11	15	2.1	0.69

[α] one death

Table 7.4

Spring calves born 1975 (Group One): Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
6/9/75	20	18	2	0	0	0	0
9/10/75	29[α]	25	4	0	0	0	0
11/11/75	28[β]	18	10	0	0	0	0
16/12/75	28	13	15	0	0	0.1	0
27/1/76	12[ψ]	0	12	0	0	0	0
27/3/76	12	0	12	0	0	0.1	0
26/4/76	9[φ]	0	9	0	0	0.1	0
2/6/76	9	0	9	0	0	0.1	0
11/8/76	9	0	9	8	8	2.4	0.89
7/10/76	9	0	1	1	9	2.6	0.11

α 9 births

β 1 death

ψ 16 sold

φ 3 sold

Table 7.5

Spring calves born 1975 (Group Two): Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
6/9/75	6	5	1	0	0	0	0
9/10/75	8[α]	7	1	0	0	0	0
11/11/75	8	7	1	0	0	0	0
16/12/75	8	6	2	0	0	0.1	0
27/1/76	8	6	2	0	0	0	0
27/3/76	8	0	8	0	0	0.1	0
26/4/76	8	0	8	0	0	0.1	0
16/6/76	8	0	8	0	0	0.1	0
11/8/76	8	0	8	0	0	0	0
26/8/76	8	0	8	2	2	2.6	0.25
2/9/76	8	0	6	3	5	3.3	0.38
9/9/76	8	0	3	1	6	7.0	0.12
16/9/76	8	0	2	0	6	9.7	0
30/9/76	8	0	2	2	8	9.7	0.25

[α] 2 births .

Table 7.6

Autumn calves born 1976: Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
27/4/76	22	7	15	0	0	0	0
2/6/76	22	8	14	0	0	0	0
30/7/76	21[α]	0	21	0	0	0	0
5/10/76	18[β]	0	18	0	0	0	0
13/12/76	18	0	18	17	17	0.3	0.94
26/1/77ψ	18	0	1	0	17	0.1	0
10/3/77	18	0	1	1	18	0.1	0.06

α 1 death

β 3 deaths

ψ Group dispersed on this date and remaining susceptible run with new group.

Table 7.7

Spring calves born 1976: Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
13/10/76	20	13	7	0	0	0	0
16/11/76	20	8	12	0	0	0	0
17/12/76	20	3	17	0	0	0	0
26/1/77	20	0	11[α]	0	0	0.3	0
10/3/77	20	6[β]	11	0	0	0.1	0
20/4/77	20	3[β]	11	0	0	0.3	0
12/5/77	20	1[β]	11	0	0	0.3	0
27/5/77	20	1[β]	11	0	0	1.0	0
10/6/77	20	0	11	0	0	0.8	0
24/6/77	20	0	11	0	0	0.8	0
8/7/77	20	0	11	0	0	0.4	0
15/7/77	20	0	11	0	0	2.1	0
22/7/77	20	0	11	0	0	2.1	0
29/7/77	20	0	11	0	0	0.8	0
5/8/77	20	0	11	1	1	0.5	0.09
13/8/77	20	0	10	0	1	0.5	0
20/8/77	20	0	10	0	1	0.6	0
3/9/77	20	0	10	2	3	0.7	0.18
10/9/77	20	0	8	4[ψ]	7	1.1	0.27
23/9/77	20	0	5	0	7	2.0	0
2/11/77	20	0	5	6[ψ]	13	2.5	0.46
17/11/77	20	0	0	0	13	-	-
27/1/78	20	0	0	0	13[φ]	-	-

α 9 calves vaccinated
β vaccinal titres
ψ includes 1 vaccinate
φ 7 vaccinates failed to become infected by end of study period

Table 7.8

Autumn calves born 1977: Summary of the appearance of new cases within the group and exposure to other infective cattle.

Test Date	Number in group	Number with colostral titres	Number of susceptibles during period	Number of new cases during period	Cumulative total of cases	Exposure Ratio $\frac{\text{infectives}}{\text{susceptibles}}$	Incidence Ratio $\frac{\text{new cases}}{\text{susceptibles + old cases}}$
20/4/77	17	11	6	0	0	0	0
13/5/77	17	11	6	0	0	0	0
24/6/77	17	7	10	0	0	0	0
20/7/77	17	2	15	0	0	0	0
3/9/77	17	0	17	0	0	0	0
2/11/77	17	0	17	12	12	0.2	0.70
18/11/77	17	0	5	1	13	2.4	0.06
27/1/78	17	0	4[α]	1	14	3.3	0.06

[α] 4 susceptibles remained at end of study period

aged one to eleven days by dark-field examination or culture. These calves were sampled on two occasions 30 days apart at a time when all but three possessed colostral antibodies.

Urine samples were collected from 83 cows and three bulls with ages ranging from two and a half to thirteen years during the course of this study. Only one case of leptospiruria was detected. This was in a two and a half year old cow which had sero-converted one year earlier, and in which leptospiruria had been observed before.

The other 33 leptospiruric animals observed during this study were between eight and 22 months old. Urines from 26 of these animals were cultured and isolates obtained from 14. In the first series of cultures only two isolates were obtained from 56 urines taken from eight animals which were observed to shed leptospires intermittently. One was obtained by culture and hamster inoculation, the other by cultural isolation alone. The isolate obtained by hamster inoculation came from a urine which was negative by dark-field examination. The isolate obtained by direct culture was from a dark-field positive urine. This series of urines had been cultured by direct inoculation of 0.5 ml of whole, or diluted, urine into 5 ml of EMJH or EMJH plus 5FU or by Swinnex filtration into the same media. The culture media and supplements used in this series were commercially prepared. The cultural results from this series are summarised in Table 7.9.

Subsequently the culture procedure was altered. Swinnex filtration was discontinued and only undiluted or 1:10 diluted urine tested by inoculating one to three drops (approximately 50 µl) into 5 ml tubes of media. Media and supplement prepared in this laboratory containing either 200 µg or 400 µg/ml of 5FU was used routinely. This culture system was used on all urines cultured apart from the 56 samples discussed above. In this second series 166 urine samples from cattle between eight and 22 months of age were cultured. Sixteen of these were positive by dark-field examination. Twelve of these 16 samples produced isolates and an additional 22 isolates were obtained from dark-field negative samples. The cultural results from this series are summarised in Table 7.10.

Table 7.9

Results of urine[56] cultures obtained from eight yearlings which sero-converted in September 1976.

		Direct inoculation						Swinnex filtration	
Urine dilution		0		1:10		1:100			
Media additive		5FU[α]	None	5FU	None	5FU	None	5FU	None
Dark-field positive urines	positive	0	1	0	0	0	0	0	0
	contaminated	4	7	2	6	0	1	0	2
	negative	12	0	6	2	8	7	16	14
Dark-field negative urines	positive	0	1	0	0	0	0	0	0
	contaminated	4	17	2	12	1	7	1	5
	negative	36	3	19	9	20	14	39	35

α 200 µg/ml 5FU

Table 7.10

Results of urine[166] culture from yearling cattle at various times before and after sero-conversion.

	Semi-solid EMJH[α] Medium					
Urine Dilution	0		1:10		1:10	
Media Additive	400 µg/ml 5FU		400 µg/ml 5FU		200 µg/ml 5FU	
Dark-field Result	+ve	-ve	+ve	-ve	+ve	-ve
Culture Result						
Positive	10	17	10	17	5	7
Contaminated	3	35	1	31	4	68
Negative	3	98	5	102	7	75

α EMJH medium prepared in this laboratory according to the method of Johnson and Seiter (1977) (Appendix V).

Pairs of hamsters were inoculated with urine from 20 of the animals studied in the first series of urine examinations. Four samples were dark-field positive and the remaining 16 negative. There were no deaths and only one of a pair of hamsters inoculated with dark-field negative urine sero-converted, with a titre of 1:192, 21 days after inoculation. An isolate, later typed as *hardjo* by the W.H.O. Laboratory, C.D.C., Georgia, was recovered from the kidneys of this hamster. No other hamster yielded isolates on kidney culture and this method of isolation was discontinued owing to its lack of sensitivity.

Three isolates were lost when they became overgrown with contaminants. Of the remaining 31 isolates two were sent to the W.H.O. Laboratory, C.D.C., Georgia and were confirmed as serovar *hardjo*. These two isolates and the other 29 were typed as *hardjo* using cross-absorbed anti-*balcanica* and anti-*hardjo* antisera at the Massey University laboratory.

The length of leptospiruria was studied in 15 calves in which leptospiruria was first detected by dark-field examination or culture within a few weeks of sero-conversion. For all 15 animals leptospiruria at the limit of the shedding period was confirmed by cultural isolation and typing. These animals were sampled at various times for up to 14 months after sero-conversion though some animals were not available for sampling for the entire period. The mean shedding time observed was 215 ± 26 days and the maximum time observed was 410 days. Leptospiruria in the animal shedding for 410 days, and in others still shedding 207 to 358 days after leptospiruria was first observed was still continuing at the time of final sampling (Table 7.11). Some of these animals may have become infected up to 60 days before leptospiruria was first detected as they had not been sampled in the two months prior to the test at which leptospiruria was first detected.

During the study period none of the cattle on the No. 1 Dairy Farm were observed to sero-convert to serovars *ballum*, *copenhageni*, *pomona* or *tarassovi*, nor were any isolates of serovars other than *hardjo* recovered from cattle.

Table 7.11

Duration of leptospiruria in cattle with natural *hardjo* infections.

ID	Period for which leptospiruria observed (days)	Titre when leptospiruria last detected
01	165	1:96
07	207*	1:48
08	410*	1:96
011	152	1:272
013	263*	1:96
015	270*	1:96
016	165	1:192
017	270*	1:48
021	284*	1:192
022	165	1:136
G9	358*	1:384
G39	101*	1:768
G55	101*	1:768
G62	274	1:384
G63	47	1:768
mean ± S.E.	215 ± 26	GMT 1:192

* still leptospiruric when last sampled.

4. *Associations with climatic factors.*

Weekly mean meteorological data covering the period during 1/1/76 to 1/2/77 was obtained for grass minimum temperature, hours of sunshine, maximum air temperature, rainfall and pan evaporation. The rainfall and evaporation figures were combined to provide an estimation of net changes in moisture build-up in the environment. Data obtained from the groups of calves in which new outbreaks occurred, were analysed to determine periods during which there had been no transmission of infection, even though there had been contact with infectives. This analysis was based on the assumptions that no transmission occurred later than five days before the start of a period during which there was no sero-conversion, and that no animal sero-converted within less than five days of becoming infected.

The combined meteorological and transmission data is shown in Figure XXIV. This figure indicates that transmission apparently failed to occur in drier, warmer periods. All the major outbreaks occurred when the cumulative moisture was near peak levels indicating an association between transmission and saturated soil rather than rainfall *per se*. Statistical associations between the various meteorological parameters and the corresponding logarithms of incidence ratios were tested by correlation analyses (Table 7.12).

Table 7.12

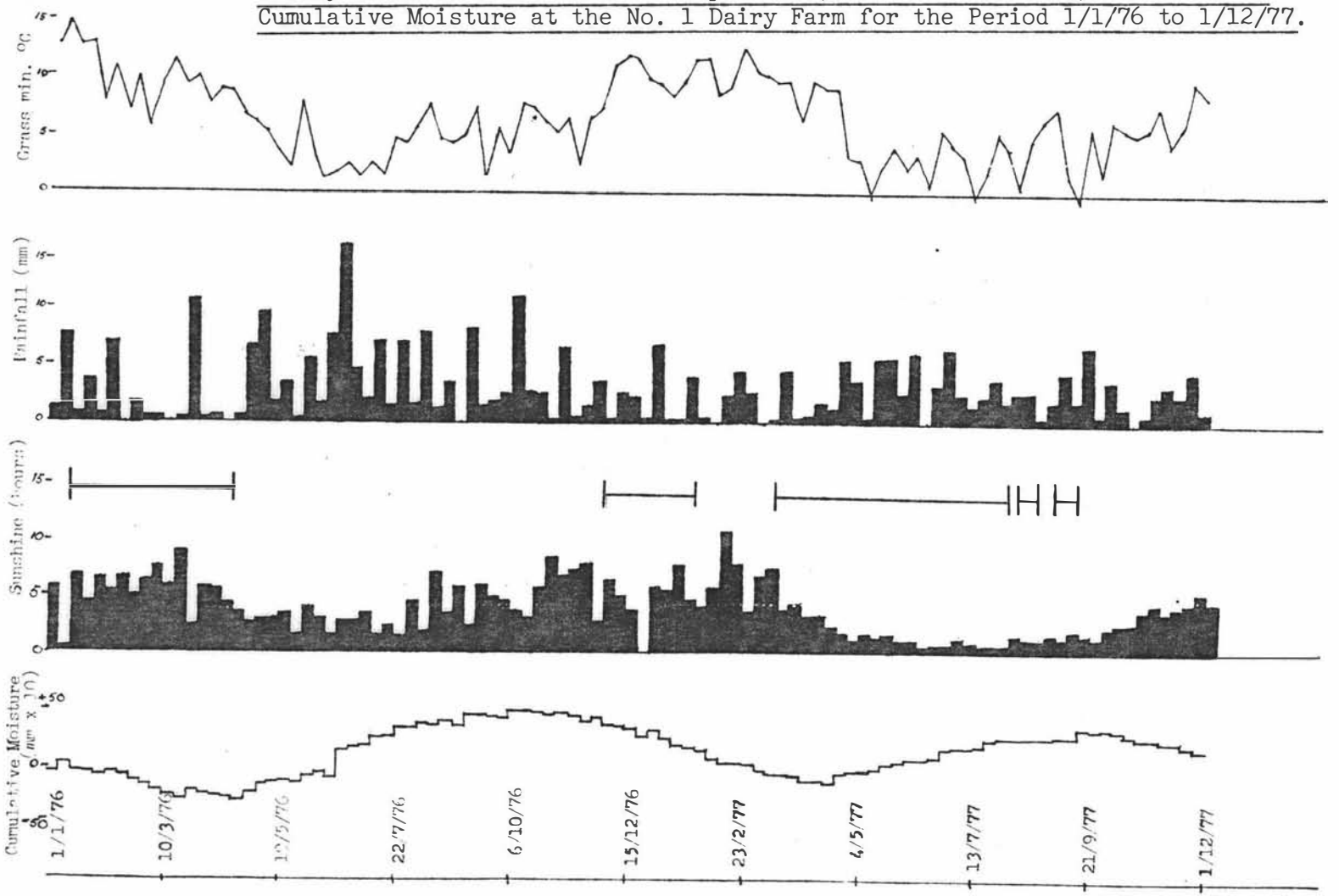
Correlation coefficients between mean four-weekly meteorological parameters and their corresponding incidence ratios (Log_{10}).

Meteorological parameter	Correlation Coefficient (15df)	Significance Level
Rainfall (mm)	0.15	N.S.
Sunshine (hours)	-0.21	N.S.
Maximum air temperature (^{o}C)	-0.50	< 0.02
Grass minimum temperature (^{o}C)	-0.34	N.S.
Net environmental water level (mm)	0.87	< 0.001

N.S. = not significant

Figure XXIV

Weekly Means of Grass Minimum Temperature, Hours of Sunshine, Rainfall and Cumulative Moisture at the No. 1 Dairy Farm for the Period 1/1/76 to 1/12/77.



= Periods during which no transmission occurred.

Owing to a more frequent sampling regime in 1977 more detailed transmission was available for that year. This data is shown graphically with the associated daily meteorological records in Figure XXV. The two periods when transmission did not occur coincided with two periods in which frosting and above average sunshine hours occurred.

