Leptospirosis in Canada. III. A Study of the Importance of the Disease in Cattle as Shown in Combined Serological, Clinical and Bacteriological Investigations.

Paul Boulanger, Douglas Mitchell, A. N. Smith and Christine E. Rice[1]

A PRELIMINARY serological survey, made in 1954, indicated that leptospiral infection was or had been present in many herds of cattle in Ontario and Quebec (1). A complement-fixation technique with a commercially-prepared *Leptospira pomona* antigen was utilized in this first survey while in subsequent survey made in 1956 the haemagglutination-lysis test was applied (2). This second serological survey detected 218 reactors among a total of 2695 cattle tested, these reactors being distributed throughout 68 of the 113 herds represented. However, among the 218 reactors, only 36 animals, from 21 herds, had agglutination-lysis titres of 1:1000 or higher, titres which are considered to be suggestive of presently-active or of fairly recent infection.

This percentage, 8.1 per cent, of serologically reactive animals and the proportion of herds involved, would suggest a fairly wide distribution of leptospirosis in these provinces, higher than would have been anticipated on the basis of clinical reports alone.

In weighing the importance of such serological reactions it was fully realized, however, that a considerable percentage of leptospiral infections are subclinical. An evaluation of the true economic loss attributable to such a clinically variable infection as leptospirosis cannot accordingly be based on serological findings alone but must take into consideration the relation of these to the incidence and severity of any clinical manifestations in the same cattle population.

When the veterinarian is dealing with a disease characterized by abortion or other reproductive difficulties, he may be inclined to think in terms of brucellosis. Leptospirosis, however, differs from brucellosis in many respects particularly in relation to the importance of the serological reactor in disseminating the disease throughout the herd. In brucellosis the reactors are thought of as carrier animals which sooner or later will infect other animals through contact. The importance of the carrier state in leptospirosis is ill-defined and problematical for as yet there is no serological test to distinguish a carrier animal from one which is convalescent or cured. In other words, an animal with antibodies in its serum, particularly if these are of titres up to 1:1000, may be a recovered subject which is no longer shedding leptospirae in the urine and is no longer a potential source of infection in the herd. The possibility should not be overlooked, however, that in a very few apparently-recovered animals the persistence of leptospiral antibodies may indicate the presence of a latent focus of infection with *L. pomona*, and that such animals might occasionally shed these organisms in the urine. Such cases are thought to be rare, but further investigations of the carrier state in leptospirosis are needed.

[1]Animal Pathology, Health of Animals Division, Canada Department of Agriculture, Animal Diseases Research Institute, Hull, P.Q.

The present report deals with epizootiological studies of 20 local herds with reproductive and other problems, special attention being given to the diagnosis, symptomatology, morbidity and mortality associated with leptospirosis. This investigation was begun in the fall of 1956 and material presented includes that collected up to the early summer of 1957.

METHODS

Selection of Herds

Contact with suitable problem herds was made through extension workers and veterinary practitioners. In such herds preliminary tests were made for brucellosis and vibriosis as well as for leptospirosis. In all herds where leptospiral reactors were detected a complete clinical history was obtained from the veterinarian or from the herd owner. This was supplemented by our own personal observations. A record was kept on each individual animal, of the sanitary condition of the farm and of possible contact of the cattle with other species of animals. When possible, visits were made to these leptospirosis problem herds during or soon after the acute stage of the infection. The more severely infected herds were then visited at monthly intervals thereafter.

The difficulties encountered in the leptospirosis negative herds were studied further and the results will be the subject of a separate report.

Collection of Blood Samples

In individual herds, the first blood samples were taken only from animals with clinical signs suggestive of leptospiral infection. When reactions were obtained, all cattle in the herd were bled. In some instances other species of animals which might be implicated in the spread of infection on the farm, were also bled. Herds with both clinical and serological evidence of infection were bled at monthly intervals thereafter. A few herds with serological but no clinical manifestations of leptospirosis were bled at intervals of two or three months.

Serological Methods

The agglutination-lysis test (3) using viable cultures of leptospira grown in Korthof's medium (4) was employed throughout the study. The following serotypes of leptospira were included in tests on most of the herds: *Leptospira pomona* and *L. canicola* strains obtained from Dr. R. J. Avery of our Branch Laboratory, Pacific Area; *L. bovis*, received from the late Dr. T. Moore of this Institute; *L. icterohemorrhagiae*, *L. sejroe* and *L. grippotyphosa* furnished by the Walter Reed Army Medical Centre, Division of Veterinary Medicine, Washington, D.C.

A screening test was first run on all sera using 0.1 ml. of a 1:100 serum dilution plus 0.1 ml. of antigen consisting of a 7 to 14 day-old motile culture of leptospira. When a reaction was obtained in this dilution, the test was repeated using two-fold dilutions from 1:100 to 1:800 inclusive, and then tenfold dilutions from 1:1000 to 1:1,000,000 inclusive. The tests were incubated for four hours at 37°C and the reactions evaluated as described in our previous paper (2).

Parallel complement-fixation tests were made on all sera using sonic vibrated suspensions of *L. pomona* and *L. sejroe* as antigens (5). The sera were tested in serial dilutions from 1:5 to 1:100. Three 50 per cent haemolytic units of complement were used. All reagents of the test were added in 0.1 ml. amounts. The period of preliminary incubation was 18 hours at approximately 9°C., that of secondary incubation after the addition of the sensitized sheep red cells, was 30 min. at 37°C. The serum titre was recorded as the highest dilution with which 50 per cent haemolysis was obtained in the presence of antigen.

A series of tube and plate agglutination tests were made with killed *L. pomona* and *L. sejroe* antigens. The results with these will be presented in a later paper.

