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BOVINE LEPTOSPIRA POMONA INFECTION: THE DISEASE IN CATTLE INFECTED DURING AN EXPERIMENTAL OUTBREAK

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SUMMARY

A clinically mild disease syndrome was observed in the majority of 44 cattle that were naturally infected with *Leptospira pomona* during an experimentally induced outbreak. The mean maximum rectal temperature associated with leptospirosis was 104·6 ± 0·2°F. The duration of the febrile reaction was directly related to the subsequent degree of leptospiruria. Three calves suffered a febrile relapse during leptospiruria, associated with a rise in serum antibody titre and a drop in the level of excretion. One of five pregnant animals aborted and three undernourished steers died during leptospiruria.

All infected cattle developed specific serum agglutinating antibody to *L. pomona*. The lowest maximum serum antibody titre was recorded at a dilution of 1:300 from the one animal that did not excrete leptospiras in the urine. Antibody was detected before leptospiruria in 10 heifers and steers bled daily from fever to detection of antibody, but not in three calves in which leptospiruria commenced very soon after the febrile reaction. Maximum titres were first recorded up to 8 weeks after antibody was initially detected; the mean interval was 3·6 ± 0·1 weeks.

The lowest maximum serum antibody titre from any animal that excreted leptospiras in the urine was recorded at a dilution of 1:1,000. The longest duration of leptospiruria was 118 days, the least 10 days and the mean 36·4 ± 3·1 days. The highest level of excretion of leptospiras was usually seen during the first half of the leptospiruria period. The degree of leptospiruria tended to decrease as age of animals at infection increased.

I. INTRODUCTION

The course of *Leptospira pomona* infection in experimentally inoculated cattle has been the subject of numerous detailed investigations, reviewed by Fennestad (1963). However, no intensive study has been reported of the disease syndrome in the individual bovine animal contracting leptospirosis during an outbreak.

This paper records clinical, bacteriological and serological data for cattle infected at pasture during the experimental outbreak described by Doherty (1967*b*).

II. MATERIALS AND METHODS

Mode of infection.—All cattle contracted *L. pomona* infection as a result of natural spread of the disease in a herd at pasture. The leptospirosis outbreak in this susceptible herd was initiated by introduction of a few cattle that had been inoculated with *L. pomona.* The history of the strain of *L. pomona*, the disease syndrome in inoculated animals and the dynamics of spread of infection in the herd have been described in previous communications (Doherty 1967*a*, 1967*b*).

Experimental cattle.—The naturally infected cattle considered in this paper comprised 20 heifers approximately 2 years of age and 20 steers approximately 8 months of age at the time of exposure to infection and four calf progeny born into the herd during the experiment. Seven other similar steers (Doherty 1967*b*) have been excluded from the data because they were treated with streptomycin (Doherty 1965) during leptospiruria.

The cattle were of dairy type, predominantly Australian Illawarra Shorthorn. The heifers and steers did not have serum agglutinating antibody to *L. pomona* or *L. hyos* at time of exposure. The calves did not receive detectable colostral antibody to either of these leptospira serotypes.

Sampling and laboratory examination.—The sampling and laboratory examination techniques were as described previously (Doherty 1967*a*, 1967*b*).

Rectal temperatures were taken each morning and a urine sample was collected concurrently. Blood samples were collected weekly from all animals, and at daily intervals from 13 cattle during the interval from fever to detection of antibody and from two calves during a secondary febrile reaction.

Leptospiruria was determined by dark-ground examinations of centrifuged formalinized urine samples and the level of leptospiruria graded from + (less than one organism per microscopic field) to + + + + + + (each microscopic

field obscured by a tangled mass of leptospiras). Serum samples were titrated for agglutinating antibody to *L. pomona* to a maximum dilution of 1:300,000 (Winks 1962). Urinary antibody was determined by titrating non-formalinized urine supernates to a maximum dilution of 1:1,024. Antibody titre was expressed as the reciprocal of the highest dilution of serum or urine which gave a 50% agglutination of leptospiras.