5. *Associations with location.*

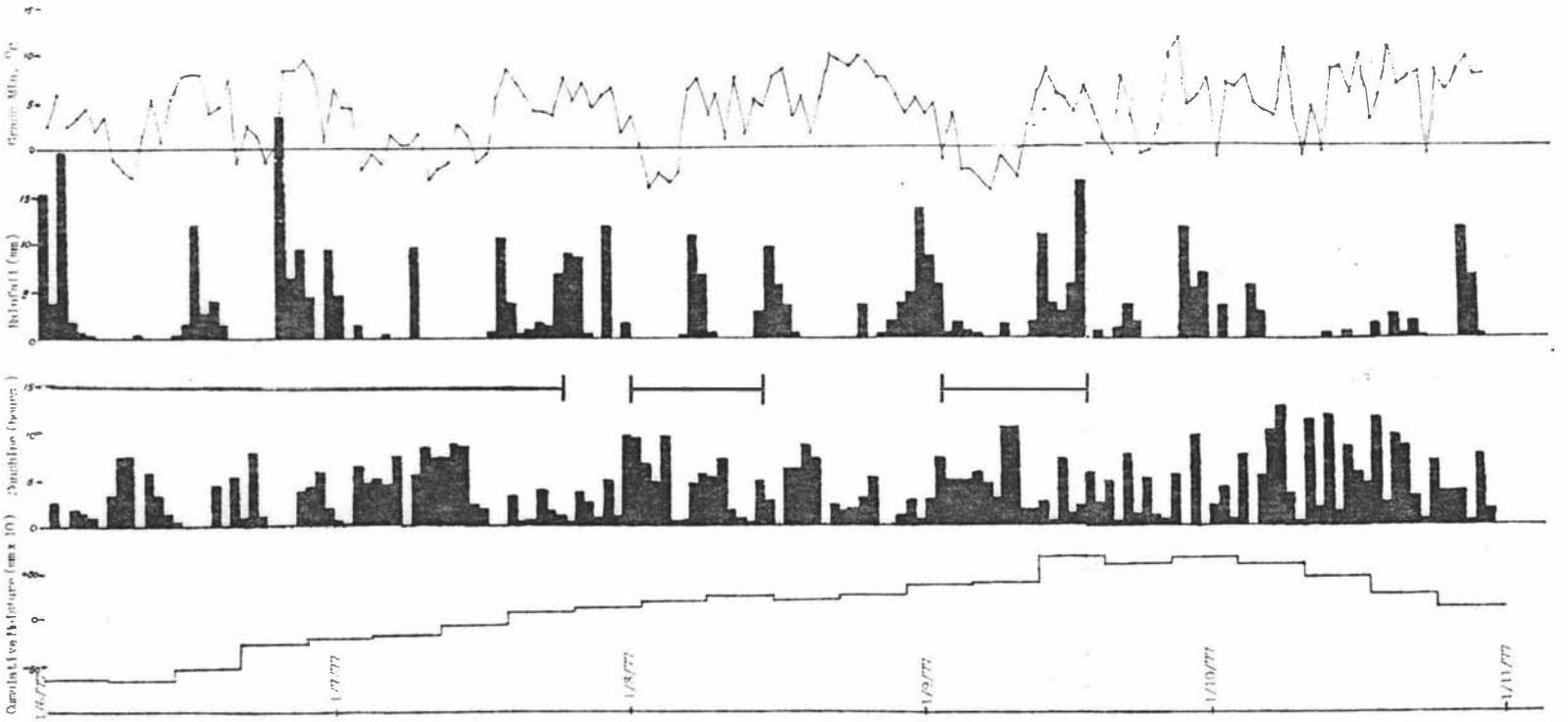
It appears that the occurrence of new cases was not associated with particular paddocks. New cases occurred while susceptibles were pastured in a number of paddocks on different parts of the farm (Figure XXII). Some of the paddocks on which stock were grazing when new cases occurred (28d, 28e, 28f, 28g, 87 and 90) were low-lying and tended to be swampy in the winter but others (24, 34a, 34b, 34c, 34d, 36, 44, 56, 57, 58, 60, 78 and 87) were drier and free-draining. One paddock (the Kennel Area), in which susceptibles were grazed in contact with shedders for about 10 weeks in May, June and July without new cases occurring was elevated, sandy and extremely free-draining. No new cases occurred until stock were moved into wetter paddocks in August.

Two series of soil and water samples were taken from the farm environment in 1976 and 1977. In 1976 samples were collected from various sites adjacent to the piggery and about the dairy farm. A *pomona* isolate was obtained from a water sample collected in an eddy of the small stream running between paddocks 13 and 54. This isolate was collected shortly after a period of heavy rain.

During June, July and August 1977, 20 susceptible calves born in spring 1976 were pastured with cattle shedding *hardjo* in the Kennel Area. On two occasions in June and August soil and water samples were taken from six separate locations in this paddock. One leptospiral isolate was obtained in pure culture from a low-lying area in this paddock. This isolate did not agglutinate with antisera against serovars *australis, autumnalis, ballum, bataviae, biflexa, canicola, copenhageni, grippotyphosa, hardjo, pomona, pyrogenes* or *tarassovi* and was non-pathogenic for hamsters. It was presumed to be a saprophytic form similar to

Figure XXV

Daily Records of Grass Minimum Temperature, Hours of Sunshine and Rainfall, and Weekly Means of Cumulative Moisture at the No. 1 Dairy Farm for the Period 1/6/77 to 1/11/77.



= Periods during which no transmission occurred.

those isolated at the piggery by Ryan (1978).

6. Associations with other animals.

During the two periods when outbreaks occurred, in winter-spring 1976 and 1977, there was no close contact with other species of domestic animals. No sheep were grazed in the sheep farm area but sows were being grazed at pasture adjacent to the piggery buildings. Some of these sows were shedding *pomona*. Serovar *pomona* was also recovered from areas of standing water in the sow paddock which were immediately adjacent to areas grazed by cattle (Ryan, 1978). Wildlife trapped within the dairy farm perimeter included 15 brown rats, 11 black rats, 16 hedgehogs, 53 mice, seven possums, two feral cats, one stoat and approximately 100 ducks. There was no evidence of *hardjo* or *pomona* in any wildlife except for a low *pomona* titre in one feral cat. Two possums yielded *balcanica* isolates, and *ballum* isolates were obtained from four black rats, ten mice and two hedgehogs (Hathaway, 1978).

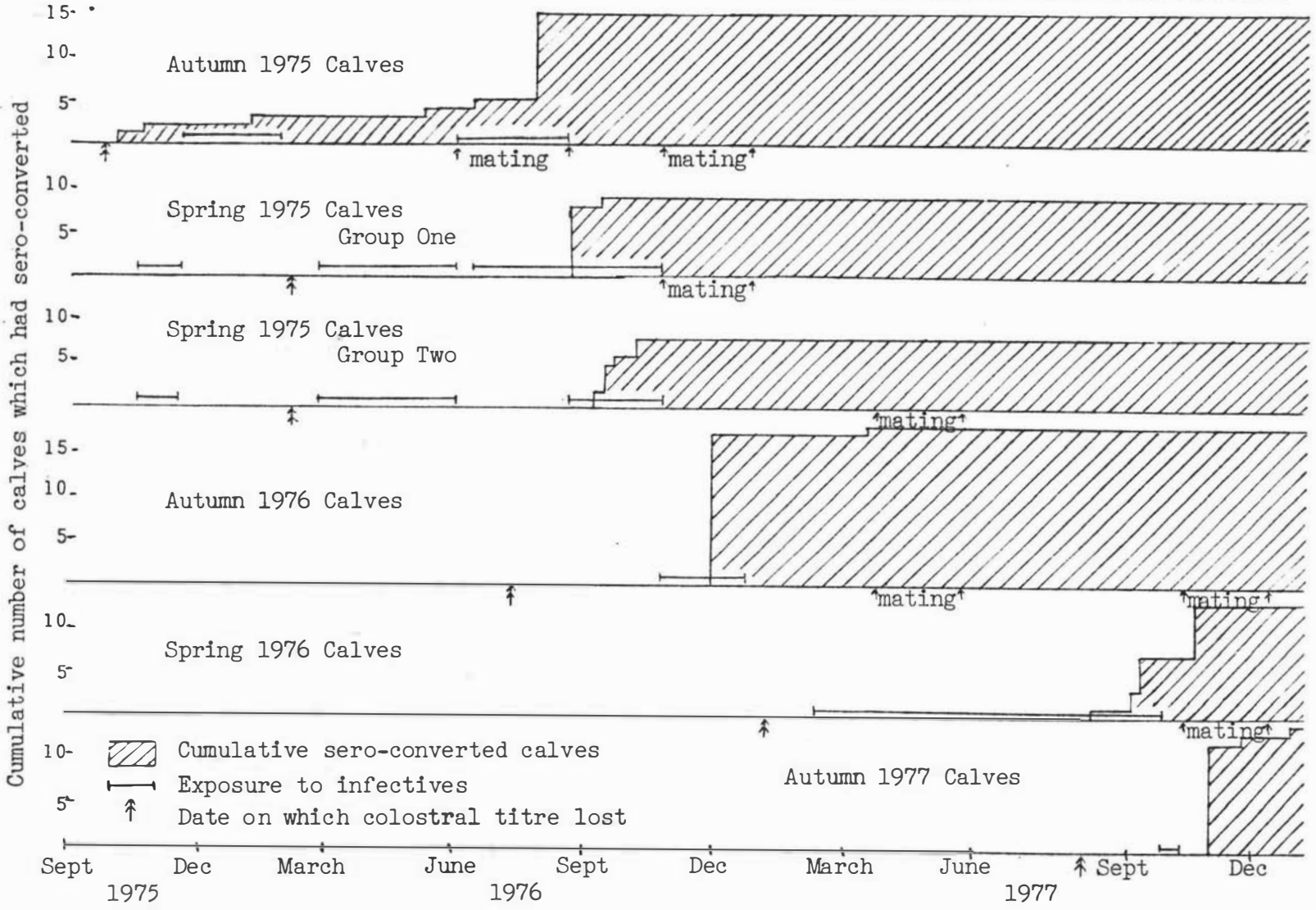
In two cases, with those calves born in autumn 1975 (Table 7.3) and with some of the calves born in 1976 (Table 7.4) mating coincided with the onset of propagating epidemics. In the other five epidemics observed mating occurred three to six months after sero-conversion of all group members (Figure XXVI). Leptospiruria was not observed in any of the bulls or teaser-bulls sampled during the study period.

Discussion.

This investigation essentially consisted of a series of prospective cohort studies, conducted on successive groups of calves born on the Massey No. 1 Dairy Farm. Each cohort was kept under serological and bacteriological surveillance while it was subjected to naturally occurring changes in immune status, environmental influences and exposure to *hardjo* infection. In order to mathematically define events involved in the establishment and maintenance of a propagating epidemic two new measurements were needed; Exposure Ratio and Incidence Ratio.

Figure XXVI

Occurrence of *hardjo* Epidemics in Groups of Calves at the No. 1 Dairy Farm.



The use of exposure ratios (i.e. ratios of infectives to susceptibles) allows comparisons to be made between the varying degrees of infective challenge to which cohorts were subjected at different times. Similarly, incidence ratios were used to define the rate of appearance of new cases in this study. This was necessary since it became apparent that the use of the measurements "crude attack rate" or "incident rate", which are more commonly used for this purpose (MacMahon and Pugh, 1970), is mathematically inappropriate for the definition of propagating epidemics occurring in small populations.

Attack rates or incident rates are calculated by expressing the number of new cases in a population as a proportion of the number of susceptibles in that population. In large populations the number of susceptibles remains relatively constant or declines slowly as individuals become infected, but in small populations each new case significantly reduces the number of susceptibles. Thus in a large population the attack rate provides a relevant measure of the dynamics of an epidemic, higher attack rates implying larger numbers of new cases, but in small populations, in which most or all individuals eventually become infected, as the epidemic progresses high attack rates may be based on only a few new cases. A simple example illustrates this point: in a population of 20 animals the first two cases occur with an attack rate of 2/20 = 10%, but the last two with an attack rate of 2/2 = 100%.

The pattern of hardjo infection in the No. 1 dairy herd.

A surveillance programme conducted on all ages and classes of cattle at the No. 1 Dairy Farm clearly demonstrated that the only groups of cattle experiencing new cases of *hardjo* infection consisted of six to eighteen-months-old calves. Similar observations have been made by Hanson *et al* (1964) and Cacchione *et al* (1968). A consistant pattern of infection was observed with each group of calves with the exception of those born in autumn 1975 (Figure XXVI). Each group of calves still included some animals with colostral titres for up to six months after birth and in no group were new cases observed until all members had lost their colostral titres, even when contact with infectives had occurred. New infections were noted within a few weeks to seven and a half months later

though most calves were at least eight months old before they became infected. Two calves became infected at a younger age. One, infected at five months of age had been born to a sero-negative dam in autumn 1975 and the other, infected at seven months of age, was born to a sero-negative dam in 1977 and at no stage acquired a colostral titre. These observations, and the fact that the autumn 1975, and apparently the autumn 1974 calves, did not experience propagating epidemics during their first winter, indicate the possible persistance of low levels of protective colostral antibody, even when passively-acquired agglutinating antibody is no longer detectable.

Of the 88 calves reared on the farm during the course of this study, only ten had failed to become infected at its conclusion. Seven of these had been vaccinated twice with an experimental *pomona/hardjo* bacterin, and the other three, born in autumn 1977, had been the youngest calves in that group and had lost their colostral titres only two months prior to contact with infectives. This observation, the fact that the autumn 1974 and 1975 calves failed to become infected shortly after the loss of detectable colostral antibody, and the failure to experimentally infect calves of a similar age with *hardjo* (reported in Chapter Nine) lend support to the hypothesis suggested by Hathaway (1978) that protective levels of antibody persist after the loss of agglutinating titres. Hathaway observed, in young possums, that after the loss of maternal antibody there was a further period of up to two months during which they continued to remain refractory to experimental challenge with *balcanica*. Using a modification of the method of Tripathy, Hanson and Mansfield (1973) he demonstrated that sera, taken from these animals, inhibited the growth of *balcanica in vitro*. It has been demonstrated that animals can have antibody protecting them against leptospiral infection even though they possess no detectable agglutinating antibody (Negi, Meyers and Segre, 1971). Hanson (1973) has noted that vaccinated cattle are frequently protected in the absence of agglutinating titres and this phenonomen was also observed in the present study in some of those calves, born in spring 1976, which were vaccinated prior to contact with infectives (Table 7.7).

New cases consistently occurred in the winter and spring with very few occurring in the summer and autumn. Thus spring-born calves, which had lost detectable passively-acquired protection in the early summer failed to become infected, even though in contact with infectives, until the following winter and spring. Autumn-born calves, on the other hand, lost their colostral titres in the winter and became infected shortly afterwards, in the early spring, on the first occasion on which they were in contact with infectives.

The calves born in autumn 1975 did not experience an epidemic during their first winter as susceptibles and, on the basis of group GMT's, it similarly appears that many of the calves born in autumn 1974 failed to become infected until 18 months of age. These twin occurrences are probably explained in part by a management change which occurred at the time that this study commenced. During the period of this study there was a transition in the mean autumn calving date from May to February. This transition is reflected in the fact that some autumn 1975 calves still had colostral titres in late September whereas in subsequent years these had been lost by the beginning of August. Also in 1976 and 1977 there were more frequent periods of contact between susceptible calves and infectives present amongst their immediate predecessors.

Each group of calves, or heifers as they grew older, was generally first exposed to infectives from within the calf or heifer group which was six months older. Epidemics in the younger aged groups normally followed contact with the older heifers if this contact occurred in the winter and spring months, but not if it occurred in summer and autumn. For this pattern of propagating epidemic infection to occur every winter, it is apparent that a significant number of animals must remain as infectives at least from one spring to the following winter. From the data presented in Table 7.11 it appears that approximately 50% of calves infected the previous winter and spring fulfil this requirement.

The observation of this epidemic cycle amongst calves and heifers indicates the possibility that if susceptibles fail to

become infected until they enter the adult milking herd then the cycle may occur one year later. Susceptibles which became infected after introduction into the milking herd would then act as the reservoir of infection for the following year's susceptibles. Such a cycle would be expected under management systems which prevented contact between consecutive age groups until entry into the adult herd. Ellis and Michna (1976a and 1976c) have reported such a pattern of management in Scottish dairy herds experiencing *hardjo* abortion storms. It can reasonably be argued that the zoonotic risk and certainly the risk of bovine abortion will be substantially greater in herds experiencing propagating epidemics in young adults rather than in calves.