Cultural Methods

Specimens of urine, milk or tissue which are to be examined for the presence of leptospira are usually heavily contaminated with miscellaneous bacteria which grow copiously in leptospira media and check the growth of the leptospira. Such contaminants can be screened by guinea-pig inoculation, the method adopted throughout this study. Young guinea-pigs (200 to 300 grams) were tagged, their temperatures taken and brought to the farm where 1 to 2 ml. volumes of fresh milk, blood or urine were inoculated intraperitoneally. Temperatures were taken daily for 12 days thereafter. If a rise of temperature was recorded at three to eight days, the animals were bled aseptically by heart-puncture and six to eight drops of blood inoculated directly into a tube of Korthof's medium. The cultures were examined after four days incubation at 30°C, then at weekly intervals for four weeks. At the end of this period if the cultures were negative, the guinea-pigs were bled and their sera tested for presence of antibodies by both agglutination-lysis and complement-fixation methods.

RESULTS

The 20 herds with reproductive and other difficulties that were studied during the course of this investigation, have been divided into leptospirosis positive, questionable, and negative. The serological and bacteriological findings in all leptospirosis-infected herds are considered here in relation to the clinical observations, but a full clinical study of each herd will be deferred to a later paper.

I. Positive Herds

A total of six herds in which leptospirosis was recognised clinically, serologically and in one case bacteriologically were studied. In these herds, 223 individual animals were tested and a total of 682 tests performed at various intervals. Since the disease took a different course in most of these herds, each herd will be described individually.

Herd No. 3.

This herd of 47 cattle was maintained on a 175 acre farm through which flow two rivers to which cattle have access. The herd consists of one bull, 25 cows, 10 heifers, 5 steers, and 8 calves. All breeding has been done naturally. The young bull, No. 1, has been used on only three or four cows since July 1956. The rest of the cows have been bred to a shorthorn bull No. 51 belonging to a brother of the herd owner.

This farm experienced a brucella "storm" eight years ago and all cattle were then disposed of and replaced by new stock. Since that time all calves have been vaccinated with *Brucella abortus* strain 19. All adult cattle are now negative in brucellosis tests but two horses, Nos. 48 and 49, the latter having a previous history of fistulous withers, are positive. Both horses were on the farm at the time of the brucella outbreak.

In summer of 1954 a cow calved and three weeks later died suddenly about 12 hours after she was first noticed to be ill. No further trouble was noted until Oct. 28, 1956, when cow No. 18 showed a drop in milk, increased respiration and haemoglobinuria. Her temperature was 103°F when taken by the local practitioner the following day. The haemoglobinuria persisted for 6 days and the milk remained yellow and thick for a few days, after which it returned to normal. This cow was not in calf at the time of this episode, and has since been bred once. The results of the first complete herd test for leptospirosis made on Nov. 14, 1956, are given in Table I. It will be noted that the serum of cow No. 18 had an agglutination-lysis titre of 1:10,000 and a complement-fixation titre of 1:100.

A second cow, No. 2, aborted a 5-month foetus on Nov. 9, 1956, while out on pasture. The foetus was not found, but the placenta which was manually removed by the local veterinary practitioner, had the suggestive thickened avascular appearance described in lep-

TABLE 1.

Summary of serological, bacteriological and clinical investigations in herd #3, W.A.N.*

Animal Number	Age and Sex	Expected date of calving	Nov. 14/56 A.L.	Nov. 14/56 C.F.	Dec. 13/56 A.L.	Dec. 13/56 C.F.	Jan. 22/57 A.L.	Jan. 22/57 C.F.	Feb. 26/57 A.L.	Feb. 26/57 C.F.	Apr. 12/57 A.L.	Apr. 12/57 C.F.	May 28/57 A.L.	May 28/57 C.F.	
1	M. 1 y.		-	-	-	-	-	-	-	-	-	-	x	x	Used for breeding.
2.	F. 6 y.	March /57	100,000	20	1 m.	100	10,000	100	10,000	50	100,000	tr.20	1,000	20	Aborted Nov.9/56 at 5 months.
3.	F. 4 y.	March 12/57	10,000	50	10,000	20	10,000	50	x	x	x	x	x	x	Sold in February.
4.	F. 5 y.	April 9/57	200	-	100	-	100	tr.1/10	100	-	200	-	100	-	Calved May 17 (see# 69)
5.	F. 4 y.	Dec. 29/56	10,000	50	10,000	50	100,000	100	100,000	50	10,000	20	100,000	50	Aborted Nov.27/56 at 8 months.
6.	F. 7 y.	March 1/57	-	-	-	-	-	-	-	-	-	-	-	-	Calved March 10/57
7.	F. 7 y.	March 1/57	1,000	-	400	-	400	10	200	-	100	-	200	-	Calved " 8/57 (see #54)
8.	F. 7y.	Apr. 9/57	100,000	20	10,000	20	10,000	20	800	5	800	tr.10	200	tr.5	" Apr.23/57(see twin #67,68) G. pig #A.L. Nov.26/56.
9.	F. 7 y.	-	100,000	20	10,000	20	1,000	50	10,000	20	1,000	-	800	tr. 5	Bred 6 times after Feb.16/56. Not in calf. G. pig #A.L.Jan/57.
10.	F. 7 y.	Jan. 1/57	--	-	-	-	-	-	-	-	-	-	x	x	?
11.	F. 7 y.	?	10,000	10	1,000	tr.10	800	tr.10	400	-	200	-	x	x	Calved March 8/57
12.	F. 8 y.	Mar. 21/57.	1 m	50	1 m	50	100,000	100	100,000	20	x	x	x	x	Sold Mar.11/57 (not in calf)
13.	F. 4 y.	Mar.30/57	10,000	20	10,000	tr.20	1,000	50	1,000	tr 10	1,000	-	400	10	Calved April 1(see #57)
14.	F. 8 y.	Apr.5/57	10,000	20	1 m	20	100,000	100	10,000	tr 5	1,000	-	1,000	tr 5	Calved Apr.12 (see #58)
15.	F. 5 y.	April 15/57	1,000	20	10,000	20	1,000	20	10,000	tr 5	1,000	tr 5	x	x	Killed May 3/57 (Mummified fostus)
16.	F. 6 y.	--	-	-	-	--	--	-	-	-	-	-	-	-	?
17.	F. 6 y.	-	1,000	-	400	-	200	-	100	-	x	x	x	x	Sold, poor milker not in calf.
18.	F. 9 y.	-	10,000	100	10,000	50	10,000	50	1,000	50	1,000	10	1,000	tr.5	Bought 1952, Hemoglobinuria, Oct.28/57, bred repeatedly in 1956, not in calf. Positive culture and G. pig Nov.26/56.
19.	F. 7 y.	?	1,000	tr.10	1m	50	1 m.	50	10,000	-	10,000	-	10,000	tr.5	Bred 3 times,fall 1956.
20.	F. 6 y.	Nov. 17/56	400	20	1m	50	100,000	100	1,000	10	x	x	x	x	Calved Nov.23/56 (see #52). Sold, Mastitis.
21.	F. 6 y.	Nov. 9/56	-	-	--	-	-	-	-	-	-	-	-	-	Calved Nov.9/56(see #47)
22.	F. 6 y.	-	100	-	-	-	100	-	100	-	100	-	100	-	Calved Oct.31/56
23.	F.13 y.	-	-	-	-	-	-	-	-	-	x	x	x	x	Calved Apr10/56, endometritis, not in calf since.Sold.
24.	F. 5 y.	Mar.30/57	10	-	100	-	10	-	10	-	-	-	-	-	Calved Mar.16/57(see #55)
25.	F. 5 y.	Mar.15/57	1,000	tr.11	400	-	200	tr.10	10	-	-	-	-	-	Calved Mar.24/57 (see#56)
26.	F. 9 y.	Apr. 9/57	1,000	20	1,000	20	800	a.c.	200	a.c.	100	a.c.	200	a.c.	Calved Apr.23/57 (see#66)
27.	F. 8 m.		1 m	100	10,000	50	1,000	20	1,000	5	1,000	-	800	tr.5	Calf out of cow 18, born Mar.17/56
48.	F.18y.Mare		1 m	100	x	x	1 m	25	1,000	10	10,000	20	800	10	Brucella positive
49.	Gelding M. 21 y.		-	-	x	x	-	-	-	-	-	-	-	-	Brucella positive, scar of fistulous withers.
50.	F. 6y.Dog.		-	-	x	x	x	x	x	x	x	x			
51.	M. 3 y.		-	-											Brother's bull used for breeding.