Examination for leptospiraemia was made by intraperitoneal inoculation of 1–2 ml of freshly collected blood, without added anticoagulant, into a single 150–250-g guinea pig. Serum from the guinea pig was collected 21 days later and tested for presence of antibody. Demonstration of leptospiraemia was attempted in 15 of the cattle while ferbile.

Thirty-seven cattle were killed from 5 to 94 days after cessation of leptospiruria, three steers died during leptospiruria and one heifer aborted. A Warthin Starry stained kidney section was made from one kidney of each slaughtered animal, both kidneys of the three steers and the liver and kidneys of the aborted foetus. A tissue suspension was also prepared from the sampled kidney of 24 of the slaughtered animals, the kidneys of two of the steers and the liver and both kidneys of the foetus. Three samples from the slaughtered cattle were inoculated into single guinea pigs, 12 into groups of four weanling mice and nine into batches of six tubes of semisolid medium. The suspensions from the two steers were inoculated into single guinea pigs and those from the foetus into groups of three guinea pigs.

The sections were examined under both dark-field and conventional illumination. The cultures were examined weekly for 6 weeks. Sera from the guinea pigs and mice were collected 21 and 14 days respectively after inoculation and tested for presence of agglutinating antibody to *L. pomona.*

Analysis of results.—As the time of infection was not known, the maximum rectal temperature associated with leptospirosis was considered to be that which was recorded in the 14 days before commencement of leptospiruria and preceding detection of serum agglutinating antibody. This criterion was based on results of studies with inoculated cattle (Fennestad 1963; Doherty 1967*a*) where precise information as to time of infection was available. The duration of fever was measured as the number of days that rectal temperature was $>103{\cdot}5°F$ in the interval defined above. This was referred to as the primary fever. Any febrile reaction during leptospiruria was referred to as a secondary fever.

An attempt was made to correlate variations in the number of leptospiras excreted and variations in serum agglutinating antibody titre with the leptospiruria period. The duration of leptospiruria for each animal was divided into quarters and the distribution of maximum and minimum agglutinating antibody titres and

maximum levels of leptospiruria noted for each of these quarters. Only animals that showed variations (i.e. maxima and minima) in serum agglutinating antibody titre or level of leptospiruria were considered.

III. RESULTS

(a) General Observations and the Disease Syndrome in all Cattle

Data concerning the duration and magnitude of the primary febrile response and leptospiruria, and the time relationship between day of maximum rectal temperature and day of commencement of leptospiruria, are summarized in Table 1. There was no significant difference, at the 5% level, in means of maximum temperature or duration of fever recorded from heifers, calves and steers. The mean duration of leptospiruria in heifers was significantly less ($P<0{\cdot}01$) than that in steers or calves, but there was no significant difference between steers and calves. The mean maximum level of leptospiruria was significantly greater ($P<0{\cdot}01$) in calves than in heifers or steers, but there was no significant difference between heifers and steers. The mean time from maximum rectal temperature to commencement of leptospiruria was significantly less ($P<0{\cdot}001$) in calves than in heifers or steers, but there was no significant difference between heifers and steers.

The time relationships between the day of detection of serum agglutinating antibody and the days of maximum rectal temperature and commencement of leptospiruria are detailed in Table 2. The times relating maximum rectal temperature and first detection of serum antibody were similar in heifers, steers and calves. However, the three calves that were bled daily all commenced leptospiruria before serum antibody was detected, whereas in the 10 heifers and steers that were bled daily this sequence was reversed.

Quantitative aspects of the serum agglutinating antibody response are recorded in Table 3. There were no marked differences between heifers, calves and steers in the maximum serum antibody titre recorded, the time to development of maximum titre, the serum antibody titre at slaughter, or the time to slaughter. The minimum serum agglutinating antibody titre during leptospiruria was, on the average, less by a factor of 10 than the maximum titre recorded. The lowest maximum titre from any animal that also had leptospiruria was 1,000. Only one animal, a steer, developed a serum antibody titre but did not develop leptospiruria. The maximum titre from this animal was 300.