Source of bovine hardjo infection.

The wild-life surveys conducted by Hathaway (1978) and the surveillance programme conducted at the piggery by Ryan (1978) conclusively exclude either wild-life or pigs as potential *hardjo* reservoirs on the No. 1 Dairy Farm. Similarly, the absence of sheep from the sheep farm area during the period when outbreaks occurred and the absence of any other species of domestic stock, either on the farm, or about its perimeter, leaves but one reservoir of *hardjo* infection.

It is apparent from the observed shedding times (Table 7.11) that *hardjo* is well adapted to the bovine and that long-term carriers are to be expected. However, the combined evidence of a very low shedder rate in adult cattle in this study, and of a 3.4% prevalence of adult carriers in the abattoir survey reported in Chapter Two, suggests that the *hardjo* carrier state is seldom or never a life-long condition. Persistant infection or reinfection amongst adult cattle is therefore not supported by these results. Nevertheless, the maximum unterminated shedding time of 410 days recorded in this study is substantially greater than the previously recorded maximum for *hardjo*-infected cattle of 153-174 days (Farina *et al*, 1972; Ellis and Michna, 1977). It is believed that the results of this study indicate that few cattle shed *hardjo* for much longer than one year.

Factors influencing the transmission of infection.

The outbreaks observed in this study invariably followed contact with infective bovines. It has been demonstrated in this study that at least 50% of cattle sero-converting in the previous nine months can be regarded as infectives and that the infective phase may last longer. However, it is also apparent from an inspection of the data summarised in Figures XXIII and XXVI that periods of contact with infectives do not invariably result in outbreaks amongst susceptibles. Even high exposure ratios do not result in the development of propagating epidemics in the summer and autumn, but relatively low exposure ratios can trigger epidemics in the late winter and early spring (Figure XXIII).

The highest incidence ratios observed in epidemics during the present study occurred during winter and early spring. Incidence ratio was not significantly correlated with rainfall, reflecting the fact that rainfall is distributed throughout the year in the Manawatu, nor with sunshine or mean grass minimum temperature (Table 7.12). However, a highly significant positive correlation was obtained with the net environmental water level. This indicates that the greatest occurrence of new cases was at the time of the year when the soil and environment was at its wettest; in the late winter and early spring. A significant negative correlation between air temperature and incidence ratio was also obtained. It is likely that this association is non-causal and follows from the fact that outbreaks occur in the winter.

The strong tendency observed in this study for new cases of *hardjo* infection to occur when the farm environment is at its wettest is in agreement with the reports of Hoare and Claxton (1972) and Ellis and Michna (1976b). However, these findings extend the observations of these authors by specifically relating the spread of infection to a wet environment rather than rainfall *per se*. This implies a major environmental role in facilitating the spread of *hardjo* from cow to cow.

The mechanisms which may be involved include a possible build-up of organisms in a favourable environment, saturated soil, which then provides a reservoir for susceptible cattle. However, there

was no evidence in the present study of susceptibles becoming infected from the environment in the absence of known or suspected infectives, nor was *hardjo* isolated from likely environments in limited surveys. The possibility that a wet environment favours transmission by stimulating an increase in the rate of shedding through a diuretic effect fails to explain why lush autumn and spring pastures do not produce a similar effect. This is also not borne out by dark-field observations on urines taken sequentially from some animals in this study. A third possibility is that prolonged exposure to a wet environment renders the host more susceptible, possibly due to such changes as a softening of the skin above and between the hooves, favouring percutaneous transmission. It is likely that a combination of these and other factors combine to produce the increased incidence of *hardjo* infection observed.

The cessation of transmission during periods of frosting, followed by immediate ressumption when grass minimum temperatures rise (Figure XXV) again indicates that a build-up of organisms in the soil and surface water is not a necessary condition for the continued propagation of an epidemic if it is assumed that frosting has a sterilising effect. However, Ryu and Liu (1966) demonstrated 14 day survival of serovars *semarang* and *australis* at $0^{o}C$. Other workers have also reported enhanced survival of leptospires at low temperatures probably due to reduced growth of other inhibitory bacteria (Chang, Buckingham and Taylor, 1948; Michna, 1970).

On balance this combined evidence indicates that a wet environment is a major factor in facilitating the transmission of infection. It is believed that this study suggests that transmission occurs shortly after an infective animal sheds organisms into the environment, probably within only a few hours.

Although it has been demonstrated that the venereal route of infection of bovines is possible (Kiktenko *et al*, 1976) most of the outbreaks which occurred in this study preceded mating. Those outbreaks which occurred concurrently with mating can be readily explained by the presence of many other infectives in a suitable environment. The three bulls examined in this study were

serologically positive but culturally negative. While it is possible that mating may contribute to some transmission of *hardjo* infection it is apparent that the disease can be transmitted very successfully by other means.

An intriguing aspect of this study was the finding of serovars *ballum* and *balcanica* in the wild-life hosts which were widespread on the farm in the absence of any bovine infection. On a number of occasions in the present study, brown rats were observed to feed in calf meal buckets and the feed store was heavily infested with mice, also possums in a farm environment spend considerable periods grazing pasture (Hathaway, 1978). Other authors have reported both a lack of correlation between serovars infecting wild-life and cattle occupying the same environment (Clark, 1961) and an association (Martin *et al*, 1967; Schnurrenberger *et al*, 1970; Twigg *et al*, 1972). It is believed that leptospiral infection in wild-life represents a relatively minor hazard to the bovine population in this country.

Bacteriological Findings.

The cultural results in this study, particularly those from the first series, were somewhat disappointing. All but one of the 16 dark-field positive urines in the first series (Table 7.9.) failed to produce isolates and none were obtained from dark-field negative urines. Two reasons may account for the improved recovery rate in the second series (Table 7.10) where 12 of 16 dark-field positive and 22 of 150 dark-field negative urines yielded isolates. The considerably smaller inoculum of urine employed in the second series was adopted after a control study indicated that larger inoculum volumes severely inhibited leptospiral growth in culture (Appendix VI). Also media prepared and monitored in the Massey laboratory, according to the method of Johnson and Seiter (1977), routinely supported superior growth of a fastidious *hardjo* isolate compared with the commercially prepared media used earlier.

Hamster inoculation proved an insensitive technique for the isolation of *hardjo*. This finding is in agreement with Hathaway's (1978) observation that even 10^8 *hardjo* organisms inoculated into hamsters intraperitoneally failed to consistently produce infections.

It appears that the culture techniques employed in the second series are substantially superior to hamster inoculation.

Summary.

1. In a dairy herd with endemic *hardjo* infection new cases only occurred in calves six to eighteen months old.
2. All the available evidence indicates that infection is spread from cow to cow, each group of susceptibles becoming infected after contact with infectives in the chronologically preceding group of calves.
3. At least 50% of cattle infected with *hardjo* experience leptospiruria lasting at least nine months and one animal was still shedding when last observed 410 days after leptospiruria was first detected.
4. Calves are apparently protected by colostral antibodies for at least the first six months of life and probably for up to two months after the loss of agglutinating antibodies.
5. There is no evidence for continuing *hardjo* infection in a significant proportion of adult animals nor of recurrent infection.
6. Contact with infectives alone was not a sufficient condition for the establishment of propagating epidemics.
7. Peak incidence of *hardjo* infection was strongly correlated with those periods when pastures were wettest and there is some evidence that transmission is interrupted by periods of frosting. However, rainfall and sunshine were not correlated with the occurrence of new cases.
8. Venereal infection, or infection associated with mating behaviour, was considered to be relatively insignificant in the epidemiology of bovine *hardjo* infection.
9. Wild-life reservoirs of *ballum* and *balcanica* appeared to present no hazard to the bovine population.
10. The most successful cultural technique employed on bovine urine samples utilised Johnson's medium containing 1% Fraction V bovine serum albumin 0.15% agar and 400 µg/ml 5-Fluorouracil. A small inoculum of approximately 50 µl of urine undiluted or diluted 1:10 was superior to a 10% inoculum. Swinnex filtration and hamster inoculation proved to be unsatisfactory techniques.

11. The use, in this study, of the measurement "Incidence Ratio" to define propagating epidemics occurring in small cohorts, appears to be more appropriate than the use of crude attack rates and incidence rates. This epidemiological parameter may be of value in the study of other infectious conditions in small populations.

CHAPTER EIGHT

EPIDEMIOLOGY OF BOVINE *POMONA* INFECTION IN NEW ZEALAND

Introduction.

During the course of this present study of bovine leptospirosis there were only limited opportunities for investigations into the epidemiology of bovine *pomona* infection. However, those observations which were made suggest that *pomona* and *hardjo* infections of cattle have some shared and some different causal associated factors. This chapter presents a brief hypothesis of the epidemiology of bovine *pomona* infection in New Zealand with supporting evidence for some of the points raised.

In addition to these observational studies, two experimental studies were undertaken. The first of these was the establishment of a self-propagating *pomona* outbreak in a group of calves and the second a laboratory study into the survival of *pomona* in soil taken from a farm where bovine *pomona* outbreaks had occurred.

In the review of bovine leptospirosis in New Zealand, presented in Chapter One, evidence is cited to demonstrate that both the clinical and serological prevalences of *pomona* infection in cattle are declining. Conversely Ryan (1978) has demonstrated that there is a high endemic level of *pomona* infection in New Zealand pigs, with many herds having close to a 100% prevalence.

Most of the early reports of bovine *pomona* infection in this country implicated pigs as the source of infection (see Chapter One). Many overseas reports have also implicated pigs as the major source of bovine *pomona* infection. In Australia it has been stated that many outbreaks of redwater in calves were associated with pig contact (Stewart, 1934; Sutherland, Simmons and Kenny, 1949; Wellington *et al*, 1951; 1953; Spotswood, 1962; Emanuel, MacKerras and Smith, 1964). Shield (1974) reported that some outbreaks of *pomona* infection in beef cattle in Queensland were caused by contact with wild pigs. He demonstrated that pigs wallowing in cattle water holes were shedding *pomona*. One

reported outbreak of bovine abortion caused by *pomona* was attributed to the introduction of carrier cattle into a susceptible herd (Knott and Dadswell, 1970). In the United Kingdom, where there has been no published report of *pomona* infection in pigs (Ryan, 1978), there have also been no reports of *pomona* titres or clinical infection in cattle. In the United States both cattle and pigs were considered primary hosts for *pomona* by Bohl and Fergusson (1952), though they found evidence of pig-to-cattle transmission in three of five outbreaks. Burnstein and Baker (1954) experimentally demonstrated the transmission of *pomona* from pigs to calves but not from a leptospiruric calf to susceptible pigs. However, Morter and Morse (1956) did succeed in infecting a pig by contact with a shedding calf. In his extensive review of the epidemiology of leptospirosis, van der Hoeden (1958) considered that pigs were the primary reservoir of *pomona* from which cattle became infected but he stated that cow-to-cow transmission also occurred.

Bovine *pomona* infection has been frequently reported in the United States in situations where pigs do not appear to be involved. Little and Baker (1950) considered asymptomatic carriers were the main source of bovine infection. Water-borne infection, close confinement on feedlots, and a wet environment were considered the major factors involved in bovine *pomona* outbreaks by Sippel, Boyer and Chambers (1952), Reinhard (1953) and Hadlow and Stoenner (1955). Bovine *pomona* outbreaks in which no pig contact occurred were reported by Hadlow and Stoenner (1955), Stoenner *et al* (1956), Clark (1961), Clark *et al* (1961) and Hanson *et al* (1964). In three of these reports wildlife were also extensively studied and *pomona* was recovered from various wildlife species. *Pomona* infection of cattle has also been attributed to contact with infected wildlife in Europe (Borg-Petersen and Fennestad, 1956; Kmety, 1957).

Burnstein and Baker (1954) considered that *pomona* was better adapted to pigs than cattle. They based this claim on their observation that pigs shed relatively much greater numbers of organisms and for a longer period than did calves. Morter and Morse (1956) also considered pigs to be better adapted hosts than calves

since *pomona* was of greater infectivity to pigs. There have been numerous reports on the degree and persistance of leptospiruria in both pigs and cattle. Shedding rates as high as 10^8 leptospires/ml have been reported in both species (Gillespie and Kenzy, 1958; Ryan, 1978) but there is a major difference in the reported duration of shedding in each species. The reported maximum shedding times for cattle infected with *pomona* are in the range of one to four months (Table 8.1), whereas shedding times of 12 to 24 months or more have been reported in pigs (Schmid and Giovanella, 1947; Mitchell *et al*, 1966; Ryan, 1978).

Table 8.1

Duration of shedding of *L. pomona* by infected bovines.

Maximum shedding time (days)	References
28	Morter and Morse (1956)
29	Morter *et al* (1958)
35	Sleight and Williams (1961)
37	Hodges and Ris (1974)
41	Reinhard *et al* (1950)
42	Reinhard (1951)
48	Doherty (1967a)
48	Doherty (1967b)
53	Baker and Little (1948)
55	Sutherland (1950)
56	Webster (1959)
60	Doherty (1966)
69	Reinhard and Hadlow (1954)
77	Gillespie and Kenzy (1958)
84	Ringen and Bracken (1956)
86	Ringen *et al* (1955)
90	Reinhard (1953)
90	Sutherland *et al* (1949)
91	Fennestad (1963)
94	Hadlow and Stoenner (1955)
102	Fergusson *et al* (1957)
104	Kenzy *et al* (1958)
118	Doherty (1967c)

The transmission of leptospirosis has frequently been associated with wet environments and periods of high rainfall. This association has led several workers to suggest that soil survival may be a key factor in the transmission of infection to new hosts (Kirschner *et al*, 1952; Derrick, 1956; Amatredjo and Campbell, 1975). Kirschner and McGuire (1957) reported that *pomona* survived 21 days in a very wet soil (50% water by weight) which had a pH of 6.2. In the same year Okazaki and Ringen had demonstrated the survival of *pomona* for 183 days in sterile water, with a pH of 6.9, containing 25% by weight sterile soil. Smith and Self (1955) had earlier demonstrated the survival of serovar *australis A* for 43 days in a drier and more acidic soil (34% water by weight, pH 6.6). In a recent study Karaseva *et al* (1974) reported the survival of *grippotyphosa* in a soil with a pH of 7.5 and a moisture content of 70% for 279 days. Various other reports had indicated shorter survival times in a variety of soil types under a variety of conditions of pH and moisture (Okazaki and Ringen, 1957; Ryu and Liu, 1967; Karaseva *et al*, 1973). The studies of Smith and Turner (1961) demonstrated that an acidic environment, particularly below pH 6.0, is unfavourable for the survival of all serovars and they considered that this would have a major effect on the survival of leptospires in soil.

Materials and Methods.

Retrospective study of an outbreak of bovine pomona abortion.

In July 1977 four of seventeen heifers aborted on a Manawatu dairy farm serviced by the Massey University veterinary clinic. The aborting heifers were four of a group of seventeen which had been introduced into a larger group of 60 in-calf heifers from another farm 19 days before the start of this investigation.

Serum and urine samples were collected from those heifers which had aborted as well as from other members of both the smaller and the larger groups. Sera were tested by the MAT and urine samples were cultured according to the modified technique described in Chapter Seven and isolates were typed. In addition sera from two of the four cows which aborted were fractionated by gel filtration according to the method described in Chapter Six.

Additional information was obtained by interviewing the farmer to obtain a history of the movements of each group of heifers. Records were also obtained of an investigation which had been conducted at a piggery on the property where the smaller group of heifers had previously been pastured.

Observations on the natural transmission of pomona from experimentally-infected cattle.

As a consequence of the attempted experimental infections reported in Chapter Nine, the opportunity arose to observe a small outbreak of *pomona* infection. Two calves, actively shedding leptospires in their urine, were grazed with eight other calves for a four month period. Urine and serum samples were collected from all calves at 7 to 14 day intervals and examined as previously described. At the termination of this study all calves were slaughtered and urine, serum and kidney tissue were collected from each animal and subjected to bacteriological and serological examination for evidence of leptospiral infection. All isolates obtained were typed against known antisera.