* All twenty nine animals (28-47, 53-54, 58-65) up to two years and a half of age, were negative throughout the experiment and are not included in the table; six of these were pregnant.
Eight young calves (52, 55-57, 66-69) that showed some reactions are the subject of table II.

tospirosis. The cow herself had shown no clinical symptoms but rectal examination revealed a marked pyometritis. On Nov. 14, the serological titres were 1:100,000 (a.l.,) and 1:20 (c.f.) and rose to 1:1,000,000 (a.l.,) and 1:100 (c.f.) during the next month. No. 2 was bred naturally on February 5, 1957, and is in calf.

A third animal, No. 5, aborted an 8-month foetus on Nov. 27, 1956. The serum titres of this animal were 1:10,000 (a.l.,) and 1:50 (c.f.,) one month before abortion occurred, then rose to 1:100,000 (a.l.,) and 1:100 (c.f.,) during the following month. No. 5 was bred artificially Feb. 2, 1957, and is in calf.

A fourth animal, No. 15, was found to be carrying a mummified foetus diagnosed by rectal examination on April 5 and confirmed at post-mortem on May 3, 1957.

Thirteen other adult cows showed serological titres above 1:1000 some as high as 1:1,000,000. They were for the most part in the fifth or sixth month of pregnancy at the time of our first visit, with the exception of one animal which was at the ninth month gestation. None of these cows aborted or showed other clinical evidence of leptospiral infection. This illustrates the frequency with which subclinical infection may occur in a leptospirosis outbreak, a point which, as mentioned earlier, should be remembered when interpreting the results of serological tests. Sterility was observed in five cows, Nos. 9, 12, 17, 18, 19, that showed a serological reaction. Otherwise this herd appeared entirely normal.

(See Table I)

Isolation of the causative agent by guinea pig inoculation of milk and urine was attempted on Nov. 26, 1956, Jan. 8, April 26 and May 3, 1957.

The results were as follows:

Nov. 26 Urine and milk from Nos. 2, 3, 5, 8, 12 and 18. Blood from Nos. 18 and her calf No. 27.

Culture *L. pomona* obtained from guinea pig inoculated with urine from No. 18. Sera of the guinea pigs inoculated with urine of No. 8 and 18 reacted in both agglutination-lysis and complement-fixation tests. Others negative.

Jan. 8 Urine from cows Nos. 2, 5, 9, and 18.

Guinea pig inoculated with urine from No. 9 developed a serological reaction. Others negative.

Jan. 11 Urine from cows Nos. 3, 8, 11, 12, 14, 15, 19 and 20, calf No. 27, mare No. 48.

All guinea pigs negative.

April 26 Urine from Nos. 2, 5, 9, 11, 15 and 18.

All guinea pigs negative.

May 3 Kidney and uterine exudate from Nos. 11 and 15.

All guinea pigs negative.

Histological examination of kidney specimens taken from these last two cattle, however, revealed a chronic glomerulonephritis, periglomerular and interstitial lymphocytic infiltration and fibrotic thickening of the arterial walls. The collecting tubules contained hyaline casts.

It seems noteworthy that serum from mare No. 48 produced agglutination-lysis of *L. pomona* at a dilution 1:1,000,000 and complement-fixation at 1:100, although no clinical manifestation of leptospirosis was observed, nor did guinea pigs inoculated with urine develop leptospiral antibodies.