The distributions of maximum and minimum serum agglutinating antibody titres and maximum levels of leptospiruria are related to the leptospiruria period in Table 4. The greatest distribution of maximum serum antibody titre was found after the end of leptospiruria. The greatest distribution of minimum serum antibody titres during leptospiruria was found in the second quarter of leptospiruria. The distribution of maximum levels of leptospiruria was greatest during the first half of leptospiruria.

TABLE 1

CLINICAL AND BACTERIOLOGICAL DATA FOR CATTLE INFECTED WITH *L. pomona* DURING THE COURSE OF AN EXPERIMENTALLY INDUCED OUTBREAK

Component of the Syndrome	Heifers		Steers		Calves		All Animals			
	No.*	Mean ± S.E.	No.*	Mean ± S.E.	No.*	Mean ± S.E.	No.*	Mean ± S.E.	Maximum	Minimum
Maximum morning rectal temperature (°F) in the 14 days before start of leptospiruria	20	104·5 ± 0·3	18	104·7 ± 0·2	4	105·0 ± 0·4	42	104·6 ± 0·2	106·8	102·2
Number of days rectal temperature was above 103·5°F in the above interval	20	1·7 ± 0·4	18	1·6 ± 0·2	4	2·0 ± 0·4	42	1·7 ± 0·2	8	0
Days from maximum rectal temperature to start of leptospiruria	20	8·2 ± 0·2	17	8·9 ± 0·2	4	2·5 ± 1·2	41	8·0 ± 0·2	14	0
Duration of leptospiruria (days)	20	28·7 ± 1·7	17	43·4 ± 2·0	4	45·0 ± 6·6	41	36·4 ± 3·1	118	0
Maximum level of leptospiruria (+ = 1)** ..	20	2·3 ± 0·3	17	2·7 ± 0·4	4	5·8 ± 0·8	41	2·8 ± 0·3	6	0

* The number of animals varies because complete data were not available for all cattle for each component of the disease syndrome; e.g. some animals died during leptospiruria.

** The maximum level of leptospiruria was graded from + to ++++++.

TABLE 2

TIME RELATIONSHIP BETWEEN THE DETECTION OF SERUM AGGLUTINATING ANTIBODY AND THE FEBRILE REACTION AND COMMENCEMENT OF LEPTOSPIRURIA

Factors Related (time in days)	Frequency of Blood Sampling*	Heifers		Steers		Calves		All Animals			
		No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	No.	Mean ± S.E.	Maximum	Minimum
Interval from maximum rectal temperature during fever to first detection of serum antibody	Daily	6	1·8 ± 0·7	4	2·8 ± 0·6	3	3·0 ± 0·6	13	2·4 ± 0·9	4	0
	Weekly	14	5·0 ± 0·9	12	6·8 ± 1·1	1	12	27	6·7 ± 0·7	14	0
Interval from first detection of serum antibody to commencement of leptospiruria	Daily	6	4·8 ± 0·8	4	7·5 ± 1·7	3	−1·7 ± 0·3	13	4·2 ± 1·1	11	−2
	Weekly	14	3·5 ± 1·2	14	3·6 ± 1·0	1	−6	29	3·2 ± 0·8	10	−7

* Some animals were bled once daily over the interval from maximum febrile reaction to first detection of serum antibody. The remainder were bled once weekly over this interval.