Soil survival of pomona

The soil survival time for *pomona* was determined in a soil found in many parts of the Manawatu district. The soil used (Manawatu sandy loam) was an acidic soil, with a pH of 5.5, and was representative of the soils present on the farms where *pomona* epidemiology was investigated in this study.

The soil was dried in a vaccuum oven at 40^{o}C for three days and packed into six sterile aluminium pots. Twelve perforated cellulose tubes, each measuring 10mm diameter by 50mm long, and containing 10g of dried soil, were buried in each pot. Sufficient sterile distilled water was added to produce pairs of pots with 75%, 100% and 125% of the saturated soil-water level respectively. These water levels, equivalent to 23%, 29% and 33% of the total wet soil weight, were maintained throughout the experiment.

An eight-day culture of a low-passage *pomona* isolate was washed by ultra-centrifugation and resuspension in sterile physiological saline. Inocula, each of 5 x 10^{8} of this organism,

were added to each cellulose tube. The pots were sealed and held in a controlled environment laboratory under simulated Manawatu winter conditions, for the duration of the experiment. The daily cycle in the laboratory was 10 hours of artificial sunlight at a temperature of 12.5°C and 31% relative humidity followed by 14 hours at 7.5°C and 61% relative humidity in darkness.

Cellulose tubes were withdrawn from each pot at various times during the following 70 days. They were examined by suspending the soil in 20ml of sterile distilled water, centrifuging the suspension for 5 minutes at 3000G, and inoculating the supernatant into culture media and hamsters. One type of media, EMJH plus 0.15% Agar, was used either without additives or containing either 400 µg/ml 5FU or a mixture of antibiotics (SNA). These antibiotics were 50 µg/ml sulfathiazole, 5 µg/ml neomycin and 0.5 µg/ml actidione prepared according to the technique of Cousineau and McKiel (1961). The soil-water supernatants were not inoculated directly into culture media. They were subjected to membrane filtration of two types; positive pressure filtration through 0.22 µm x 13mm cellulose membrane filters held in plastic filter holders (Swinnex), and floating membrane filtration according to the method of Fowler (1970). Thus six replicates of each soil-wash supernatant were cultured, each of three media being inoculated using both filtration techniques.

Pairs of hamsters were inoculated intra-peritoneally with soil-wash supernatants collected on alternate sample days. They were cultured from kidney, liver, blood and urine either at the time of death or on the 45th day after inoculation. Tissues were also preserved for histopathological examination and sera collected for serological examination.

Results.

Retrospective study of a natural outbreak of bovine pomona abortion.

Four heifers, from a group of 17, aborted between 12 and 18 days after they were introduced into a larger group of 60 heifers. All 77 heifers were at a late stage of gestation.

Urine and blood samples collected from 12 of the 17 introduced

heifers, including the four which had aborted, and also from 12 of the 60 resident heifers were examined for evidence of leptospiral infection. Three urine samples were positive on dark-field examination, and were estimated to contain between 10^6 and 10^8 leptospires per ml. Two of these samples and one other yielded isolates on culture (Table 8.2). All four leptospire-positive urine samples came from the recently aborted heifers. The isolates were typed as members of serogroup *Pomona*.

The serological results summarised in Table 8.2 clearly indicate that all but one of the sample of introduced heifers had *pomona* titres while none of the sample of resident heifers had any evidence of infection. Sera taken from two of the heifers which had aborted (G6 and G15) were fractionated by gel filtration and the relative amounts of agglutinating activity in their IgM and IgG immunoglobulin components were estimated. Heifer G6 had 86.5% of its agglutinating activity in the IgM class and heifer G15 had 71.3%.

The group of 17 new heifers had spent three months prior to their introduction grazing an area adjacent to a piggery. Inspection of laboratory records pertaining to this piggery indicated that there was a high level of endemic *pomona* infection in the pig herd. The piggery was not specifically investigated as part of this study.

The four heifers which aborted were immediately isolated from the main group of heifers, and following this investigation were all culled. In-contact heifers were vaccinated with a commercial *pomona* bacterin on the same day that the diagnosis was made and no further cases of abortion occurred. Unfortunately there was no opportunity to continue investigations with this group of heifers but a random sample of 24 lactating cows from the farm were sampled three months later and no *pomona* titres >1:24 were detected. The farmer reported that he had disposed of most of the 17 heifers and could no longer identify those remaining.

Table 8.2

Serological and cultural findings on two groups of heifers sampled on 8/7/77.

	Identification	*pomona* titre	Dark-field	Culture
Sample of heifers introduced on 19/6/77.	G3	1:384	-	-
	G5	1:1536	-	-
	G6[α]	1:6144	+	+
	G7	<1:24	-	-
	G9	1:1536	-	-
	G10	1:192	-	-
	G12	1:384	-	-
	G13[α]	1:2172	+	C[β]
	G15[α]	1:1086	+	+
	G18[α]	1:1536	-	+
	G24	1:68[illegible]	-	-
	G47	1:1536	-	-
Sample of the heifers resident on the farm 19/6/77.	F1	<1:24	-	-
	F2	<1:24	-	-
	F3	<1:24	-	-
	F4	<1:24	-	-
	F5	<1:24	-	-
	F6	<1:24	-	-
	F7	<1:24	-	-
	Y2	<1:24	-	-
	Y5	<1:24	-	-
	Y6	<1:24	-	-
	Y13	<1:24	-	-
	Y15	<1:24	-	-

α aborted during the period 4/7/77 to 8/7/77.

β all tubes contaminated

Observations on the natural transmission of pomona in a small group of calves.

Two calves shedding an estimated 10^5 to 10^7 leptospires per ml of urine were run at pasture with eight other calves which were sero-negative to *pomona*. Six new cases were detected by sero-conversion 16, 50, 50, 62, 71 and 71 days later. The two remaining calves were removed from the group after 85 days and were still sero-negative when they were slaughtered after a further 46 days.

The serological and cultural observations made on these calves during the study period are summarised in Table 8.3. In none of the calves which sero-converted were peak MAT titres less than 1:6144 and in two (VG1 and VG9) peak titres of 1:98,304 were detected. Leptospires were observed by dark-field microscopy at estimated counts ranging from 10^4 to 10^8 per ml in seven calves and *pomona* isolates were obtained from all seven. No shedding was observed in the other calf which sero-converted nor were isolates obtained by urine or kidney culture of this animal.

Using the data reported in Table 8.3 estimates were made of the minimum and maximum times after infection when leptospiruria was detected in each calf (Table 8.4). Since the actual dates of infection were not known for all calves these estimates were based on the assumption that there was an average five-day period between infection and sero-conversion (see Chapter Nine). Two of the animals which shed *pomona* were still leptospiruric approximately three months after becoming infected and a third still had infected kidneys at this time. The actual duration of shedding detected by repetitive urine examination and culture, ranged from 13 to 69 days with a mean of 45 ± 9.2 days. Maximum shedding times ranged from 38 to 94 days p.i. with a mean of 67.0 ± 8.5 days.

Weather records were consulted for the period during which these animals were run together. Only one frost occurred, on 25/10/77, and throughout the period the net environmental water level (see Chapter Seven) was falling rapidly as evaporation generally exceeded rainfall during this period. Three periods

Table 8.3

Summary of cultural and serological observations of an outbreak of *pomona* infection.

Test Date	VG10		VG7		VG5		VG1		VG9		VG4		VG3		VG8		VG2		VG6	
	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria	Coded Titre	Lepto-spiruria
17/10/77	7½	+	7	+	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
21/10/77	8	+	9	+	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
25/10/77	9	+	8	+	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
2/11/77	8	-	7	+	6	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
13/11/77	8	+	8	-	8	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
23/11/77	8	-	8	-	9	+	0	-	0	-	0	-	0	-	0	-	0	N/S	0	-
6/12/77	7	-	7	+	7	+	5½	+	5	N/S	0	-	0	-	0	-	0	N/S	0	-
18/12/77	7	N/S	7	-	6½	-	7½	+	9	+	2½	+	0	-	0	-	0	N/S	0	-
27/12/77	6½	-	6½	-	7	-	13	+	8	+	10	+	6	-	8	-	0	-	0	-
2/1/78	6	-	5	-	6	-	11½	+	11½	+	9	Cont	5	+	9	Cont	0	-	0	-
10/1/78	6½	-	5½	-	6	-	12	-	12	Cont	8	+	7	-	6	Cont	0φ	-	0φ	-
20/1/78	5½	N/S	4	-	5	N/S	13	-	13	N/S	6	+	11½	+	4	N/S				
30/1/78	5	-	5	-	5	-	13	-	13	Cont	6	+	9½	-	4	-				
13/2/78	5½	-	5	-	5	-	12	+	11	+	6	Cont	8	-	4½	-				
28/2/78	5½	-	4½	-	5	-	10	+ε	8	+	6	+	7½	-	4	-				

+ = leptospiruria, - = no leptospiruria detected.
N/S = No sample.
Cont = All culture tubes contaminated.
φ = Removed from group and run in isolation after 10/1/78
ε = *pomona* isolated from kidney tissue only.

Table 8.4

Estimated times after infection when the shedding of *pomona* commenced and ceased.

Animal Identification	Possible Infection Date Earliest	Latest	Date Leptospiruria Detected First	Last	Duration of Leptospiruria(days)	Days p.i. when leptospiruria last detected. Minimum	Maximum
VG10	26/9/77 α	26/9/77	13/10/77	13/11/77	31	48	48
VG7	26/9/77α	26/9/77	17/10/77	6/12/77	50	71	71
VG5	20/10/77	28/10/77	23/11/77	6/12/77	13	39	47
VG1	18/11/77	1/12/77	6/12/77	13/2/78 β	69 β	74 β	87 β
VG9	18/11/77	1/12/77	18/12/77	23/2/78	67 ψ	84 ψ	94 ψ
VG4	1/12/77	13/12/77	18/12/77	23/2/78	67 ψ	72 ψ	84 ψ
VG3	13/12/77	22/12/77	2/1/78	20/1/78	18	29	38
VG8	13/12/77	22/12/77	-	-	N/A	N/A	N/A
					45.0 ± 9.2$^{\phi}$	59.6 ± 7.8$^{\phi}$	67.0 ± 8.5$^{\phi}$

α Experimentally infected on that date.

β *pomona* recovered from kidney tissue 10 days later.

ψ Still shedding when finally sampled.

\- N/A = Not applicable as leptospiruria not observed in this animal.

ϕ Mean ± standard deviation.

of heavy rainfall occurred but only one coincided with a period when transmission of *pomona* infection was occurring. On no occasion was there standing water on the area where the calves were grazing and they obtained water from cattle troughs supplied directly from an artesian well. The calves had no contact with any other species of domestic animal and the only wildlife observed in the area were occasional brown rats, hedgehogs and mice.

Studies on the survival of pomona in the soil.

The results of the study to determine survival times of *pomona* in soil are summarised in Table 8.5. *Pomona* was recovered from soil containing both 23% and 33% water by weight on the 42nd day after the soils were inoculated with washed *pomona* cultures. The soil held at the intermediate water level, of 29% by weight, yielded a *pomona* isolate 31 days after inoculation.

As indicated in Table 8.5, there was close agreement between isolation results achieved using direct culture and hamster inoculation. Although the hamsters which were inoculated with soil-wash supernatants obtained from the wettest and driest soils on day 10 did not yield isolates, they developed titres to *pomona* in the range of 1:3072 to 1:12288 and leptospires were observed in Warthin-Starry stained sections from their kidneys.

A comparison of the cultural techniques used in this investigation is summarised in Table 8.6. The data presented in this table indicates that the addition of antibiotics (SNA) to the EMJH semi-solid medium considerably reduced the number of contaminated tubes but also suppressed the growth of leptospires when compared with tubes containing 400 µg/ml 5FU. The addition of 400 µg/ml of 5FU to the semi-solid EMJH medium appeared to have little effect on the isolation or contamination results.

Similar numbers of isolates and contaminated tubes were recorded in both the medium containing 5FU and the one that did not. However, unique isolates were obtained by the use of each medium so their combined use was considered to be justified.

Table 8.5

Leptospiral isolations made by culture from soil-washings and from hamsters inoculated with soil-washings.

Time (days)	Soil Culture % of water by weight			Hamster Culture % of water by weight		
	23%	29%	33%	23%	29%	33%
1	c	+	+			
3	+	+	+	+	+	+
6	+	c	+			
10	+	+	+	s	+	s
15	+	+	+			
22	+	+	+	+	+	+
31	+	+	+			
42	c	-	+	+	-	+
49	-	-	-			
56	-	-	-	-	-	-
63	-	-	-			
70	-	-	-	-	-	-

+ = isolation

- = no isolation

c = contaminated

s = serological and/or histopathological evidence of infection but culturally negative.

Table 8.6

Comparison of isolation and contamination rates using different media additive and filtration methods.

Days elapsed	EMJH additive	Direct Filtration Positive*	Direct Filtration Contaminated	Floating Filtration Positive	Floating Filtration Contaminated	Total Positive	Total Contaminated
1 - 15	SNA	1/30	3/30	0/30	19/30	1/60	22/60
	5FU	9/30	18/30	16/28	15/28	25/58	33/58
22 - 70	SNA	0/42	7/42	1/42	20/42	1/34	27/34
	5FU	2/42	15/42	6/42	37/42	8/84	52/84
	None	5/42	12/42	2/42	12/42	7/84	52/84
1 - 70	-	17/186	55/186	25/184	131/184		

* Tubes yielding isolates, some of which were also contaminated.

The two filtraton methods each resulted in unique isolations and although an overall higher isolation rate was achieved using the floating membrane technique (13.6% compared with 9.1%) a much higher contamination rate also resulted from this technique (71.2% compared with 29.6%).

Discussion.

The retrospective study of a natural outbreak of *pomona* abortion clearly indicates that the aborting animals were infected before they came onto the farm. The serological findings from the two samples of heifers (Table 8.2) show that almost all of the introduced heifers had moderate to high titres whereas there were no titres in any of the resident heifers. The detection of high proportions of IgM agglutinating activity in sera obtained from two of the heifers which aborted, suggests that these particular animals had sero-converted approximately three weeks earlier. It appears likely that they became infected in the last week or two before they were introduced into the larger group of heifers. Circumstantial evidence suggests that the piggery near to which they had been grazing at that time was the source of infection. Laboratory records indicate that this piggery had a high level of endemic *pomona* infection. An intriguing aspect of this outbreak is that it was apparently controlled by the removal of infectives and vaccination of susceptibles in spite of the fact that climatic conditions were theoretically favourable to further transmission.

The study of the outbreak in the calves that were exposed to two experimentally-infected peers clearly demonstrates the ease with which this disease can be transmitted between cattle. There was no other apparent source of *pomona* infection to which these calves may have been exposed. The infection was apparently transmitted by direct contact and it is believed that urine sampling was the most likely mode of infection since it was consistently dry throughout the study period, and environmental factors were not thought to play a part in facilitating transmission. This conclusion is in agreement with the findings of Blackmore *et al* (1976) who observed an outbreak of *pomona* in young bulls under similar conditions.

The shedding times observed in these calves were all within the range previously reported (Table 8.1) and the mean shedding time of 45 ± 9.2 days observed in this study is similar to the 36.4 ± 3.1 days reported for 44 cattle observed by Doherty (1967c). Agglutinating titres to *pomona* in these calves were substantially greater than the homologous titres observed in *hardjo*-infected calves (Chapter Four). This consistent observation in naturally-infected cattle is in agreement with previous reports in this country (Anon, 1975c; Hodges, 1975). Taking these two observations together, it appears likely that *pomona* leptospiruria is generally associated with moderate to high *pomona* titres in contradistinction to the situation observed with *hardjo*.

The experiment to determine the period for which *pomona* could survive in acidic Manawatu soils provided interesting results. The soil, climate, laboratory conditions and a range of soil water-levels were all chosen to represent conditions experienced during a Manawatu winter, ranging from relatively dry to saturated conditions. The results obtained in this study indicate that *pomona* could survive in these soils, and remain infective, for at least six weeks during the winter, and that variations within the climatic range normally experienced in the Manawatu are unlikely to significantly alter this survival time. One objection to this hypothesis is the fact that no periods of artificial frosting were applied in the controlled-environment laboratory. It has already been shown in Chapter Seven that periods of frosting appear to interrupt *hardjo* epidemics and that this is probably attributable to an effect on some leptospires in the environment. It is likely that the top 5-10mm of soil would be affected by Manawatu frosts (Brook, 1978).