Table II gives results of serological tests on the sera of eight calves that reacted when tested shortly after birth. The low grade and transient serological reactions observed were interpreted as due to the passive transmission of antibodies through colostrum or milk, since two calves, Nos. 54 and 58 bled before suckling, showed no antibodies.

(See Table II)

HERD NO. 5

This herd consisted of 1 bull, 16 cows, 5 heifers, 9 calves and 1 steer. Little buying and selling of animals has been done, the last purchase being one year

TABLE II

Results of serological tests on new-born calves the progeny of leptospirosis-reacting cows in Herd No. 3.

Number of Calf	Dam	Date of Birth	Serological Titres*											
			Nov. 26/56		Dec. 13/56		Jan. 22/57		Feb. 26/57		Apr. 12/57		May 28/57	
			A.L.	C.F.	A.L.	C.F.	A.L.	C.F.	A.L.	CF.	A.L.	C.F.	A.L.	C.F.
52	No. 20	Nov. 23/56	1,000	50	—	10	—	5	—	—				
54	No. 7	Mar. 8/57	1st bl. March 8/57 (A.L. = −)								—	—		
55	No. 24	Mar. 16/57	" " " 17/57 (A.L. = 400)								100	—	10	—
56	No. 25	Mar. 24/57	" " " 25/57 (A.L. = tr 10)								—	—	—	—
57	No. 13	Apr. 1/57	" " April 4/57 (A. L. = 100)								200	—	—	
58	No. 12	Apr. 12/57	" " April 12/57 (A.L. = −)								—	—		
66	No. 26	Apr. 23/57	" " " 26/57 (A.L. = 100)										—	—
67	No. 8	Apr. 23/57	" " " " (A.L. = 200)										10	—
68	"	"	" " " " (A.L. = 400)										—	
69	No. 4	May 17/57	" " May 17/57 (A.L. = 100)										10	—

*Agglutinin-lysis titres of first bleeding are given in brackets in centre column.

earlier. All cattle had been vaccinated with *Br. abortus* strain 19 and all adult animals, except cow No. 10, were negative to brucellosis tests. The breeding has been natural, the bull being pastured with cows in spring and summer. This explains the lack of breeding records.

Cow No. 6 was thought to have aborted in September 1956. At that time it was febrile, inappetant, showed changes in milk and haemoglobinuria; the milk became normal in appearance within two days and the urine after five or six days. The level of lactation which had been markedly reduced also returned gradually to normal. A blood sample from this animal collected on our first visit No. 19, had an agglutination-lysis titre of 1:1,000,000 and a complement-fixation titre of 1:50. The results of the serological test on all animals reacting in subsequent herd tests are given in Table III. Six animals reacted in various degree to *L. pomona* and two, Nos. 10 and 11 to *L. sejroe* in titres up to 10,000. Nevertheless, no subsequent clinical symptoms have been reported in this herd. On Jan. 15, 1957, attempts to isolate the organisms by inoculation of guinea-pigs with urine from cows Nos. 6 and 11 and calf No. 23 proved negative.

This herd No. 5 is an example of one in which the infection has not apparently spread but has remained localised in one or a few animals.

(See Table III)

HERD NO. 7.

This herd was located on a level, well-drained, 100-acre farm crossed by a river to which the cattle have access. It was composed of 1 bull, 31 cows, 3 heifers, 3 calves; 2 horses, 1 sow and 1 dog were also on the premises. A new bull has been bought each year but no females have been bought for three or four years. Breeding has been natural, the bull being pastured with the cows during summer with intention of having all females calve in spring. All cattle have been vaccinated with *Br. abortus* strain 19 although the herd was not listed. No abnormalities were reported by the owner apart from occasional retained placenta and a single abortion four or five years ago.

In Sept. 1956, one cow, No. 26, showed symptoms suggestive of leptospirosis. Changes in milk were first noticed and later haemoglobinuria. Two days after treatment with antibiotics by the veterinary practitioner, the milk reverted to normal and the urine became clear in four or five days. A blood sample taken Nov. 16, 1956 had a *L. pomona* agglutination titre of 1:10,000. Two other animals in the herd gave significant serological reactions but no other clinical manifestations of the disease were observed thereafter (Table IV). Two horses, Nos. 39, 40, also showed traces of reactivity in agglutination-lysis tests.

Attempts to isolate the organism from the urine of cow No. 26 by guinea-pig inoculation on Jan. 15, 1957, gave negative results as might be anticipated from the interval that had elapsed since the appearance of symptoms.

(See Table IV)

HERD NO. 10.

This herd was located on a 150 acre farm with no bush or creek. It was composed of 25 cows, 5 heifers and 3 calves. Breeding was done by artificial insemination except for a number of heifers bred naturally at pasture. The herd was almost self-contained, the only recent acquisition being one cow, No. 30, bought last spring. The cattle have been brucellosis-listed for 13 years.

In 1954, considerable breeding difficulty had been experienced but no abortion was noticed. Apart from two cows, Nos. 4 and 17, which had to be bred several times, no further trouble was reported until late in 1956 when two abortions occurred. The first cow, No. 10, aborted on Dec. 10 at eight months gestation. The placenta was retained and removed manually. The calf was alive but so small and weak, that it was destroyed. A blood sample taken Dec. 17 was negative both for leptospirosis

TABLE III

Summary of results of serological and clinical investigations in herd No. 5

Number of Animal	Age	Sex	Serological Titres								Remarks
			Nov. 19/56		Jan. 15/57		March 4/57		May 10/57		
			A.L.	C.F.	A.L.	C.F.	A.L.	C.F.	A.L.	C.F.	
1	9y.	F	10	—	—	—	—	—	—		Calved Feb. 15/57
3	6y.	F	1,000	25							Sold
4	4y.	F	10	—	—	—	—	—	—	—	Calved Feb. 28/57
6	7y.	F	1 m	50	1m	100	1,000	tr 10			Haemoglobinuria Sept. 56, febrile abortion?
22	8m.	F	—	—	10,000	10	200	—			
23	6m.	F	10,000	tr 10	1,000	—	800	tr 10			Calf from No. 15 (neg.)
31	2y.	F			10,000	—	400	—			
10	5y.	F	1,000	—	10,000	tr 5	1,000	—			Positive Brucella agglut.
11*	7y.	F	1,000	25	800	50	1,000	20	1,000	tr 10	

*Titres for Nos. 10 and 11 indicate *L. sejroe* reactions given by all bleedings from these two animals. No. 11 gave a slight cross reaction with *L. pomona*. All other titres in this table are reactions with *L. pomona*.

tr = trace of reaction in complement-fixation test.

