

BOVINE LEPTOSPIRA POMONA INFECTION: THE DISEASE IN INOCULATED CATTLE

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SUMMARY

The mild disease syndrome produced by inoculating a recently isolated Australian strain of *Leptospira pomona* into 21 cattle is described.

Eight of nine previously unexposed animals that were injected with calf or guinea pig organ suspensions containing *L. pomona* became febrile and one had haemoglobinuria. Except for two killed for passage, all of the cattle developed serum agglutinating antibody to *L. pomona* to a high titre. One died of lantana poisoning 18 days after inoculation. The six survivors all developed marked leptospiuria. However, no febrile reaction was observed in any of nine cattle inoculated with bovine urine microscopically positive for leptospiras, and leptospiuria was seen in only four animals, though all developed serum antibody. Agglutinating antibody to *L. pomona* was found in the urine of the two cattle examined, from the sixth week of the disease.

Three animals that were relatively resistant to inoculation with guinea pig organ suspensions containing *L. pomona* had been previously exposed in a leptospirosis outbreak without contracting the disease. These included two calves that had maternally derived serum agglutinating antibody to *L. pomona* for 114 and 142 days, but were seronegative for 114 and 60 days before inoculation.

I. INTRODUCTION

Previous studies of experimental bovine *Leptospira pomona* infection were recently reviewed by Fennestad (1963). Sutherland, Simmons, and Kenny (1949) and Sutherland (1950) injected a total of three calves with *L. pomona* strains that had been isolated in Queensland. Spradbrow and Seawright (1963) made a detailed investigation of the acute disease syndrome in 22 calves inoculated with three Australian strains of *L. pomona*. Laurie-Rhodes (1960), in Victoria, experimentally infected 12 control calves in a vaccination trial, but used an imported strain of *L. pomona*.

This paper describes the disease syndrome in 21 cattle, including nine animals one year of age or older, which were observed for as long as 222 days after inoculation with a recently isolated Australian strain of *L. pomona*.

II. MATERIALS AND METHODS

Inocula.—The strain of *L. pomona* was isolated by inoculation of guinea pigs with pooled brain, liver and kidney material, submitted to the Animal Research Institute, Yeerongpilly, from a heifer that died with haemoglobinuria on May 25, 1963. This date was taken as day 1 of the experiment. The organism was maintained by animal passage (Table 1). It was serologically identified as *L. pomona* by comparison with the standard "Staines" strain.

TABLE 1
DETAILS OF INOCULA AND EXPERIMENTALLY INFECTED CATTLE

Animal			Inoculation			
No.	Age	Sex	Day* of Experiment	Volume (ml)	Route†	Nature and Source of Inoculum
H1 ..	1 year	Female	14	10	I/M	Guinea pig organ suspension, second passage from original isolate
C1 ..	2 weeks	Female	56	10	I/M and I/P	Urine from H1
H2 to H6 ..	2 years	Female	71	10	S/C	Urine from C1
C2 ..	2 weeks	Male	69	10	I/M	Guinea pig organ suspension, 12 guinea pig passages from original isolate
C3 ..	3 weeks	Male	74	20	I/M	Kidney suspension from C2
C4 ..	3 weeks	Female	84	15	I/M	Kidney suspension from C3
H7 ..	2 years	Female	116	50	I/M	Urine from C4
M1 ..	1 year	Male	116	50	I/M	Urine from C4
H8 ..	2 years	Female	155	50	I/M	Urine from H7
S1, S2, S3	32 weeks	Male (steer)	168	10	I/M	Guinea pig organ suspension, one natural bovine passage (Doherty 1965) and one guinea pig passage from M1
H29 ..	2 years	Female	366	10	I/M	Guinea pig organ suspension after three guinea pig passages from cattle infected at pasture (Doherty 1965)
C10 ..	28 weeks	Male	366	10	I/M	
C11 ..	24 weeks	Female	366	10	I/M	
S31 and S32	32 weeks	Male (steer)	366	10	I/M	

* Day 1 was May 25, 1963, and this was the day that the heifer from which the strain was isolated died in the field.

† I/M = intramuscular; I/P = intraperitoneal; S/C = subcutaneous.

Cattle were injected (Table 1) with freshly voided bovine urine microscopically positive for leptospiras or with 10% calf or guinea pig organ suspensions from infected animals (Doherty and Baynes 1967). Guinea pigs were inoculated intraperitoneally with urine or with organ suspensions (Doherty 1966).

Experimental cattle.—Dairy breed cattle, which were serologically negative to *L. pomona* and *L. hyos*, were purchased. One animal, H1, had been splenectomized before use in this experiment.

None of the cattle had serum agglutinating antibody to *L. pomona* or *L. hyos* at time of inoculation (Table 1). Subsequent to inoculation, H1, C1, C2, C3, C4 and M1 were confined in separate concrete-floored pens. The remainder were pastured with an experimental herd of cattle (Doherty 1965). Two heifers, H2 and H3, were approximately 205 and 175 days pregnant at time of inoculation and later gave birth to C10 and C11. These two calves and H29 were exposed in an *L. pomona* outbreak (Doherty 1965) for at least 200 days before inoculation.