Since most cows which become infected with *pomona* remain sero-positive for life (Chapter Five) and since cows which are shedding will have become infected recently, and will have moderate to high titres, it seems that most of the *pomona* titres detected in serological surveys of cattle in this country are convalescent titres. This interpretation would indicate, based on Table 2.4, that the annual incidence rate of bovine *pomona* infection is not more than 8% and probably less than 5%.

It is believed that, in this country, bovine *pomona* infection can be described as a sporadic epidemic disease which generally results from direct or indirect contact between susceptible cattle and shedding pigs. Ryan (1978) has confirmed the continuing existence of a major *pomona* reservoir in the New Zealand pig population whereas the studies of Hathaway (1978) have failed to detect any significant wildlife reservoir of this serovar, even in environments heavily contaminated with *pomona* organisms. These findings are in total agreement with earlier reports from workers in this country (Chapter One) though they are not in accord with many overseas reports. This difference between New Zealand and most other countries may be attributable to the paucity of wild and feral mammalian hosts in the New Zealand environment.

Based on all available evidence it is clear that the two major hosts for *pomona* in New Zealand are pigs and cattle. While it is accepted that *pomona* infection may cycle for short periods in cattle it is argued that bovine infection can almost invariably be traced to a porcine source. This opinion is in contrast with those of Salisbury (1954) in New Zealand and Stoenner *et al* (1956) and Clark *et al* (1961) in the United States.

The reptrospective study of an outbreak of bovine *pomona* infection, reported in this chapter, revealed recent contact with pigs and evidence of *pomona* infection in these pigs. This report demonstrates how, following more detailed investigation, an apparent case of endemic infection in cattle can be explained by epidemic spread resulting from contact with pigs. The recovery of a *pomona* isolate from a water-way near the piggery on the No. 1 Dairy Farm (Chapter Seven) indicates how readily *pomona* can escape from shedding pigs and present a risk to susceptible cattle. Circumstantial evidence for bovine *pomona* infection occurring by such a method has been reported previously (Bruere, 1952; Gillespie *et al*, 1957; Gillespie and Ryno, 1963).

As discussed, the other potential source of bovine infection is infected cattle. The propagating epidemic established in a small group of calves following the introduction of two infective

calves clearly demonstrates the ease of transmission of *pomona* between cattle. These observations are in agreement with other studies in which cattle were the only demonstrable source of infection for new cases of *pomona* infection (Webster, 1959; Doherty, 1967a; Blackmore *et al*, 1976). However, it is argued that *pomona* is by nature a self-limiting disease in cattle.

The evidence reviewed and presented in this chapter shows that cattle will normally shed *pomona* for two to three months after infection and very occasionally for four months (Table 8.1). For an epidemic to continue once all susceptibles in a group have become infected, new susceptibles must be introduced within two to four months of the last animal becoming infected. As the epidemic proceeds, many animals are likely to cease shedding even before all the susceptibles have become infected (Table 8.3) and thus the total infective challenge to new susceptibles will start decreasing as the epidemic continues. This situation was observed in a bovine *pomona* epidemic by Doherty (1967a). It is also likely that when conditions become very dry little or no transmission will occur and epidemics will terminate (Doherty, 1967a). The climatic conditions which applied during the present study were sufficiently favourable to allow transmission to proceed; rainfall was sporadic throughout the study period, but extremely dry conditions, which may prevent transmission, did not occur.

In the course of the serological survey of Manawatu dairy cattle (Chapter Two) only one herd was detected, amongst the 40 tested, which appeared to have a high level of *pomona* infection. This herd had a 67% prevalence of *pomona* titres and several sera had titres of 1:200 or more. Investigation of this herd revealed that there was a piggery with endemic *pomona* infection on the dairy farm and that frequent contact between pigs and cattle occurred. Apart from this one herd, the prevalence of *pomona* titres in *pomona* positive herds ranged from 5% to 20% and titres were generally less than 1:200. The serological pattern in these herds appeared to be similar to that in the Massey No. 1 herd. It is believed that this serological pattern represents the convalescent pattern in a herd which has experienced a self-limiting epidemic. This certainly occurred in the Massey No. 1 herd which had experienced an epidemic

several years ago, but subsequently had no further cases (Chapter Five). Other authors have also reported that bovine *pomona* outbreaks are self-limiting (Ensor and McClure, 1953; Stoenner *et al*, 1956).

Brockie (1976) demonstrated that human cases of *pomona* infection occur throughout the year in New Zealand, showing no seasonal variation in incidence, whereas there is an extremely marked rise in the incidence of human *hardjo* cases in October and November each year. This observation indicates a significant difference in the epidemiology of human infection by these two serovars. As discussed in Chapter Seven, the cyclical changes in the incidence of human *hardjo* infection are probably related to the endemic cycling of *hardjo* in the bovine population. However, it appears that either another source of infection, presumably porcine, is responsible for human *pomona* infection or, if a substantial amount of human *pomona* infection is also acquired from cattle, that the pattern of *pomona* infection is different from that of *hardjo* infection.

If human *pomona* infection comes from cattle, then the temporal distribution of new human cases suggests that there is no marked seasonal variation in bovine *pomona* infection. Salisbury (1954) was of the opinion that this was the case. Two alternative hypotheses could account for this. If *pomona* is cycling endemically in the bovine population, without contact with leptospiruric pigs, then there must be frequent introductions of new susceptibles into a group of cattle experiencing a propagating epidemic. It is believed that under New Zealand management conditions this situation is very much the exception. The other possibility is that bovine outbreaks occur throughout the year following direct or indirect contact with pigs and that human infection results from these outbreaks.

Summary.

1. In a retrospective study of an outbreak of bovine *pomona* infection, pigs were incriminated as the primary source of infection.
2. The ability of cattle to transmit *pomona* infection to

susceptible cattle was demonstrated by running susceptible calves with experimentally-infected leptospiruric calves.

3. Calves were observed to shed *pomona* for up to 94 days p.i. and the mean observed period of leptospiruria was 45.0 ± 9.2 days.
4. The survival of *pomona* for 42 days in soil, under simulated Manawatu winter conditions, was demonstrated.
5. An argument is presented to define the epidemiology of bovine leptospirosis in New Zealand. The main features of this hypothesis are:
   - that pigs represent the major reservoir of bovine *pomona* infection in this country.
   - Outbreaks of *pomona* infection in cattle are likely to be self-limiting except in the unusual situation when there are frequent introductions of susceptibles into a group experiencing an outbreak.
6. It is suggested that human *pomona* infection in this country either results from direct contact with pigs or from contact with groups of cattle experiencing epidemics following recent direct or indirect contact with pigs.
7. Based on the results of this study and the results of the serological survey of New Zealand cattle sera presented in Chapter Two it is estimated that the annual incidence rate of bovine *pomona* infection in New Zealand is about 5%.

CHAPTER NINE

EXPERIMENTAL INFECTION STUDIES

Consequent upon the studies of bovine leptospirosis reported in the preceding chapters, were a number of questions requiring experimental investigation. In particular, four observations which have important epidemiological implications in respect of bovine leptospirosis needed to be studied further.

The isolation of *balcanica* from possums at the No. 1 Dairy Farm raises the question as to whether bovine infection with this serovar occurs on that farm. This serovar has been isolated from cattle in the U.S.S.R. in the course of an abattoir survey (Zemenova, 1965). Following the recovery of *balcanica* from New Zealand possums by the Massey University group (Marshall *et al*, 1976) its ubiquitous distribution in this host has been demonstrated by Hathaway (1978). In many situations in this country possums and cattle live in close association and there is strong evidence for the transmission of mycobacterial infection between these species (Anon, 1977a). However, if bovine infection with *balcanica* occurs there is no simple method by which it can be detected. Titres to different members of the *Hebdomadis* serogroup cannot be distinguished by routine methods and the antibody absorption technique for serological distinction between infections to related serovars is expensive and time-consuming and is not definitive (Alexander and Evans, 1962). It was therefore decided to investigate the possibility of experimentally infecting cattle with this serovar and also to establish if there were any clinical, biochemical or serological differences in the infected animals which would allow a distinction to be made between bovine *balcanica* and *hardjo* infection.

Observations reported in Chapter Seven suggest that, in young calves with colostral titres, there may be a period, after the loss of detectable agglutinating titres, when calves are still refractory to infection. While most calves have lost detectable colostrum-derived titres by 120 days of age and all have by 190 days of age (Chapter Three), *hardjo* infection rarely occurs before seven to eight months of age (Chapter Seven). It has been argued that this

observation could be partly explained by environmental causal associated factors. However, as Hathaway (1978) has demonstrated, young possums are resistant to experimental infection for two to three months after they have lost maternally-derived MAT titres. It was therefore decided to experimentally challenge calves both at two to three months and at five to six months after the loss of passively-acquired titres in an attempt to determine whether this phenomonon also occurred in calves.

It can be argued that animals observed to be leptospiruric many months, or even years, after first becoming infected with a particular serovar may not be experiencing a persistant infection, but instead have become reinfected, even though they still possess convalescent titres. There is evidence that vaccines of the bacterin type may prevent clinical disease, though not preventing leptospiruria (Stalheim, 1971; Hanson *et al*, 1972). This indicates that the degree of protection against leptospiral infection may vary. Therefore an experiment was undertaken in an attempt to determine whether reinfection of animals with convalescent titres can occur in the field. This study was conducted in two parts. The first part involved rechallenging the animals used for the comparitive *balcanica/hardjo* study and the second part involved the challenge of adult cows with naturally-acquired convalescent *hardjo* titres.

The final observation which was subjected to further investigation in this chapter was the fact that cattle in the convalescent stage of leptospiral infection have relatively constant levels of agglutinating titres. Since it is very likely that cattle at this stage of infection will have frequent contact with other cattle still in the leptospiruric phase, it is possible that they may experience anamnestic rises in titre. As part of the study of the effects of experimentally challenging cattle with convalescent titres, the serological response was studied in order to determine what changes occur.

Materials and Methods.

Experimental Animals. I. Calves.

Ten Friesian calves were selected at birth for this study. Blood samples were taken from calf and dam, and colostrum samples

were taken when the dam was first milked. The calves were born between 13/4/77 and 8/5/77 and were kept indoors, individually penned, and fed on milk and calf meal until 25/5/77. They were then reared as a single group on pasture, and were isolated from all other stock from 25/5/77 until 19/9/77 when they were again housed prior to experimental challenge. Throughout this time they continued to receive a meal supplement. During the first four weeks of the experiment they were housed in individual pens and were fed dairy meal, chopped hay and some dried grass and received water from nipple waterers. Following this period they were again run at pasture, remaining in isolation from other stock for a further four months. They were slaughtered at an abattoir when the experiment was terminated on 23/2/78 and kidneys and urine were collected for cultural examination.

Experimental Animals. II. Adult Cows.

Seven adult dairy cows, of various ages ranging from two to seven years, were made available by a commercial dairy farmer. These cows were crosses of the Friesian and Jersey breeds. Throughout the study period, between 20/12/77 and 17/1/78, these cows were run at pasture isolated from other stock, and at the end of the experiment were slaughtered at an abattoir where blood, urine and kidneys were collected for further examination.

Infecting Leptospiral Serovars.

Strains of three serovars were used in this study. All three serovars had been successfully used in transmission studies with other host species. The strain of *balcanica* used (*MU1*) was a low passage isolate from a possum trapped on the No. 1 Dairy Farm. This isolate had been subjected to serial passage through hamsters just prior to this study. A dose of $5x10^7$ organisms consistently infected hamsters causing death approximately 14 days p.i. The strain of *pomona* used (*T116*) was a low passage isolate recovered from a pig at the Massey Piggery. This isolate had been subjected to 15 serial hamster passages prior to this study and at a dose of approximately $5x10^2$ organisms it was shown to be consistently lethal for hamsters. The strain of *hardjo* used (*G 39/1*) had been isolated from a calf at the No. 1 Dairy Farm about one year previously. This isolate had been initially recovered from infected

urine by hamster inoculation and it remained infective for hamsters when administered intra-peritoneally at a high dose rate ($>10^8$ organisms) although it never produced symptoms or death in hamsters. The identity of all three isolates had been confirmed by the cross-absorption agglutination test conducted at the W.H.O. Reference Laboratory, Centre for Disease Control, Georgia. Each isolate was recovered from hamster kidney tissue and passaged for three to five times in liquid and semi-solid EMJH media to establish dense growth. The *hardjo* strain, *G 39/1*, was inoculated twice, three months apart, and was maintained by four passages in semisolid medium and seven passages in liquid medium during that three month interval.

The infecting doses of organisms were assessed, by dark-field examination in a Petroff-Hauser counting chamber, to be between 10^8 and 10^9 organisms/ml. These were all fresh, dense, actively-growing cultures. On each occasion that infection was attempted 5ml of semisolid culture was inoculated intramuscularly and 10ml of liquid culture was given subcutaneously. These doses represented approximately 10^8 organisms per Kilogram live weight for the calves when first inoculated and $5x10^7$ organisms per Kilogram when rechallenged, and approximately $2x10^7$ organisms per Kilogram live weight for the cows.

Experimental Procedures. I. Calves.

Blood samples were taken from the calves at frequent intervals from a few days after birth, and urine samples were also collected on several occasions and examined by dark-field microscopy as previously described (Chapter Seven). During the four week period for which the calves were housed for experimental challenge, blood and urine samples were collected every second day. After they were released onto pasture, samples were collected at seven to fourteen day intervals.

The calves were housed for five days before they were inoculated, in order to allow them to become accustomed to their new environment. Throughout the period during which the calves were housed, twice-daily rectal temperatures were taken, at 6 a.m. and 6 p.m., and any clinical changes were noted.

The housing used for this experiment was a large room with concrete floors and walls, which was divided by galvanised steel partitions into twelve pens, each of which had a wooden slatted floor laid over a concrete base. Each pen drained into centrally-located collecting drains, not into adjacent pens. The room was rodent-proof and the drains were kept sealed and were treated with disinfectant (Medol) and were emptied twice-daily while the animals were being examined and tended. The ten calves were individually penned with the two spare pens being used as additional barriers to prevent contact between groups inoculated with different serovars.

At the time of the first challenge the calves weighed an average 100 Kilograms (range 82 to 117 Kg) and were an average five months of age (range 141 to 166 days). Two calves were inoculated with *pomona*, three with *hardjo* and three with *balcanica*, and two, which were not inoculated, were left as controls.