TABLE IV

Summary of results of serological and clinical investigations in herd No. 2

Number of Animal	Age	Sex	Serological Titres								Remarks
			Dec. 3/56		Jan. 21/57		March 6/57		May 10/57		
			A.L.	C.F.	A.L.	C.F.	A.L.	C.F.	A.L.	C.F.	
16	4y.	F	10	—	—	—	—	—	—	—	
19	4y.	F	800	—							
22	7y.	F	—	—	—	—	100	—	—	—	
24	7y.	F	10	—	—	—	—	—	—	—	
26	8y.	F	1m	100	1,000	25	1,000	10	1,000	tr 10	Clinical leptospirosis Sept. /56 (Nov. 16, A.L. = 10,000)
29	7y.	F	10,000	tr 5	200	tr 10	400	—	400	—	
35	3y.	F	10,000	tr 5	200	tr 10	400	—	400	—	
36	6m.	M	—	—	—	—	—	—	—	—	Calf from No. 35
39	8y.	Gelding	200	—	10	—					
40	21y.	Gelding	10	—	—	—					

tr = trace of complement-fixation

TABLE V

Summary of results of serological and clinical investigations in herd No. 12

Number of Animal	Age	Sex	Expected month of calving	Serological Titres				Remarks
				Jan. 28/57		March 14/57		
				A.L.	C.F.	A.L.	C.F.	
4	5y.	F	—	10,000	20	1,000	20	Aborted Nov. 26/56 at 2 mos.
9	7y.	F	June /57	10,000	10	1,000	10	
17	8m.			—	—			Calf from No. 9
34	2y.	F	June/57	1,000	10	400	10	Bought Feb./55
35	4y.	F	Apr./57	1,000	10	400	tr 10	
43	2y.	F	July/57	200	—			
31 Horse	6y.	M		1,000	—	200	tr 10	

TABLE VI

Summary of results of serological and clinical investigations in herd No. 15

Number of Animal	Age	Sex	Expected date for calving	Serological Titres				Remarks
				March 21/57				
				L. pomona		*L. sejroe*		
				A.L.	C.F.	A.L.	C.F.	
1	9y.	F	Dec. /57	—	—	800	—	
2	5y.	F	"	—	—	200	tr	
3	7y.	F	Fr.	—	—	100	—	Not bred (April 11/57)
4	6y.	F	Aug./57	—	—	1000	tr	

5	8y.	F	Fr.	—	—	100	—	Not bred
6	3y.	F	July/57	—	—	400	—	
7	5y.	F	May/57	—	—	200	—	
8	5y.	F	Apr./57	—	—	200	—	
9	11y.	F	—	—	—	100	—	Calved in fall—bred March 20 & April 2
10	5y.	F	May/57	—	—	100	—	
11	2y.	M		—	—	200	—	On loan since March 13/57
12	4m.	M		—	—	100	—	
13	4m.	M		—	—	100	—	Calf of No. 3
14	5m.	F		—	—	—	—	" " " 9
15	5m.	F		—	—	—	—	" " " 6
16	4m.	F		—	—	100	—	
17	4m.	M		—	—	—	—	
18	2y.	F	Jan./57	—	—	—	—	
19	1y.	M		—	—	100	—	
20	2y.	F		—	—	800	tr	
21 Horse	20y.	F		—	—	—	—	On farm since 1943
22 Horse	21y.	F		800	—	—	—	On farm since 1943 Poor condition, oedema abdominal wall

tr = trace of reaction in complement fixation tests.

and brucellosis. On Dec. 15, a second cow, No. 11, aborted at six months gestation. A blood sample collected Dec. 17 had an agglutination-lysis titre of 1:1,000 and a complement-fixation titre of 1:40 with *L. pomona* antigens. Therefore, only one of the two abortions could seemingly be attributed to leptospirosis, the cause of the other, was not ascertained.

Every animal in the herd was blood tested for leptospirosis on Jan. 10, Feb. 28 and May 10, 1957. With the exception of cow No. 11 they were negative at all tests. The blood titres of this cow, which were 1:1,000 (a.l.) and 1:50 (c.f.,) on Dec. 17, 1956, had risen to 1:1,000,000 (a.l.,) and 1:100 (c.f.,) by Jan. 10, 1957, but had fallen to 1:800 and 1:400 (a.l.,) by Feb. 28 and May 10 respectively.

Guinea pigs were inoculated with urine from cow No. 11 on Jan. 15, 1957, but the causal organism was not isolated and the inoculated guinea-pigs did not react serologically.

In herd No. 10 as in Nos. 5 and 7, the clinical infection apparently remained localized in one single animal despite the absence of any alteration in the routine management of the herd.

HERD NO. 12.

This herd was located on a 175-acre farm crossed by a river to which cattle have access. Drinking water was also available from a creek and an old quarry containing water all year round. There were 16 cows, 2 heifers, 17 calves, 5 steers and 3 horses on the premises. The farm was also infested with rats. Beef calves have been bought each year, but only cows Nos. 9 and 34 have been purchased recently. Some of the cows had been bred by artificial insemination, others by a neighbour's bull. All animals had been vaccinated with *Br. abortus* strain 19.