TABLE 3

SEROLOGICAL DATA FOR CATTLE INFECTED WITH *L. pomona* DURING THE COURSE OF AN EXPERIMENTALLY INDUCED OUTBREAK

Component of the Serological Reaction	Heifers		Steers		Calves		All Animals			
	No.	Mean ± S.E. or Average	No.	Mean ± S.E. or Average	No.	Mean ± S.E. or Average	No.	Mean ± S.E. or Average	Maximum	Minimum
Weeks from first detection of serum antibody to first detection of maximum titre	20	3·7 ± 0·5	14	3·6 ± 0·9	4	3·0 ± 0·7	38	3·6 ± 0·1	8	1
Maximum serum agglutinating antibody titre recorded*	20	Between 100,000 and 300,000	14	Between 30,000 and 100,000	4	Between 100,000 and 300,000	38	Between 100,000 and 300,000	300,000	300
Minimum serum agglutinating antibody titre recorded during leptospiruria	20	Between 10,000 and 30,000	14	Between 10,000 and 30,000	4	10	38	Between 10,000 and 30,000	100,000	0
Weeks from first detection of serum antibody to slaughter	18	11·3 ± 0·7	12	11·1 ± 1·3	4	11·8 ± 2·2	34	11·3 ± 0·6	18	6
Serum agglutinating antibody titre at slaughter ..	18	Between 30,000 and 100,000	12	Between 30,000 and 100,000	4	Between 10,000 and 30,000	34	Between 30,000 and 100,000	300,000	300

* Maximum dilution tested was 1 : 300,000. Titres are expressed as the reciprocal of the maximum dilution giving 50% agglutination of leptospiras.

TABLE 4

DISTRIBUTION OF MAXIMUM SERUM AGGLUTINATING ANTIBODY TITRE, MINIMUM SERUM AGGLUTINATING ANTIBODY TITRE DURING LEPTOSPIRURIA AND MAXIMUM LEVEL OF LEPTOSPIRURIA RELATIVE TO THE LEPTOSPIRURIA PERIOD, FOR CATTLE SHOWING MAXIMA AND MINIMA IN THESE FACTORS

Component of the Disease Syndrome in which Variation was Observed	Type of Animals	Number of Animals	Leptospiruria					
			Pre-	First Quarter	Second Quarter	Third Quarter	Fourth Quarter	Post-
Number of animals showing a maximum serum agglutinating antibody titre	Heifers	18	3	3	3	0	3	11
	Steers	12	3	5	1	0	4	4
	Calves	4	0	1	1	1	2	3
	All animals	34	6	9	5	1	9	18
Number of animals showing a minimum serum agglutinating antibody during leptospiruria	Heifers	16	..	8	11	8	6	..
	Steers	8	..	8	8	6	4	..
	Calves	4	..	4	3	0	0	..
	All animals	28	..	20	22	14	10	..
Number of animals showing a maximum level of leptospiruria ..	Heifers	12	..	2	8	3	1	..
	Steers	13	..	9	4	1	1	..
	Calves	4	..	1	4	0	0	..
	All animals	29	..	12	16	4	2	..

A direct relationship was found between duration of fever and subsequent maximum level of leptospiruria (Table 5). There was a trend for such a relationship between duration of fever and duration of leptospiruria. The maximum serum agglutinating antibody titre was directly related to the maximum level of leptospiruria, but not to the duration of fever or leptospiruria or the interval from first detection of antibody to first detection of maximum titre (Table 6).

TABLE 5

RELATIONSHIP BETWEEN DURATION OF FEVER AND LEPTOSPIRURIA FOR ALL CATTLE

Leptospiruria	Duration of Fever (days)			
	0	1	2	>2
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Duration (days)	20·5 ± 4·7	39·1 ± 6·9	34·4 ± 2·7	42·6 ± 4·7
Maximum level (+ = 1)*	1·0 ± 0	2·1 ± 0·3	3·5 ± 0·5	3·9 ± 0·8

* The maximum level of leptospiruria was graded from + to ++++++.

TABLE 6

RELATIONSHIPS BETWEEN MAXIMUM SERUM AGGLUTINATING ANTIBODY TITRE RECORDED AND DURATION AND MAGNITUDE OF LEPTOSPIRURIA, DURATION OF FEVER, AND INTERVAL FROM FIRST DETECTION OF SERUM ANTIBODY TO FIRST DETECTION OF MAXIMUM TITRE FOR ALL CATTLE

Component of the Disease Syndrome	Maximum Serum Agglutinating Antibody Titre Recorded (titre as reciprocal)			
	30,000	30,000	100,000	300,000
	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
Duration of leptospiruria (days)	29·8 ± 3·8	40·5 ± 5·7	36·1 ± 4·3	33·7 ± 3·6
Maximum level of leptospiruria (+ = 1)	1·4 ± 0·8	2·6 ± 0·4	3·1 ± 0·5	3·5 ± 0·5
Duration of fever (days)	2·6 ± 0·4	1·5 ± 0·4	1·7 ± 0·4	2·5 ± 0·4
Weeks from first detection of antibody to first detection of maximum titre ..	1·8 ± 0·5	3·0 ± 0·6	3·6 ± 0·8	3·0 ± 0·9

No leptospiras were demonstrated in the kidneys of the 37 slaughtered cattle that had ceased leptospiruria from 5 to 94 days previously.