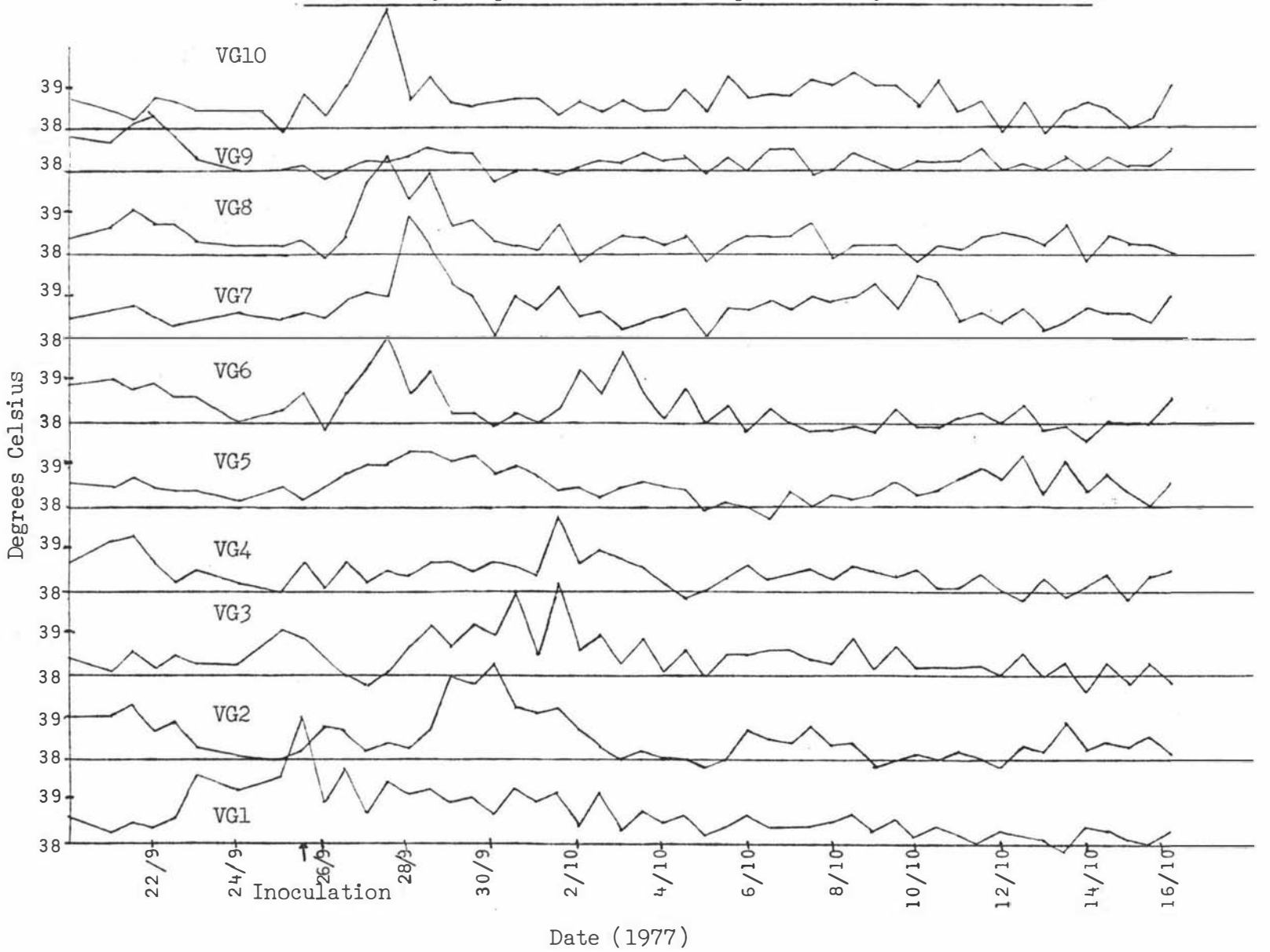
Three months after the first experimental challenge all ten calves, which then weighed an average 200 Kilograms (range 183 to 229 Kg) and were, on average, eight months old (range 232 to 257 days), were challenged with the same total dose of *hardjo* as had been given originally. Since their weight had doubled during the intervening three months this represented a 50% reduction in the number of organisms inoculated per Kilogram live weight.

Eight weeks after the second experimental inoculation the calves were killed and serum, urine and kidney tissue were collected at the abattoir. The kidney samples were cultured using the techniques described in Chapter Two.

All urine samples collected were examined by dark-field microscopy and were cultured using the techniques described in Chapter Seven. The pH, protein, glucose and haemoglobin content (Multistix) specific gravity and gamma glutamyl transferase content (Merck γGT Kit) of each urine sample were also assessed. In addition heparinised blood samples were collected from the calves every second day during the time they were housed in order to determine the packed cell volume and haemoglobin concentration.

Figure XXVII

Twice-Daily Temperatures of Ten Experimentally-Infected Calves.



All serum samples were examined by the MAT and all isolates obtained were typed using the procedures described in Chapter Two.

Experimental Procedures. II. Adult Cows.

Blood and urine samples were collected from the seven dairy cows one week prior to their inoculation, again on the day of the inoculation and 7, 14 and 21 days after inoculation. Additional urine samples were also collected five days prior to inoculation. Four weeks after inoculation the cows were slaughtered and serum, urine and kidney samples were obtained from each. On each occasion that urine samples were collected they were examined by dark-field microscopy as well as being inoculated into culture media. Kidney samples were examined culturally using the techniques described in Chapter Two.

Results.

Experimental infection of calves with serovars balcanica, hardjo and pomona.

1. Initial challenge.

Eight of the ten calves were born to sero-positive dams and these calves acquired titres after suckling; the two calves born to sero-negative dams remained sero-negative after suckling (Table 9.1). The sero-positive calves lost detectable MAT titres at times ranging from 43 to 160 days before the date of experimental challenge (Table 9.1). One calf (VG8) had acquired a *pomona* titre after suckling. This titre was lost after 26 days, which was 127 days before its experimental challenge with serovar *balcanica*.

On 26/9/77 eight of the ten calves were inoculated with either *balcanica, hardjo* or *pomona*, the other two calves remaining as controls. The serological and bacteriological observations made on these calves are presented in Tables 9.2 and 9.3 respectively and their temperature recordings are shown graphically in Figure XXVII. No significant changes were observed in the P.V.C. or haemoglobin concentration of any blood sample nor in the pH, protein content, specific gravity or gamma glutamyl transferase content of any urine sample. All these parameters from all calves remained within normal limits throughout the four week observation period.

Table 9.1

Coded *hardjo* titres in experimental calves between birth and date of infection.

	VG1	VG2	VG3	VG4	VG5	VG6	VG7	VG8	VG9	VG10
Birth date	13/4	15/4	17/4	19/4	22/4	24/4	25/4	26/4	24/4	8/5 (1977)
Dam titre	3	1	4½	0	2	0	2	2	½	4
Post-suckle titre	6	3	6	0	5	0	5	3½	1	5½
15/4/77	6	3	-	-	-	-	-	-	-	-
22/4/77	5½	1	6	0	5	-	-	-	-	-
29/4/77	5	1	5	0	4	0	4	3½	1½	-
5/5/77	5	1½	5	0	5	0	4	3	0	-
13/5/77	5½	0	5½	0	4	0	4	3	0	6
20/5/77	3½	0	3½	0	1	0	3	1	0	3½
27/5/77	3	0	3	0	½	0	1½	1½	0	3
10/6/77	3	0	3	0	0	0	3	1½	0	3½
24/6/77	2½	0	3	0	0	0	1	1	0	1½
20/7/77	1	0	1	0	0	0	½	0	0	½
14/8/77	½	0	0	0	0	0	0	0	0	0
10/9/77	0	0	0	0	0	0	0	0	0	0
26/9/77 β	0	0	0	0	0	0	0	0	0	0
Days from last recorded titre to experimental challenge	43	144	68	160α	122	155α	68	94	150	68

α never had detectable titre.

β date of experimental infection.

\- not tested.

Table 9.2

Coded *hardjo, balcanica* and *pomona* titres of calves inoculated with serovars *balcanica* or *hardjo*.

	Calf 1			4			5			3			6			8		
	H	B	P	H	B	P	H	B	P	H	B	P	H	B	P	H	B	P
26/9/77*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27/9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
28/9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29/9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30/9	0	0	0	½	0	0	0	0	0	0	0	0	1½	1	0	2½	1½	0
1/10	0	0	0	2	0	0	2½	0	0	½	1	0	4	3½	0	5½	2	0
2/10	0	0	0	4	1	0	3	0	0	2	1½	0	5½	5½	0	6½	4½	0
3/10	0	0	0	6½	4½	0	3	1½	0	3	1½	0	7	6½	0	7	5½	0
5/10	0	1	0	8	6½	0	2½	1½	0	3½	3	0	8	9	0	6½	3½	0
7/10	0	1	0	7½	5½	0	2½	2	0	3	3	0	8	7½	0	5½	5½	0
9/10	½	1½	0	7	5½	0	2	2	0	3½	4	0	8½	8	0	6½	5½	0
11/10	2½	3	0	9	6	0	4	3	0	4½	3½	0	9	8	0	6	5½	0
13/10	1	3	0	8	6½	0	3½	2	0	5	4	0	9	6	0	6	6	0
15/10	2	2	0	7	5½	0	3	4	0	4½	4½	0	8	8	0	6	5½	0
17/10	1½	2	0	6½	5	0	3½	3	0	4½	4	0	8	8	0	6½	6½	0
21/10	1	2	0	5	5	0	3	3	0	5	4½	0	7½	7	0	6	6	0
25/10	2	3	0	5	4½	0	3½	3½	0	4½	5½	0	6	7	0	5	5	0
2/11	2	1½	0	4	5	0	4	3½	6α	5	4½	0	6	7	0	5	5	0
13/11	2	1½	0	4	4	0	3	2½	8	4½	6	0	4	7	0	4½	5	0
23/11	2	1½	0	4	5	0	3½	2½	9	4	5	0	3½	6	0	4½	5	0
6/12	2	1	5½α	3½	4	0	2½	2	7	3	4	0	4	5	0	4½	4½	0
18/12	2	1	7½	3	3	2½α	2½	3	6½	3	4	0	4	5½	0	4	4½	0
27/12	1	1	13	2	2½	10	1	1½	7	3	4	6α	3	4½	0	3	4½	8α
2/1/78+	1½	½	11½	2	2	9	1	1	6	2	4	5	3	4	0	3	5	9
10/1	2	1	12	3	2	8	3	1½	6	3½	3½	7	4	4½	0	2	4½	6
20/1	2½	1½	13	3	2½	6	3	2	5	3	3	11½	4	5½	0	4	5	4
30/1	2	1	13	2½	1	6	2½	1	5	2	3	9½	3	4½	0	3	4	4
13/2	1	½	12	2½	1	6	3	1	5	2	3	8	3	3½	0	3	4	4½
23/2	1	1	10	2	1	6	2	½	5	2	3	7½	2½	4	0	2½	4	4
initial challenge serovar	*hardjo*			*hardjo*			*hardjo*			*balcanica*			*balcanica*			*balcanica*		

* date of initial challenge
\+ date of second challenge with *hardjo*
α natural infection with *pomona*

H,B,P. = *hardjo, balcanica, pomona*.

Table 9.2 (contd.)

Coded *balcanica*, *hardjo* and *pomona* titres of calves inoculated with serovar *pomona*, and uninoculated controls.

	Calf 7			10			2			9		
	H	B	P	H	B	P	H	B	P	H	B	P
26/9/77*	0	0	0	0	0	0	0	0	0	0	0	0
27/9	0	0	0	0	0	0	0	0	0	0	0	0
28/9	0	0	0	0	0	0	0	0	0	0	0	0
29/9	0	0	0	0	0	0	0	0	0	0	0	0
30/9	0	0	0	0	0	0	0	0	0	0	0	0
1/10	0	0	5	0	0	4	0	0	0	0	0	0
2/10	T	0	5	T	0	5	0	0	0	0	0	0
3/10	T	0	7	T	0	7	0	0	0	0	0	0
5/10	T	0	8	T	0	8	0	0	0	0	0	0
7/10	T	0	8	T	0	8	0	0	0	0	0	0
9/10	T	0	7½	T	0	7	0	0	0	0	0	0
11/10	T	0	7½	½	0	7½	0	0	0	0	0	0
13/10	T	0	8	T	0	7	0	0	0	0	0	0
15/10	0	0	9	T	0	6½	0	0	0	0	0	0
17/10	½	0	7	T	0	7½	0	0	0	0	0	0
21/10	½	0	9	1	0	8	0	0	0	0	0	0
25/10	T	0	8	1	0	9	0	0	0	0	0	0
2/11	2	0	7	2½	T	8	0	0	0	0	0	0
13/11	1½	T	8	2½	T	8	0	0	0	0	0	0
23/11	½	0	8	0	0	8	0	0	0	0	0	0
6/12	0	0	7	0	0	7	0	0	0	0	1	5α
18/12	0	0	7	0	0	7	0	0	0	0	1	9
27/12+	0	0	6½	0	0	6½	0	0	0	0	T	8
2/1/78	6½	6½	5	0	0	6	0	0	0	0	1½	11½
10/1	7	5½	5½	5	4	6½	5	4½	0	3	3	12
20/1	6	6	4	4½	3	5½	6½	7	1	5	5½	13
30/1	5	5½	5	3½	4	5	5½	6	0	4½	4	13
13/2	5½	4	5	4	4	5½	6	4½	0	4	4	11
23/2	6	4½	4½	4	3	5½	5½	5	0	4	3	8
initial challenge serovar	*pomona*			*pomona*			control			control		

* date of initial challenge
\+ date of challenge with *hardjo*
α natural infection with *pomona*

Table 9.3

Summary of bacteriological findings in experimentally-infected calves.

Date of sampling	VG1	VG2	VG3	VG4	VG5	VG6	VG7	VG8	VG9	VG10
19/9/77	-	-	-	-	-	-	-	-	-	-
26/9†	-	-	-	-	-	-	-	-	-	-*
27/9	-	-	-	-	-	-*	-*	-	-	p*
28/9	-	-	-	-	-	b* -	p* -	-	-	p
29/9	-	-	-	-	-	-	-	-	N/S	-
30/9	Cont-	-	Cont-	-	-	-	Cont-	-	-	-
1/10	-	-	-	-	Cont-	-	-	-	-	-
2/10	-	-	-	-	-	-	-	-	-	-
3/10	-	-	-	-	-	-	-	-	-	-
5/10	-	N/S	-	-	-	-	-	-	-	-
7/10	-	-	-	-	-	-	-	-	-	-
9/10	-	-	-	-	-	-	-	-	N/S	-
11/10	-	-	Cont-	-	-	-	-	Cont-	Cont-	-
13/10	Cont-	-	-	-	-	-	Cont-	-	N/S	+[4]
15/10	-	N/S	Cont-	-	-	Cont+[4]	-	-	-	p+[6]
17/10	-	-	-	-	-	-	p+[4]	-	-	Cont+[6]
21/10	-	-	-	-	-	-	p+[4]	-	-	Cont+[6]
25/10	-	-	-	-	-	-	Cont+[4]	-	-	p+[7]
2/11	-	-	-	-	-	-	p+[5]	-	-	-
13/11	-	-	-	-	-	-	-	-	-	+[4]
23/11	-	N/S	-	-	+[5]	-	-	-	-	-
6/12	p+[6]	N/S	-	-	p+[4]	-	p+[4]	-	N/S	-
18/12∇	p+[5]	N/S	-	p+[5]	-	-	-	-	p+[8]	N/S
27/12∇	p+[6]	-	-	p	-	-	-	-	+[7]	-
2/1/78	p-	-	+[5]	Cont-	-	-	-	Cont	p+[5]	-
10/1	-	-	-	+[5]	-	-	-	Cont	Cont-	-
20/1	-	-	p+[4]	p	N/S	-	-	N/S	N/S	N/S
30/1	-	h-	-	p+[5]	-	-	h-	-	Cont-	-
13/2	p-	N/S	-	Cont-	-	-	-	-	p-	h-
23/2	p-α	h-α	-	p-	-	-	h-α	-	p+[4]	Cont-

\+ = leptospires seen by dark-field microscopy
Cont = all tubes contaminated
b = *balcanica*, p = *pomona*, h = *hardjo*, cultured and typed.
N/S = no sample
* = blood isolates
† = date of initial challenge
∇ = date of second *hardjo* challenge
α = isolated from kidneys only
\- = negative findings by dark-field microscopy

$^{4} = 10^{4}-10^{5}$ leptospires/ml
$^{5} = 10^{5}-10^{6}$ "
$^{6} = 10^{6}-10^{7}$ "
$^{7} = 10^{7}-10^{8}$ "

Seven of the eight inoculated calves developed titres on the fourth of fifth day p.i.; the first detectable titre in the remaining calf was detected on the ninth day. Titres rose rapidly and reached high levels within three to six days of first appearing. Peak homologous titres occurred between 6 and 25 days p.i. although peak homologous *hardjo* and *balcanica* titres appeared earlier than peak homologous *pomona* titres. In the six calves challenged with either *hardjo* or *balcanica*, peak *balcanica* titres ranged between 1:96 and 1:6144. There was no consistent difference between *hardjo* and *balcanica* titres in these calves regardless of which serovar they were inoculated with. The peak *pomona* titre was 1:6144 in both animals challenged with that serovar. Cross-reaction titres to *hardjo* were observed in the calves inoculated with *pomona* but not *vice versa*.