In 1955, three cows had presented difficulty in natural breeding, but all three became pregnant to first service after changing to artificial insemination. In 1956, the only difficult breeder was No. 11 that had a retained placenta after calving in August.

In the fall of 1956 a 5-year old cow, which had calved in June began to show a drop in lactation and progressive weakness. A few days afterwards, changes were apparent in milk and the presence of blood was seen in both milk and urine. The animal died a week after the onset of symptoms, about two or three days after blood appeared in urine. This was one of the three cows in which breeding difficulty had been found in 1955. Her 1956 calf never throve and died a few days after birth. Another cow, No. 4, aborted a 2-month foetus in November 1956.

The herd was bled and tested on Jan. 28 and March 14, 1957. The results are given in Table V. Only five cattle and a horse showed *L. pomona* reactions. Cow No. 4 which aborted was among the reactors. Guinea-pig inoculation of the urine of cows Nos. 4 and 9 were made Feb. 9, but the causal organism was not isolated.

(See Table V)

HERD NO. 15.

The hygiene and management of this farm left much to be desired. The herd was composed of 20 scrub beef animals: 2 bulls, 10 cows, 2 heifers and 6 calves. One cow died in Dec. 1956, six days after the onset of acute symptoms resembling leptospirosis, severe diarrhea, icterus, haemoglobinuria, sudden drop in milk yield, fever and inappetance. The veterinary practitioner reported that postmortem examination revealed marked icterus, anemia and widespread haemorrhage. All other cows were in good health and there had been no breeding problems.

The entire herd of 20 cattle along with two horses were bled March 21, 1957. All adult cattle and a few of the young animals were found to react in the agglutination-lysis tests with *L. sejroe* antigen with titres up 1:1000 (Table VI). The only *L. pomona* reaction obtained was given by one of the two horses, No. 22, which was in poor

condition and showed oedema of the ventral abdominal wall.

(See Table VI)

II. Questionable Herds

In this group are included a total of 113 cattle from four herds in which a few cattle showed leptospiral serological reactions of varying degree. The herds were classified as "questionable" because, (a) the serological titres were of a low order, or (b) the animals with high titres in the particular herd did not or had not shown reproductive or other manifestations associated with leptospirosis. In two of the four herds there were concurrent brucella reactions; in another herd there was serological evidence of brucellosis and vibriosis as well as leptospirosis. In the remaining herd, a poor breeder accounted for part of the reproductive difficulties.

Studies of such questionable herds re-emphasizes the complexity of our reproductive problems in cattle, and illustrates the dangers inherent in a hasty diagnosis based on a few superficial clinical or laboratory examinations. Therefrom it might be concluded that a particular infectious agent was the cause of all of the difficulties observed, whereas their real basis could be ascertained only through a more detailed study not only of the common infectious conditions but also of nutritional, physiological, and possibly genetic factors. A brief summary of the picture in these four herds is given below.

HERD NO. 4.

This was a self-contained herd of 34 cattle, 1 bull, 16 cows, 9 heifers and 8 calves, which had experienced a breeding problem for the past five years, manifested as prolonged oestral periods of up to 3 months and repeated breedings. Thirteen females and the present bull are descendants from one cow. Six other female descendants have been sold for beef because of breeding difficulties. The bull presently being used had never impregnated a cow and examination of his semen showed only a few degenerated spermatozoa. The 16 nonpregnant females, that showed breeding difficulties had been bred as follows:

Nos. 4, 5, 8, 11, 12, 13, 14, 23 bred repeatedly to herd bull
Nos. 1, 6, 16 bred repeatedly to herd bull then once A.I.
Nos. 2, 3, 15 bred repeatedly to herd bull then twice A.I.
No. 21 bred repeatedly to herd bull then three times A.I.
No. 22 bred repeatedly to herd bull then five times A.I.

Sixteen adult cattle, the bull and three calves were bled and tested for leptospirosis and brucellosis. All were brucellosis negative. Sera of cows Nos. 3, 6 and 16 showed agglutination-lysis reactions with *L. pomona* antigen in dilutions ranging from 1:10 to 1:400. From reactions of such low order, it would not seem justifiable to conclude that the breeding difficulties in these animals are attributable to leptospirosis.

Samples of cervical mucus taken from the 16 females listed above were all negative to the *Vibrio foetus* mucus agglutination test.

Four of these cows were sold. Since purchasing a new bull all but two of the remainder have conceived.

HERD NO. 9.

This self-contained herd had experienced considerable breeding difficulty in 1956. Only one abortion had been noticed, which was in cow No. 8 at 40 to 50 days pregnancy. However, of 19 cows which have been bred, eight were not in calf even after repeated breeding. A new bull was normally used each year, but in 1956 a three-year old animal had been kept for a second season, no breeding difficulties having occurred in 1955. This herd is not brucellosis listed but all animals had been vaccinated with *Br. abortus* strain 19 and were found to be negative in agglutination tests.

Twenty-one cattle were bled and tested for leptospirosis. Five cows reacted with *L. pomona* antigen in titres of 1:400, 1:10,000, 1:200, 1:100 and 1:100 respectively. Two of these animals, Nos. 3 and 9, and one additional cow reacted to *L. sejroe* in titres of 1:400, 1:400 and 1:200 respectively. Except for one cow, *L. pomona* a.l. titre 1:100, none of these reactions were in animals that had experienced abortion or repeated breedings.

Vaginal mucus from six females was tested for the presence of *V. foetus* agglutinins but all samples were negative.

HERD NO. 16.

The present owner bought this farm two years ago and has purchased pedigree stock from various sources in building up a Shorthorn herd. All breeding has been natural. The herd is made up of 31 cows, 21 calves and 3 bulls. During the past 18 months there has been marked incidence of repeated breedings among cows of all ages although conception has usually occurred eventually. Repeat breeding and infertility were reported in five cows. One cow gave birth to a full term dead calf. Another aborted at seventh months gestation.