(b) Limited Observations and Differences in the Disease Syndrome in Individual Cattle

Leptospiraemia was demonstrated on three of four days (1, 2 and 6 but not 4) of the primary fever in one heifer, one of two days (2 but not 4) in another, and in 9 of 12 attempts from 12 other cattle.

Two calves, which were aged 46 and 40 days at commencement of leptospiruria, had a secondary febrile reaction. This lasted for 8 days in each case and began 12 and 28 days respectively after commencement of leptospiruria. Maximum rectal temperatures of 107·0°F and 106·0°F respectively were recorded, compared with maximum rectal temperatures of 104·8°F and 105·8°F respectively during the primary fever. Leptospiraemia could not be demonstrated in the first calf on the first, third or seventh day of the secondary fever. This recrudescence of fever was accompanied by a rise in serum agglutinating antibody titre from 1,000 to 300,000 over 4 days and from 3,000 to 300,000 over 5 days respectively. There was an accompanying permanent fall in level of leptospiruria from +++++ to + in 1 day and ++++ to + over 5 days respectively. Leptospiruria ceased in the second animal on the last day of this febrile reaction. A similar phenomenon, but to a lesser degree, was observed in a third calf which was 39 days of age at the commencement of leptospiruria.

Only one animal, a heifer, had haemoglobinuria. This occurred on the day that serum antibody was first detected. One of five heifers in advanced pregnancy aborted 16 days after the first febrile reaction and 12 days after the start of leptospiruria. No leptospiras could be identified in the silver-stained sections of foetal liver and kidney and *L. pomona* was not isolated from either of these organs.

Three steers died during the course of the disease at 8, 12 and 18 days after commencement of leptospiruria. They did not suffer severe febrile reactions but were in very poor condition at the time of infection. In the two steers sampled, leptospiras were seen in silver-stained kidney sections and *L. pomona* was demonstrated by guinea pig inoculation of kidney tissue.

There were no other specific clinical symptoms in any of the 44 cattle. Serous nasal and ocular discharges were observed in some animals concurrent with fever. These were not constant, and in pastured cattle could have been caused by a number of other factors.

Urinary antibody was measured in 12 animals on the day before slaughter. Ten were positive, at titres from 8 to 256, from 11 to 73 days after the end of leptospiruria. The two negative heifers, killed 54 and 69 days after the end of leptospiruria, had only mild clinical, bacteriological and serological responses to *L. pomona*.

IV. DISCUSSION

The disease syndrome produced by contact infection of cattle with this strain of *L. pomona* was clinically mild, and similar to that observed in animals inoculated with the same strain (Doherty 1967*a*).

All reports of *L. pomona* infection in cattle in Australia, reviewed by Doherty (1967*a*, 1967*b*), have described severe symptoms, especially in calves. However, Bell, Rice, and Connor (1953) and Hughes and Keech (1960) in North America and Te Punga and Bishop (1953) in New Zealand have described outbreaks where abortion was the only obvious symptom. Several authors have indicated this in a percentage of herds in survey studies (Wellington, Ferris, and Stevenson 1953; Morse *et al.* 1955*a*, 1955*b*; Stoenner *et al.* 1956; Schnurrenberger *et al.* 1961). There is serological evidence that at least 10% of cattle in Queensland (Winks 1962; Spradbrow 1964; Lucas 1966) and New South Wales (Keast, Forbes, and Wannan 1964) have antibody to *L. pomona.* It is possible that the essentially mild syndrome seen in the experiment described here is a common form of the disease.