Leptospiraemia was detected in only three calves; in both calves inoculated with *pomona* and in one of the three calves inoculated with *balcanica* (VG6) (Table 9.3). Leptospiruria was first detected on the 17th and 18th days respectively in the two calves inoculated with *pomona* and a number of isolates, subsequently typed as *pomona*, were obtained from them (Table 9.3). Leptospires were observed by dark-field microscopy in urine obtained from one calf inoculated with *balcanica* (VG6) but all culture tubes inoculated with this urine were contaminated (Table 9.3). No calves inoculated with *hardjo* on 26/9/77 developed leptospiruria.

All ten calves were run together in isolation after 17/10/77 and a further six calves became naturally infected with *pomona* by 23/2/78 (Chapter Eight). Isolates typed as *pomona* were obtained from five of these calves.

11. Second challenge.

All ten calves were challenged with *hardjo* on 27/12/77. The six calves which had previously been inoculated with *hardjo* or *balcanica* showed only small rises in titre, the largest increase, two doubling dilutions from 1:24 to 1:96, occurring in one calf. The four calves which had not been inoculated previously with *hardjo* or *balcanica* all sero-converted, developing peak *hardjo* titres between 14 and 24 days p.i. These titres ranged from 1:384 to 1:1536.

Leptospiruria was observed by dark-field examination in one of these calves (VG9) but *pomona* was the only serovar recovered from this calf; it had been shedding *pomona* at the time of challenge (Table 9.3). However, serovar *hardjo* was recovered from urine samples from the other three calves and from the kidneys of two of them. All culture tubes inoculated from the kidney of the third (VG10) were contaminated (Table 9.3).

Experimental challenge of adult cows with hardjo.

Six of the seven cows had *hardjo* titres ranging from 1:48 to 1:136 when they were inoculated with that serovar and the remaining one had a titre of 1:17 seven days earlier (Table 9.4). They all showed a transient elevation in titre following inoculation with *hardjo* though no titre rose higher than 1:192 (Table 9.4). No leptospires were observed in any urine sample from any cow, nor were leptospires isolated from urine or kidney samples obtained when the animals were slaughtered three weeks p.i. (Table 9.5).

Discussion.

The two attempts to infect young calves with *hardjo* produced different results although they were challenged with the same strain of *hardjo* on both occasions. For both experimental challenges serologically negative calves were inoculated, by the same routes, with cultures which were shown to successfully infect hamsters. Identical isolation and cultural methods were employed on both occasions. In spite of this, the three calves which were challenged at five months of age sero-converted without developing detectable leptospiraemia or leptopsiruria, whereas all four calves challenged at eight months of age sero-converted with at least three developing leptospiruria. The fourth may also have shed *hardjo* organisms in its urine but this may have been concealed by the fact that this animal (VG9) was shedding *pomona* at that time.

Two of the three five-month-old calves in which *hardjo* infection was not established (VG1 and VG5) had lost colostral titres 43 and 122 days prior to challenge. The third (VG4) had never had a detectable colostral titre. Following inoculation with *hardjo* the peak serological responses of VG1 and VG5 were substantially lower (1:68 and 1:192 respectively) than that of VG4 (1:6144).

Table 9.4

Coded *hardjo* titres of seven cows inoculated with *hardjo* on 27/12/77.

	Identification						
	9	16	39	54	91	115	191
20/12/77	½	3½	2	3	2	2	3
27/12/77*	0	3½	2	3½	1½	2½	2
3/1/78	4	4½	4	4	2½	4	1
10/1/78	2	3	3½	4	3	1½	3
17/1/78	2	3½	3	3	2	2½	3

* date of experimental challenge

Table 9.5

Summary of barcteriological findings in seven cows inoculated with *hardjo* on 27/12/77.

	Identification						
	9	16	39	54	91	115	191
20/12/77	-	-	-	-	-	-	-
24/12/77	-	-	-	-	-	-	-
27/12/77*	-	-	-	-	-	-	-
3/1/78	C	-	-	-	-	-	-
10/1/78	-	-	-	-	C	-	-
17/1/78α	-	-	-	-	-	-	-

\- = urine dark-field and culture negative

C = all tubes contaminated

α = kidneys cultured on this date negative

* = date of experimental challenge

This provides some evidence for the presence of persistent colostral antibody after the loss of detectable agglutinating titres. This result is in agreement with the observations of Doherty (1967b) who showed that calves were still refractory to challenge with *pomona* two months after they had lost detectable colostral antibody to that serovar. It has also been demonstrated that vaccinated animals can be protected against leptospiral infection even when their titres are very low or no longer detectable, although in some cases there is protection against clinical disease only (Negi *et al*, 1972; Stalheim, 1971; Hanson, 1973). The presence of colostral antibody titres has been shown to protect against leptospiral infection (Chapter Seven).

The fact that five-month-old calves did not become infected may be due to a non-specific age-associated factor rather than a specific persistent immunity. Two other calves of the same age which did become infected when challenged with *pomona* did not have *pomona* titres at any stage and neither did their dams. This observation strengthens the argument that some specific factor was protecting the calves against *hardjo* infection.

It is possible that there were different reasons for the failure of any of the three calves challenged with *hardjo* to develop leptospiruria. The high titre detected in calf VG4, which never had a colostral titre, indicates that it mounted a far greater response to experimental challenge than did the other two calves (VG1 and VG5), which had possessed colostral titres. Failure to produce leptospiruria in some or all of groups of cattle experimentally challenged with *hardjo* has been reported previously (Hanson and Brodie, 1967; Sullivan, 1970a; Ellis and Michna, 1977).

The attempt to infect calves with *balcanica* produced equivocal results. Although all three of the five-month-old calves which were challenged with *balcanica* sero-converted, with peak titres of 1:786, 1:1086 and 1:6144 respectively, only a single isolate was obtained from one of these calves (VG6). The culture used for these inoculations was infective for hamsters, causing death. Since this isolate was obtained from a culture of blood taken just 48 hours after inoculation it does not prove that infection was

established. It is quite possible that inoculated organisms had not been cleared from the blood by that time. One urine sample from this calf taken 17 days later was positive on dark-field microscopy but all culture tubes inoculated with that sample were contaminated and no isolate was obtained.

Two of the three calves challenged with *balcanica* had lost detectable colostral titres against *hardjo* two to three months prior to challenge. The third calf (VG6), which had never had a colostral *hardjo* titre, developed the highest peak titre and yielded the only isolate. These facts tend to support the observations and comments relating to the possible extension of the protection afforded by colostral antibodies to *hardjo*. Certainly the failure to produce leptospiruria with *balcanica* in this study does not prove that this serovar will not infect cattle. A comparison between the results obtained from the calves inoculated with *hardjo* or *balcanica* indicates that they are similar.

There was a marked similarity between the serological responses of calves inoculated with these two serovars. The coded titres to *hardjo* and *balcanica* shown in Table 9.2 are virtually identical at all stages of the serological response regardless of whether the calves were inoculated with *hardjo* or *balcanica*. The only exception was that more frequent and longer *hardjo* cross-reaction titres were observed in the two calves inoculated with *pomona*.

The virtual identity of the serological responses in the calves inoculated with either *hardjo* or *balcanica* is in contrast to the results obtained in possums by Hathaway (1978). He found a highly repeatable and marked difference between *hardjo* and *balcanica* titres in possums inoculated with *balcanica*, with the heterologous titre being substantially higher than the homologous titre in the early stages of infection. The observations made in this study clearly indicate that should bovine *balcanica* infection occur naturally it would be serologically indistinguishable from bovine *hardjo* infection. Thus the question of whether *balcanica* infection can escape from the possum population into the cattle population can be resolved only by extensive cultural surveys

followed by serotyping of isolates using cross-absorbed antisera.

A number of reports have shown that experimental inoculation with some strains of *pomona* can produce marked biochemical changes in the urine of the host including elevated bilirubin and protein levels (Fennestad, 1963; Millar *et al*, 1977). Farina *et al* (1972) could not demonstrate any marked changes in the blood or urine biochemistry of cattle following experimental *hardjo* infection. Preliminary studies on the effects of experimental infection of hamsters with *balcanica* at Massey University have indicated that, in this species, this serovar is more pathogenic than *hardjo* and the possibility of changes in blood and urine biochemistry has been considered (Hathaway, 1978; Manktelow and Marshall, unpublished). Shaw (1976) has demonstrated that urinary gammaglutamyl transferase levels are elevated in sheep following damage to kidney tubular epithelium. Therefore it was decided to assay urinary levels of this enzyme in order to determine whether elevations occurred following leptopiral infection. It was considered that if biochemical changes were detected it may be possible to make a distinction between infections by the three serovars used in this study. However, there were no significant changes in any blood or urine chemistry parameter measured in any calves during the course of this study.

The possibility that cattle with convalescent titres may become reinfected and recommence shedding could be used as an argument to explain the lengthy shedding times observed in cattle naturally infected with *hardjo* reported in Chapter Seven. Since these animals were in contact with other known shedders at various times after they had sero-converted it is clear that they were re-exposed to infection on many occasions. The present attempts to establish infections in both calves and cows with convalescent titres were all unsuccessful. The strain of *hardjo* used to challenge these sero-positive animals was one of proven infectivity for cattle since sero-negative calves challenged with the same dose of the same culture on the same day became infected and developed leptospiruria. It is believed that this provides strong evidence that cattle in the convalescent phase of leptospiral infection are refractory to further challenge.

Furthermore it is believed that this resistance is likely to persist at least as long as convalescent titres remain; probably for life in most animals (see Chapter Five). It has been shown that convalescent titres protect possums against further experimental challenge with *balcanica* (Hathaway, 1978). This result should not be considered surprising when considered in the light of the evidence reviewed earlier that colostral titres, and to some extent vaccinal titres, are also protective.

None of the calves or cows with convalescent *hardjo* titres which were challenged with that serovar on 27/12/77 showed marked anamnestic rises in titre. The rises observed were in the order of one or two doubling dilutions though one animal which was sero-negative at 1:24 developed a titre of 1:192 after challenge. Considering that these animals were given a massive challenge, far in excess of that likely in the field, it appears that natural exposure to reinfection will produce only slight perturbations in titre.

Summary.

1. Evidence is presented which indicates that the protective effect of colostral antibody against leptopsiral infections persists after calves have lost detectable MAT titres.
2. It appears likely that cattle are susceptible to *balcanica* infection and that the extensive reservoir of this infection in the possum population in New Zealand may present a hazard to cattle.
3. The serological responses resulting from experimental challenge of cattle with *hardjo* or *balcanica* are indistinguishable by the use of microscopic agglutination test.
4. It was not possible to distinguish between experimental challenge with *balcanica*, *hardjo* or *pomona* on the basis of biochemical changes in blood or urine.
5. Evidence is presented which indicates that animals with convalescent leptospiral titres are not susceptible to further challenge with the homologous serovar.
6. Experimental challenge of cattle with convalescent leptospiral titres failed to produce marked anamnestic rises in agglutinating titres.

7. It is considered that the findings reported in this chapter support the contention that bovines are likely to shed *hardjo* only during the period of their lives which commences two to three months after the loss of maternal antibody and terminates approximately one year after sero-conversion.

SUMMARY AND CONCLUSION

The investigations reported in this thesis can be divided into four main areas: surveys, serological and immunological investigations, epidemiological observations and experimental infections.

The random survey of New Zealand bovine sera conducted in this study represents the first reported attempt to assess the true national prevalence of titres to the five serogroups representative of the six serovars known to occur here. It is believed that the estimates of the reported prevalences of *hardjo*, *pomona* and *tarassovi* titres in New Zealand cattle of 60%, 18% and 9% respectively are good estimates of the true situation. These estimates largely confirm the findings of earlier workers although they suggest that bovine *pomona* infection is becoming less prevalent in New Zealand. It is believed that this decline is related to changes in farming patterns which have considerably reduced the degree of contact between cattle and pigs in this country. There is little evidence that infection with serovars other than these three is of more than minor significance in the New Zealand bovine population.

Bovine infection with serovar *hardjo* is extremely prevalent in this country especially in the North Island. This finding is in agreement with the situation occurring in most other parts of the world. Although infection with this serovar was first reported in New Zealand about ten years ago there is no evidence to indicate that it has not been prevalent for many more years. However, it is possible that the prevalence has increased in recent years in association with changes in farm management, in particular the trends towards larger herds and higher stock densities and increased movements of cattle about the country. The high prevalence of bovine *hardjo* infection observed here and in other countries undoubtedly reflects a marked host-parasite adaptation.

The epidemiology of bovine *hardjo* infection has been investigated in this study by conducting a detailed examination of a single herd with a known high level of endemic infection. Based on a series of prospective cohort studies on groups of calves, heifers and cows, a consistent pattern of infection was observed. Most adult cows were serologically positive and in the convalescent stage of infection. Very few individuals in the milking herd were still in the leptospiruric state; the only ones detected in this study were heifers which had recently calved for the first time. Cows transferred agglutinating antibody to their calves in their colostrum. There was no evidence that calves experienced *in utero* infection. Calves with colostral titres remained sero-positive for up to six months after birth. New infections were first observed in calves aged seven to eight months, although the mean age at infection was approximately 12 months. Infected calves or heifers were observed to shed *hardjo* in their urine for an average of nine months, though several shed for more than one year and one was still shedding when last sampled, over 14 months after infection. Following the cessation of leptospiruria most animals remain sero-positive, with slowly declining titres, for life.

All *hardjo* outbreaks occurred following close contact between susceptible calves and known or suspected shedder cattle; generally contact between consecutive cohorts. Thus one cohort of heifers containing a number of leptospiruric individuals acted as the source of infection for the following one or two cohorts of younger heifers or calves. In spite of intensive study no wild, feral or other domestic species was demonstrated to act as a reservoir for *hardjo* infection.

These findings indicate that there is a bovine endemic cycle of *hardjo* infection. Cattle become infected by close contact with other older cattle shedding *hardjo* in their urine. Causal associated factors were not fully determined in this study but it is apparent that both age and climate are important. In herds with a high level of endemic infection, calves do not appear to become susceptible until they have lost all maternally-derived antibody, at seven to eight months of age. However, infection

results only following contact between susceptibles and infectives when climatic factors are favourable, most particularly when the degree of soil-water saturation is near its peak winter levels between the months of June and September.

The feature of the host-parasite relationship favouring the establishment and maintenance of this cycle is the significant proportion of infected cattle which continue shedding *hardjo* for at least a year after infection. There is no evidence that any other serovar occurring in New Zealand is similarly adapted to cattle though similar associations occur between other host species and their naturally-infecting serovar. It is argued that for the maintenance of a particular serovar in a particular host population the main condition is that a significant proportion of infected animals remain carriers sufficiently long to infect each new generation or cohort of susceptibles.

It is suggested that the natural cycle of *hardjo* infection observed in this study could be postponed for 12 months under management systems which maintain adequate segregation between young susceptible calves and older infective heifers and cows. Under these conditions the endemic cycle would become established in the adult milking herd. When susceptible heifers enter the adult herd after their first calving, they would become infected by exposure to the previous year's infective heifers and then act as the reservoir of infection for subsequent introductions of susceptibles 12 months later. If such an association can be demonstrated then the risk to the farmer of *hardjo* infection may be substantially reduced by the simple expedient of encouraging calfhood infection by ensuring contact between young susceptibles and known shedders at least 12 months before these animals enter the adult milking herd. This possibility represents an obvious and important area for further study.

The other prospect offered by the demonstration of this endemic cycle is that of control and eradication, through management practices supplemented with vaccination. The experimental infection studies reported in this investigation support the contentions that cattle with convalescent titres are protected

against reinfection and that the leptospiruric state is rare in this age group. Thus the protection of only one or two successive cohorts against infection may be sufficient to break the cycle of infection and allow a herd to become free of *hardjo* infection. This hypothesis is most worthy of further investigation.

Although cattle clearly acted as the reservoir species for further bovine infection in this study, the role of sheep in the overall epidemiology of bovine *hardjo* infection in New Zealand was not studied. This possibility, that sheep may act as a reservoir for bovine infection, requires further study. There is extensive evidence for the occurrence in New Zealand of ovine infection with members of the *Hebdomadis* serogroup, and it seems likely that under some circumstances sheep-to-cattle transmission may occur. It is, however, possible that sheep are not well-adapted hosts for *hardjo* infection and that the *hardjo* titres observed in sheep are attributable to short-term infections accompanied by a transient leptospiruric phase which result from contact with infective cattle.