When the 55 animals were bled, three cows, and the bull showed a *L. sejroe* reaction in a titre of 1:1,000, and fourteen cows reacted to the same antigen in titres varying from 1:100 to 1:800. None of these leptospira serological reactions were given by the animals with the reproductive difficulties mentioned above.

Positive *Brucella abortus* agglutination reactions were given by four cows; two others gave a questionable reaction to same antigen. Brucellosis vaccination had been practised in this herd, but in view of the fact that these animals were all adults, would be officially regarded as infected since it is impossible to differentiate between a serological reaction due to vaccination or infection. Of 13 animals tested for vibriosis, two gave positive mucus agglutination tests for *Vibrio foetus*, a third showed a suspicious reaction to the same test. No. 21 which aborted, gave positive tests for brucellosis and vibriosis as well as a leptospiral reaction, which made it difficult to determine which infective agent was responsible for the difficulties reported.

HERD NO. 20.

In this herd of one bull, 17 cows, 2 heifers, 1 steer and 7 calves, cattle are bought and sold frequently. Three years ago a cow was bought which aborted a live calf after 7 months gestation. The cow was sold but her calf was raised. Four more cows aborted in 1956 and six in 1957. Nine of the cows in the herd when tested gave positive agglutination test for brucellosis and two gave questionable reactions. Nine of these brucellosis reactors had aborted. Calfhood vaccination had not been practiced in this herd.

In the serological test for leptospirosis, one cow had an a.l. titre of 1:10,000. This animal had not aborted. Among nine others which had low a.l. titres for *L. pomona*, eight had aborted. It seems obvious that the recent difficulties which had been experienced in this herd were due to brucellosis rather than leptospirosis since the one relatively high serological reaction with *L. pomona* was given by a cow that had shown no clinical symptoms.

III. Negative Herds

A total of 18 abortions were encountered in these 10 herds and in only one or possibly two herds, Nos. 13 and 14, could the abortion be attributed to brucellosis. In herd No. 13, three cows aborted but since only one showed a positive agglutination reaction for brucellosis, it is doubtful whether brucellosis was the only cause of abortion in this herd. All blood samples collected from 345 individual animals in these ten herds, including one goat and eight horses, were negative when tested by the agglutination-lysis and complement-

TABLE VII

Reproductive difficulties of cattle in 10 leptospirosis-negative herds.

Herd Number	Reproduction Difficulty				Number of cattle in herd		Number of samples tested**	Brucellosis Results
	Abortion		Infertility	Repeated breeding*	Young	Adult		
	No.	Time						
1	3	5m.	—	—	13	21	19	—
2	2	4m.	—	6	18	39	57	—
	1	6m.						
6	1	4m.	—	—	13	21	32	—
8	1	9m.	3	4	15	20	35	—
	1	5m.						
11	1	5m.	—	—	20	26	46	—
13	1	5m.	—	—	12	16	28	Pos. (1)
	1	6m.						
	1	7m.						
14	1	7m.	—	3	35	42	42	Pos. (8) Ques. (3)
17 & 18	2	5m.	4	4	23	50	32	Ques. (2)
21	1	4m.	2	3	17	47	46	—
	1	9m.						

*Repeated breeding = four breedings without conception.

**1 goat and 2 horses also tested in herd 2, and 2 horses in each of herds Nos. 8, 11, and 13.

fixation methods with the six leptospiral antigens listed above.

In brief, therefore, 16 abortions, nine cases of infertility and 20 of repeated unsuccessful breeding were encountered among 318 brucellosis and leptospirosis-negative cattle. Evidently the breeding problems in these herds were due to causes other than these two infective agents. Such findings clearly illustrate the care which should be exercised not to confuse such cases with leptospirosis especially in animals that have experienced leptospiral infection some years prior to the observed abortion, and might still have residual antibodies of low agglutination-lysis titre (1:100 to 1:1,000).

DISCUSSION

This survey demonstrates that leptospirosis may play an important part in the reproductive problems in certain herds of cattle in Canada. However, if the ten leptospirosis — an brucellosis-negative herds with 18 abortions, nine cases of sterility and 20 of repeated unsuccessful breedings are considered, it will be realized that attention should be focused not only on brucellosis and leptospirosis. This so-called "fashionable disease", leptospirosis, fails to account for a great part of the reproductive difficulties still remaining in herds where brucellosis is not present.

The problem becomes more complex in herds where the presence of mixed infections such as vibriosis, brucellosis or leptospirosis are detected by serological methods. In such herds, a positive leptospiral test in an individual animal might be misinterpreted by the veterinary practitioner if not considered in relation to clinical findings. To minimise the possibility of confusion, the laboratory in reporting its results on

leptospirosis serological tests, should indicate the type of test used and the titre obtained. This enables the clinician to evaluate the degree of reaction in relation to his personal observations in the individual animals and in the herd. A definitely rising titre in successive samples should constitute strong evidence of active leptospiral infection. If the titre remains unchanged there is more likelihood that residual antibody from a previous leptospiral infection is responsible for the reaction.

A thorough knowledge of the symptomatology of the disease is often sufficient to lead the clinician to suspect leptospirosis. The most striking sign of leptospirosis observed in the present study was undoubtedly that of abortion preceded by an history of depression, abnormal milk which became yellowish or bloody and thick in appearance. This was accompanied or followed shortly afterwards by haemoglobinuria. However, abortion was not always preceded by the general symptomatology that accompanies the acute infections, it often occurred abruptly without any previous manifestation in that individual animal. If leptospirosis is at times dramatic in its appearance, it can also be very insidious. In our study, many of the animals with strong serological reactions, titres of 1:10,000 to 1:1,000,000, never showed any clinical symptoms of the disease. This is well exemplified in herd No. 3 in which one cow with a titre of 1:100,000 and which was shedding leptospira in the urine, remained entirely normal throughout the period of observation. In other serologically-reactive animals of the same herd the only observable abnormalities were repeated breedings without conception.