Fennestad (1963) was able to correlate severity of the febrile reaction with subsequent degree of antibody production and leptospiruria. In our experiment it was only possible to relate febrile reaction and leptospiruria. The observation that the degree of leptospiruria tends to decrease with increasing age of the animal was also made by Fennestad (1963).

The observation that the greatest distribution of maximum serum antibody titres was found after the end of leptospiruria and the fact that the mean interval from first detection of serum antibody to development of maximum titre was over 3 weeks supports the hypothesis of Fennestad (1963) that the continued presence of leptospiras in the renal tubules is responsible for high, slow-rising antibody titres. However, this phenomenon probably depends on the number of leptospiras present over this period, for, in the experiment reported here, maximum serum antibody titre was related to the level of leptospiruria but not to the duration of leptospiruria.

The greatest distribution of minimum serum antibody titres during leptospiruria and maximum levels of leptospiruria both occurred in the second quarter of leptospiruria. There was a fall in the distribution of maximum serum antibody titres during the second and third quarters of leptospiruria. This fall in antibody titre could have resulted from loss of antibody in the urine concurrent with presence of maximum numbers of leptospiras in the tubules; however, Fennestad (1963) and Doherty (1967*a*) could not detect specific aggultinating antibody in the urine at this stage of the disease. McIntyre and Montgomery (1952) proposed that antibody in the urine might be responsible for the clumping seen during leptospiruria. If this was the case, antibody might be fixed and not detectable in the urine when there were large numbers of leptospiras present. However, Faine (1962) could not demonstrate absorption of globulin onto *L. australia* B (renamed *L. zanoni*) in the urine of mice. Therefore at the present state of knowledge, no conclusion can be made concerning the trend for serum antibody titre to fall during leptospiruria followed by a rise late in, or after the end of, leptospiruria. This phenomenon was marked in individual animals (Doherty 1965).

The time sequence of detection of fever, serum antibody and leptospiruria was similar to that described by workers with experimentally inoculated cattle (Fennestad 1963). However, in the experiment reported here three calves that were bled daily commenced leptospiruria before serum antibody was detected. This phenomenon was apparent in one of five calves inoculated by Fennestad (1963). It has not been mentioned in any of the numerous publications reviewed by Fennestad (1963) and Doherty (1965).

The secondary febrile reactions in calves have been recorded in similar inoculated animals by Gillespie and Kenzy (1958) and Fennestad (1963). Febrile relapses are well known in human leptospirosis (Thiel 1948). As, in the experiment reported here, this was associated with a marked rise in antibody titre and drop in urinary excretion of leptospiras, it would appear that this recrudescence of fever was a function of rejection of renal leptospiras by the host.

The maximum duration of leptospiruria of 118 days was longer than any previously reported. Ferguson, Ramge, and Sanger (1957) demonstrated *L. pomona* in the urine of a heifer for 102 days after inoculation, Fennestad (1963) for 91 days in a calf. The contact-infected calves of Webster (1959) excreted for 6–8 weeks. Ringen and Bracken (1956) showed leptospiruria for 7 weeks in a 12-month-old Hereford, and observed an average excretion time of 33 days in four such animals, infected by exposure of the mucous membranes and outer integument to *L. pomona.* Sutherland (1950) observed excretion for 28 and 55 days in two naturally infected calves. These results are similar to those reported here (Table 1).

The failure to isolate *L. pomona* from the aborted foetus is in accord with conclusions drawn from studies of leptospiral abortion by Ferguson, Ramge, and Sanger (1957), Morter, Langham, and Morse (1958), Fennestad and Borg-Petersen (1956) and Sleight and Langham (1962). However, *L. pomona* was isolated by Podgwaite *et al.* (1955) from a foetus aborted during an outbreak.

The death of the three undernourished steers during leptospiruria was considered to be due to the additive effects of leptospirosis and nutritional stress. This is similar to the "ill thrift" syndrome emphasized by Sutherland, Simmons, and Kenny (1949) and Stoenner *et al.* (1956). Inability to concentrate the glomerular filtrate as a result of damage to the kidney tubules (Austoni and Corà 1961) could have been a factor in these deaths.

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