The epidemiology of bovine *pomona* infection appears to be closely associated with the cycling of that serovar in the porcine population. The present study of bovine *pomona* infection was secondary to the major investigation of bovine *hardjo* infection. Further study is required to test the suggested hypothesis that bovine *pomona* infection is a sporadic self-limiting disease resulting from direct, or indirect, contact with shedding pigs. Should this hypothesis be correct, control of *pomona* infection in piggeries will be necessary if the public health risk presented by infected pigs and cattle is to be reduced.

The studies on the role of IgM and IgG immunoglobulins reported in Chapter Six suggest an area for further investigation. The observations reported in that chapter indicate that recently-acquired titres could be detected by a simple method of IgM inactivation. This could readily be performed in any laboratory equipped to carry out the leptospiral MAT. Such a test would prove an invaluable epidemiological tool in identifying recent

and convalescent cases amongst groups of sero-positive animals.

The failure to experimentally infect calves with *balcanica* reported in Chapter Nine may well have resulted from the presence of protective levels of passively acquired antibody in the experimental calves and does not prove that this serovar cannot infect cattle. The results indicate that if bovine *balcanica* infection should occur it would be serologically indistinguishable from *hardjo* infection using current methods. Further attempts to experimentally infect cattle with this serovar are clearly needed. The present study has indicated that young calves which have recently lost passively-acquired *hardjo* titres are unlikely to be suitable experimental animals.

The question of the susceptibility of the New Zealand cattle population to serovar *balcanica* must be resolved by further experimentation. The findings reported in this study of bovine *hardjo* infecion indicate that the control and elimination of this infection within a closed population of cattle may be a practical possibility. However, the close ecological association between cattle and possums in New Zealand is well known and the possibility that a *hardjo*-free bovine population could be susceptible to infection with *balcanica* must not be ignored. The introduction of *balcanica* to the national herd might have significant economic and public health implications.

APPENDICES

APPENDIX I.

Manufacturers of products identified in the text by brand names.

Atago:	Bussan Co. Ltd, Minato-Ku, Tokyo 105, Japan.
Analar:	B.D.H. Chemicals, Ltd., Poole, England.
Beckman:	Beckman Instruments Inc, Palo Alto, California, U.S.A.
B.D.H.:	British Drug House, Poole, England.
Colworth 400:	A.J. Seward & Co. Ltd., 6 Stamford St., London SE1 9UE, England.
BBL:	Becton, Dickinson & Co., Rutherford, New Jersey 07070, U.S.A.
Difco:	Difco Laboratories, Detroit, Michigan, U.S.A.
Dynatech:	Cook Laboratory Products, 900 Slaters Lane, Alexandria, Virginia 22314, U.S.A.
Isco:	Golden Retriever Model, Instrumentation Specialties Co., P.O. Box 5347, Lincoln, Nebraska 68505, U.S.A.
M & B:	May and Baker Ltd., Dagenham, England.
Medol:	Laboratory Services Ltd., 23 Botha St., Auckland 6, New Zealand.
Merk:	E. Merk, Darmstadt, Federal Republic of Germany.
Microtitre:	Dynatech Singapore (Private) Ltd., 21B Goldhill Plaza, Singapore 11.
Miles Laboratories:	Miles Laboratories Inc., Research Products, Elkhart, Indiana 46514, U.S.A.
Millipore:	Millipore Corporation, Bedford, Massachusetts 01730, U.S.A.
Multistix:	Ames Division, Miles Laboratories Inc.
Pentex:	Pentex Division, Miles Laboratories Inc.
Petroff-Hauser:	C.A. Hauser and Son, Philadelphia, U.S.A.

Pharmacia: Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Sigma: Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63178, U.S.A.

Swinnex: Millipore Corporation.

Titan Zipzone: Helena Laboratories Corp, Box 752, Beaumont, Texas 77704, U.S.A.

Vacutainer: Becton, Dickinson & Co., Rutherford, New Jersey 07070, U.S.A.

Varigrad: Buchler Instruments, Colorado, U.S.A.

Whatman: Whatman Inc., Clifton, New Jersey, U.S.A.

Yellow Springs Instrument Company: Model 31 Conductivity Bridge, Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.

APPENDIX II

Statistical methods used in this study.

All statistical analyses were conducted on coded titres. The method for coding titres was discussed in Chapter Two. The code is a logarithmic transformation of the true titre and represents the rank of the well in which the endpoint occurred. Coded titres can be converted to true titres by the formula

$$\text{Titre} = \frac{1}{12 \times (\{0.301 \times \text{Code}\})}$$

The geometric mean titre (GMT) was obtained by calculating the mean coded titre and then applying this transformation to obtain the GMT.

1. Mean coded titre:

$$\frac{\Sigma x}{n} \qquad (x = \text{coded titre, } n = \text{sample size})$$

2. Mean standard error of mean coded titre:

$$\text{S.E.} = \pm \sqrt{\frac{\Sigma(x - \bar{x})^2}{n^2 (n - 1)}}$$

3. 95% confidence limits of mean coded titre:

$$\bar{x} \pm 1.96 \times \text{S.E.}$$

Where 95% confidence limits were expressed as true titres the coded values of $\bar{x} - 1.96 \times$ S.E. and $\bar{x} + 1.96 \times$ S.E. were calculated and then decoded.

4. Student's *t* test for comparing mean coded titres of small samples:

First the variance ratio, $F = \frac{S_1^2}{S_2^2}$ was calculated to determine whether the variances of each sample were equal. Then student's *t* was calculated.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}} \qquad \text{with } n_1 + n_2 - 2 \text{ degress of freedom.}$$

where

$$s = \frac{\Sigma_1(x - \bar{x}_1)^2 + \Sigma_2(x - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

Subscripts $_1$ and $_2$ refer to data from each sample respectively.

5. Test for comparing mean coded titres from two large samples:

$$z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)}}$$

where z is the standardised normal variable.

6. One-way analysis of variance:

Source of variance	Sum of squares	Degrees of freedom	Mean square	F
Among samples	$\sum_{i=1}^{k} n_i(\bar{x}_{i.} - \bar{x})^2$	$k - 1$	$\frac{\sum_{i=1}^{k} n_i(\bar{x}_{i.} - \bar{x})^2}{k - 1} = S_b^2$	$\frac{S_b^2}{S^2}$
Within samples	$\sum_{i=1}^{k}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i.})^2$	$n - k$	$\frac{\sum_{i=1}^{k}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i.})^2}{n - k} = S^2$	
Total	$\sum_{i=1}^{k}\sum_{j=1}^{n_i}(x_{ij} - \bar{x})^2$	$n - 1$		

where k = number of sampled populations

n = total number of observations

i = index of population

j = index of observation

$\bar{x}_i$ = mean of $_i$th population

$\bar{x}$ = overall mean

x_{ij} = $_j$th observation in $_i$th population

7. Chi-square tests for frequency data:

$$\chi^2 = \Sigma\frac{(O - E)^2}{E}$$

O = observed frequency in each class

E = expected frequency in each class

8. 95% confidence limits of prevalence data:

$$95\%\ \text{C.L.} = \pm\ 1.96 \times 100\sqrt{\left(\frac{pq}{n}\right)}$$

where p = proportion affected

q = proportion not affected = (1 - p)

9. Calculation of regression line:

The regression line for y on x is $y = a + bx$

where $b = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{(x - \bar{x})^2}$

and $a = \bar{y} - b\bar{x}$

10. Calculation of correlation coefficient:

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2(y - \bar{y})^2}}$$

11. The standard error of the estimate of the regression line:

$$S_{y.x} = \frac{(n - 1)(S_y^2 - b^2 S_x^2)}{(n - 2)}$$

where n = number of samples

S_x^2 = variance of sample x values

S_y^2 = variance of sample y values

b = estimated slope of regression line

12. Significance test for comparing regression coefficients:

$$z = \frac{b_1 - b_2}{\sqrt{\left(\frac{S_{y.x_1}^2}{\Sigma_1(x - \bar{x}_1)^2} + \frac{S_{y.x_2}^2}{\Sigma_2(x - \bar{x}_2)^2} \right)}}$$

where subscripts $_1$ and $_2$ refer to statistics calculated from each population and z is the standardised normal statistic.

13. Calculation of 99% confidence limits of regression lines:

$$99\%\ \text{C.L.} = a + b(x_o - \bar{x}) \pm t_{0.995,\ N}\, S_{y.x} \sqrt{\left(\frac{1}{n} + \frac{\{x_o - \bar{x}\}^2}{\{n - 1\}S_x^2} \right)}$$

where x_o = x value at which confidence limits are being set

$t_{0.995}$ = student's t statistic for 99% level of probability

N = degrees of freedom

APPENDIX III

Preparation of Buffer.

1. Sodium Barbitone Buffer:

Dissolve 20.62g of Sodium barbitone in 1500 ml of distilled water, adjust pH to 8.6 with 0.5M HCl (about 10 ml) and make up to 2000 ml with distilled water.

2. Phosphate Buffer:

Stock solutions were prepared as follows:

Solution A: Dissolve 312.02g $NaH_2PO_4.2H_2O$ (M & B) in 2000 ml of distilled water.

Solution B: Dissolve 716.40g $Na_2HPO_4.12H_2O$ (M & B) in 2000 ml of distilled water.

1.0M Phosphate buffer can be prepared by combining x ml of solution A and y ml of solution B.

x	y	pH
45.0	55.0	6.9
39.0	61.0	7.0
33.0	67.0	7.1
28.0	72.0	7.2
23.0	77.0	7.3
19.0	81.0	7.4
16.0	84.0	7.5
13.0	87.0	7.6
10.5	90.5	7.7
8.5	91.5	7.8
7.0	93.0	7.9
5.3	94.7	8.0

0.6M, 0.1M and 0.01M phosphate buffers were prepared by diluting 1.0M buffer with distilled water.

APPENDIX IV

Preparation of antiserum against bovine serum.

A mixture of 1.0 ml Freunds complete adjuvant 0.5 ml sterile physiological saline and 0.5 ml fresh bovine serum was emulsified by repeatedly being forced through a 22 guage needle. Aliquots of 0.75 ml of the emulsified mixture were administered to each of a pair of rabbits by intramuscular injection. Seven days later the process was repeated with the exception that Freunds incomplete adjuvant was substituted in the mixture. This treatment was repeated again a further seven days later. Samples of blood were collected from the ear vein about 25 days after the intial injection and tested for antibody avtivity against bovine serum in immune electrophoresis plates. The rabbits were exsanguinated 28 days after the initial injection and their blood left to clot overnight before serum was removed by centrifugation for ten minutes at 5000G. Five millilitre aliquots of serum were held at -10^{o}C until required.

APPENDIX V.

Preparation of EMJH Medium according to the method of Johnson and Seiter (1977).

The stock solutions and media were prepared in glassware which had been thoroughly washed in an automatic laboratory washing machine and rinsed with distilled water before being autoclaved at 121°C for 20 minutes.

Fresh stock solutions of chemicals were prepared for each batch of media as follows:

		grams per 100 ml deionised water
NH_4Cl	(B.D.H.)	25.0
$ZnSO_4.7H_2O$	(M & B)	0.4
$MgCl_2.6H_2O$	(Analar)	1.5
$CaCl_2.2H_2O$	(Analar)	1.5
$FeSO_4.7H_2O$	(Analar)	0.5
$CuSO_4.5H_2O$	(Analar)	0.3
Sodium pyruvate	(B.D.H.)	10.0
Glycerol	(B.D.H.)	10.0
Tween 80	(Sigma)	10.0
Thiamine.HCl	(Sigma)	0.5
Cyanocobalamin	(Sigma)	0.02

Basal medium consisted of:

deionised water		9960 ml
KH_2PO_4	(Analar)	.3g
Na_2HPO_4	(Analar)	10g
NaCl	(Analar)	10g

plus stock solutions as follows:

NH_4Cl	10 ml
Thiamine.HCl	10 ml
Sodium pyruvate	10 ml
Glycerol	10 ml

This solution was adjusted to pH 7 using a pH meter (Beckman) and 450 batches were decanted in screw-capped bottles, autoclaved

at 121°C for 20 minutes and held in bulk.

The albumin supplement was prepared by dissolving 100g Bovine albumin fraction V powder (Pentex) in 500 ml deionised water using a magnetic stirrer in a one litre conical flask. The following stock solutions were slowly added as the powder dissolved:

$CaCl_2$	10 ml
$MgCl_2$	10 ml
$ZnSO_4$	10 ml
$CuSO_4$	1 ml
$FeSO_4$	100 ml
Cyanocobalamin	10 ml
Tween 80	125 ml

When the powder was completely dissolved the pH was adjusted to 7.4 and the solution brought to one litre by the addition of deionised water. The solution was then sterilised by filtration using a 0.22 µm filter (Millipore) and stored in 50 ml batches in sterile glass bottles.

Liquid medium was prepared by adding 50 ml of albumin supplement to 450 ml of basal medium.

Semisolid medium was prepared by adding 0.75g agar (Difco) to 450 ml of basal medium. This was autoclaved at 121°C for 20 minutes then cooled to about 40°C before the addition of 50 ml of albumin supplement.

Both media were incubated for three days at 37°C and three days at 30°C before use to check for the presence of contamination.

All new batches of basal medium and albumin supplement were tested to see that they supported the growth of a slow-growing *hardjo* isolate. Both liquid and semisolid media were prepared from samples of each new batch and approximately 10^2 organisms were inoculated into several tubes of media. These were incubated for two to three weeks at 30°C.

Stock solution of 5FU (Sigma) was prepared by adding 1.0g of 5FU to 50 ml of distilled water. This was placed in a 60°C water bath to dissolve the 5FU and the pH then adjusted to 7.6 by the addition of 1N HCl. The solution was made up to 100 ml by the addition of distilled water and was sterilised by filtration through a 0.22 µm filter (Millipore). Aliquots of the 5FU solutions were held at 4°C until required, when they were dissolved by placing in a 60°C water bath prior to addition to the prepared media.

APPENDIX VI

Effect of urine on the recovery of *hardjo* in culture.

The following experiment was conducted to test the inhibitory effect of urine on the growth of *hardjo* in semisolid EMJH medium.

Urine was collected from a known shedder and sterilised by double filtration through a 0.22µm cellulose filter followed by a final filtration through a 0.10µm filter. Dilutions of this urine were prepared in sterile physiological saline to provide final urine concentrations of:

100%
75%
50%
25%
0%

A fresh culture of *hardjo* grown in liquid EMJH medium was added to aliquots of these dilutions of sterile urine to produce counts of leptospires in each dilution ranging from approximately 10^1 to 10^5 organisms in tenfold steps.

Each of the 25 resulting combinations of urine were inoculated into paired tubes of semisolid EMJH containing 200 µg/ml 5FU at two inoculum rates; 50µl and 1000µl. The leptospiral count of the larger inoculum was adjusted so that approximately the same number of organisms was inoculated by either method. All tubes were incubated for 60 days at 30°C and examined once at the end of that period.

The results are summarised in the tables given below.

Number of organisms in the 1000µl inoculum.

Proportion of urine in the inoculum	10^1	10^2	10^3	10^4	10^5
100%	N	N	N	N	P
75%	N	N	N	C	N
50%	N	N	N	N	N
25%	N	N	N	N	P
0%	N	P	N	P	C

Number of organisms in the 50µl inoculum.

Proportion of urine in the inoculum	10^1	10^2	10^3	10^4	10^5
100%	N	N	N	P	P
75%	N	N	N	N	P
50%	C	C	N	P	P
25%	N	N	P	N	P
0%	N	P	C	P	P

N = Both replicates negative

P = at least one replicate positive

C = Both replicates contaminated

These results were taken to indicate that large volumes of urine in the inoculum were inhibiting the isolation of *hardjo*. Consequently, after this experiment, all urine cultures were carried out using 50µl inocula of urine which was either undiluted or diluted 1:10.

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