Sampling techniques.—Rectal temperatures were taken each morning with clinical thermometers and urine samples collected concurrently in 4-oz wide-mouthed jars. Urination was stimulated in females by rubbing on the escutcheon with a wet-gloved hand, and in males by also brushing the tip of the prepuce with the lip of a specimen bottle.

Until day 160 of the experiment, urine samples were held in a portable ice-box or refrigerator until processed within 12 hr of collection. After day 160, all urine samples were formalinized (Doherty 1966) and processed within 96 hr of collection.

Cattle were bled from the jugular vein. Those inoculated with bovine urine were bled once weekly, whereas cattle inoculated with organ suspensions were bled daily after inoculation until serum antibody was first detected and then weekly.

Laboratory techniques.—Urine samples were centrifuged and examined by dark-field illumination (DGE) as described by Doherty (1966). The number of organisms present in each preparation was assessed on a subjective basis and graded from + (less than one organism per microscopic field) to + + + + + (each microscopic field obscured by a tangled mass of leptospiras). This estimate was referred to as the level of leptospiuria.

Sera were stored at -20°C . All sera from the one animal were titrated on the one day for agglutinating antibody to *L. pomona* (Winks 1962). The maximum dilution tested was 1 : 300,000. Non-formalinized urine supernates from C1 and H6 were retained each day, stored at -20°C and later tested for presence of antibody (Doherty 1966) to a maximum dilution of 1 : 1024. Antibody titres were expressed as the reciprocal of the highest dilution of serum or urine in which there was 50% agglutination of leptospiras.

At slaughter, a portion from one kidney from each animal was embedded in paraffin, sectioned at 6 microns and stained with Warthin Starry's silver stain for leptospiras (United States Armed Forces Institute of Pathology 1960). Similar preparations were made from the livers of C3 and C4.

III. RESULTS

All inoculated cattle, except two killed during fever, developed serum agglutinating antibody to *L. pomona*.

None of the nine cattle that were inoculated with the microscopically positive bovine urine had a detectable fever and four did not develop leptospiruria (Table 2). Of the nine previously unexposed cattle that were inoculated with organ suspensions, eight had a fever (Table 3). The one that did not become febrile died of lantana poisoning 18 days after inoculation. Clinical reactions in febrile cattle were characterized by some degree of debility, but only one animal, S3, had haemoglobinuria, which occurred only on the day of first detection of serum antibody. Two of this group were killed for passage when febrile but the surviving six animals all developed severe leptospiruria.

The average maximum serum agglutinating antibody titre recorded from the four previously unexposed animals that did not develop leptospiruria (Table 2) was between 3,000 and 10,000. The average maximum titre recorded from 11 similar animals that did develop leptospiruria was between 100,000 and 300,000. Maximum titres were recorded at mean intervals of 18 (S.E. \pm 1.7) and 31.6 (S.E. \pm 3.7) days after inoculation in the groups without and with leptospiruria respectively. These differences in time to development of maximum titre were not significant at the 5% level. The average titre at approximately 18 days after inoculation in the group that did develop leptospiruria was approximately 30,000.

TABLE 2
BACTERIOLOGICAL AND SEROLOGICAL DATA FOR CATTLE INOCULATED WITH BOVINE URINE
CONTAINING *L. pomona*

Animal No.	Leptospiruria			Serum Agglutinating Antibody (Titre as Reciprocal*)				
	Day of First Detection	Duration (days)	Maximum Level	Day of First Detection	Maximum and Day First Recorded		Titre at Slaughter and Day	
					Titre	Day	Titre	Day
C1	11	42	+++++	7	300,000	31	NS	NS
H2	Nil	Nil	Nil	6	10,000	20	30	222
H3	Nil	Nil	Nil	6	10,000	13	30	222
H4	Nil	Nil	Nil	13	3,000	20	100	157
H5	22	10	+	13	30,000	20	300	157
H6	13	45	+	6	100,000	13	300	157
H7	11	34	+	9	300,000	51	NS	NS
H8	Nil	Nil	Nil	13	1,000	19	300	73
M1	11	26	+++	6	300,000	36	300,000	113

* Cattle were bled once weekly after inoculation.

NS Not slaughtered.

TABLE 3
CLINICAL, BACTERIOLOGICAL AND SEROLOGICAL DATA FOR PREVIOUSLY UNEXPOSED CATTLE INOCULATED WITH CALF OR GUINEA
PIG ORGAN SUSPENSIONS CONTAINING *L. pomona*

Animal No.	Fever		Leptospiuria			Serum Agglutinating Antibody (Titre as Reciprocal*)				
	Maximum Rectal Temperature (a.m.) and Days 103.5°F		Day of First Detection	Duration (days)	Maximum Level	Day of First Detection	Maximum and Day First Recorded		Titre at Slaughter and Day	
	Level (°F)	Days					Titre	Day	Titre	Day
H1	104.8	7-9	14	45	++++	9	30,000	16	30,000	63
C2	104.0	5K	K	K	K	K	K	K	K	K
C3	105.4	8-10K	K	K	K	K	K	K	K	K
C4	105.0	8-9	17	34	+++++	9	300,000	31	NS	NS
S1	104.4	8-9	13	46	+++++	10	300,000	33	300,000	163
S2	105.8	7-9	14	48	+++	10	300,000	51	30,000	125
S3	106.0	5-10	14	42	+++++	11	300,000	35	300,000	126
S31	104.4	6-7	26	T	++++	8	300,000	46	300,000	46
S32	102.4	Nil	Nil D	D	D	9	100,000	18	100,000	18

D Died from lantana poisoning 18 days after inoculation.

K Killed for passage.

T Treated with streptomycin 38 days after inoculation (Doherty 1965).

NS Not slaughtered.

* All cattle were bled daily from inoculation to development of antibody, then weekly.

None of the three cattle that had been exposed during a leptospirosis outbreak, without contracting the disease, became febrile subsequent to inoculation. Of these three animals, C10 and C11 had maternally derived serum agglutinating antibody for 114 and 142 days respectively, but had been serologically negative for 114 and 60 days respectively before inoculation. They were killed 44 days after inoculation and developed maximum serum agglutinating antibody titres of 300,000 (H29), 10,000 (C10) and 100,000 (C11). Only one (C11) developed leptospiruria and this was very slight over 14 days.

Antibody was first detected in the urine of C1 37 days after inoculation and was present consistently in daily examinations over the next 7 weeks. The maximum titre recorded was 64. The titre at autopsy 243 days after inoculation was also 64. Motile leptospiras and antibody were found in the urine concurrently. Urinary antibody was first found in H5 38 days after inoculation. The maximum titre recorded was 128.

Leptospiras were seen in silver-stained liver and kidney sections from C2 and C3 respectively. No leptospiras were seen in sections from any of the other cattle.

IV. DISCUSSION

The strain of *L. pomona* used in this experiment caused a clinically mild disease in inoculated cattle, compared with that produced by the recently isolated strains of Sutherland, Simmons, and Kenny (1949) and Spradbrow and Seawright (1963). The syndrome and sequence of appearance of fever, serum antibody and leptospiruria were similar to those reported by other workers as reviewed by Fennestad (1963).

Urine from cattle excreting leptospiras did not appear to be a suitable inoculum for producing the disease. According to Kirschner and Maguire (1957) and Fennestad (1963), undiluted voided bovine urine is a poor survival medium for leptospiras. The variation in syndrome between animals inoculated with infected urine and those inoculated with organ suspensions from febrile animals may reflect a difference in dosage of viable organisms. Reinhard and Hadlow (1954) and Fennestad and Borg-Petersen (1956) also reported difficulty in establishing leptospiruria in a proportion of cows and heifers that were inoculated with either *L. pomona* culture or infected guinea pig blood or organ suspensions.

Reinhard and Hadlow (1954) observed that serum agglutinating antibody titres were first recorded 6–7 days after inoculation and were at a maximum in a further 2–5 days. However, Hoag and Bell (1955), McDonald and Rudge (1957), Reilly, Muraschi, and Dean (1962), Fennestad (1963) and Robertson and Boulanger (1963) found a much more prolonged period of rising titre. Both Land and Morse (1959) and Fennestad (1963) have proposed that leptospiras in the renal tubules of bovine carriers continue to influence the antibody-forming cells of the host. The results reported in this paper support this hypothesis in

that the average interval from inoculation to development of maximum serum antibody titre in cattle with leptospiruria was relatively long (mean 31.6 ± 1.7 days). More tentative support is offered by the fact that animals that did not develop leptospiruria developed maximum serum antibody titres, of relatively low magnitude, more quickly than those that did develop leptospiruria. However, the pathogenesis of the acute stage of the disease may have been quite different in these two groups of cattle. This is in contrast to the evidence, cited by Babudieri (1958), that leptospiras in the renal tubules of some carrier species are no longer antigenic for the host while in this site.

The relative resistance of H29, C10 and C11 to inoculation is of interest. All had been exposed in an *L. pomona* outbreak for at least 200 days (Doherty 1965) without contracting leptospirosis. Sera from the heifer (H29) had shown intermittent agglutination at a dilution of 1:10 in weekly tests, from day 70 of the experiment, but this was regarded as non-specific by the criterion of Winks (1962). The maternally derived agglutinating antibody in the two calves disappeared well before the disease ceased to spread to susceptible cattle in the outbreak (Doherty 1965). This resistance may have been due to the presence of a protective mechanism not associated with antibody that can be detected by agglutination of living leptospiras, as proposed by Heath and Box (1965).

The presence of urinary antibody in bovine leptospirosis is well known (Hoeden 1936). Fennestad (1963) could not find agglutinins in urine before the fifth week of the disease. Specific agglutinating antibody in the serum or urine of mice infected with *L. australis* B (renamed *L. zanoi*) did not adversely affect urinary leptospiras in *in vitro* tests (Faine 1962). This may also be true of *L. pomona* infection in cattle, as motile leptospiras and antibody were present concurrently in the urine in the experiment reported here. Urinary antibody did not influence the infection rate in guinea pigs inoculated with urine microscopically positive for leptospiras (Doherty 1966).

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