Undoubtedly the period of pregnancy and the state of general health in the animals are important factors in determining the severity of an outbreak. Most observers mention leptospirosis as an infection where abortion takes place in the late months of pregnancy. This is certainly the most frequent occurrence, but in the herds reviewed here, animals have aborted at the fifth and even at the second month of gestation. Other animals in the same herds and at the same period of gestation did not abort even though infected as revealed by a high serologicacl reaction with *L. pomona* and or by guinea-pig isolation of leptospira from the urine. Furthermore, animals at the ninth month of gestation, such as cow No. 21 in herd No. 3, may not contract the infection even though in contact with infected animals.

The form of an epizootic of leptospirosis may vary from herd to herd. In certain herds such as No. 3 almost every adult animal may contract the infection whereas the young stock remain free. Yet, in other herds, such as No. 10, only one animal may become infected as shown by serological and clinical methods. In this connection, Stoenner (6) stated that during the early phase of an epizootic, most infections are inapparent, that it is only in later months or years that the dramatic picture of severe leptospirosis becomes apparent. This may be what happened in herd No. 3, where in the summer of 1954 a cow died shortly after parturition, but it was not until October 1956 that a really dramatic outbreak of leptospirosis was recognised in this herd. If such a sequence of events was seen in the majority of the herds with infection limited at first to one or two animals, then effective preventive and control measures could be instituted at this early phase of the herd infection. Thereby the most destructive form of the infection might be largely prevented.

In evaluations of the significance of laboratory reports for leptospirosis, there appears to have been in some instances a tendency to attach too much importance to the low-grade serological reaction (6, 7). Where the possibility of very early infections can be definitely excluded, such reactions should be interpreted as the presence of residual antibodies in a recovered animal, one that has experienced the infection many

months or even years previously. In the present study every animal that showed clinical signs of acute infection had an agglutination-lysis titre of at least 1:10,000 and a complement-fixation titre of 1:50 or above. Conversely, however, many animals with such titres suffered only inapparent infection.

Among the diagnostic and serological procedures both the agglutination-lysis and complement-fixation tests are reliable. Their use is, however, not practicable in some smaller laboratories by reason of technical difficulties in their application. Such difficulties would be reduced with the introduction of simple plate and tube agglutination tests performed with killed antigen. Our results with such tests are encouraging and will be published in a later paper.

SUMMARY

A total of 20 herds with reproductive problems have been studied, in six of which leptospirosis was shown to be the etiological agent. In four other herds, serological reactions with leptospiral antigens were obtained but could not be correlated with the difficulties encountered in the individual animals.

The course of the leptospiral infection varied considerably from herd to herd. In one herd the infection, as revealed by serological, cultural and clinical methods, spread to almost all of the adult cattle, even to one horse. In other herds it remained limited to one single animal.

Serological reactions and cultural isolation indicated that in most of these infected herds the *L. pomona* serotype was involved. However, reactions with *L. sejroe* were obtained in a few of the herds. ....

RESUME

Des problèmes de reproduction ont été étudiés dans vingt troupeaux et la leptospirose a été reconnue comme agent étiologique dans six d'entre eux. Des réactions sérologiques avec des antigènes leptospiriens ont été obtenues dans quatre autres troupeaux mais sans corrélation avec les difficultés rencontrées dans les cas individuels.

Le cours de l'infection leptospirienne varie considérablement d'un troupeau à l'autre. Dans un troupeau, l'infection, tel qué démontré par les méthodes sérologiques culturales et cliniques, s'est propagée à presque toutes les vaches adultes, atteignant même un cheval. Dans d'autres troupeaux, le 'trouble s'est limité à un seul animal.

Les réactions sérologiques et les isolements culturaux indiquent que, dans presques tous les troupeaux infectés, *L. pomona* est le sérotype incrimine. Cependant, des réactions avec *L. sejroe* ont été obtenues dans un petit nombre de troupeaux.

ACKNOWLEDGMENT

The authors wish to thank Miss Eliette Patry and Mr. W. A. Boyd for technical assistance in the experimental work. Appreciation is also expressed to Doctors D. A. Docksteader, A. A. Hanna, W. P. Holden, and T. Maxwell for their assistance in locating the infected herds and to Doctors D. B. Meads and R. J. Julian of Ontario Veterinary College, Extension Service, Kemptville Agricultural School, for invaluable help in collecting samples from many of the herds.

REFERENCES

1. MOORE, T. and RICE, C. E. Serological investigations of leptospirosis in Canada I. Introduction and preliminary complement-fixation studies of cattle sera with commercially-prepared **Leptospira pomona** antigen. Can. J. Comp. Med., **20**: 362-373, 1956.

2. BOULANGER, P. and SMITH, A. N. Serological investigations of leptospirosis in Canada. II. Preliminary agglutination-lysis studies of cattle sera with **Leptospira pomona** and **Leptospira canicola** antigens. Can. J. Comp. Med., **21**: 4-11, 1957.

3. SCHUFFNER, W. and MOCHTAR, A. Versuche zur Aufteilung von Leptospirenstammen mit einleitenden Bermerkungen uber den Verlauf von Agglutination und Lysis. Zentrable. f. Bakt., Abt. 1. Orig., **101**: 405-413, 1927.

4. WOLFF, W. J. Laboratory diagnosis of leptrospirosis. F. A. O. Agricultural Studies, No. **25**: 127-138, 1953.

5. RANDALL, R., WETMORE, P. W., and WIRNER, A. R. Sonic-vibrated leptospira as antigen in the complement-fixation test for the diagnosis of leptospirosis. J. Lab. and Clin. Med., **34**: 1411-1415, 1949.

6. STOENNER, H. G., CREWS, F. W., CROUSE, A. E., TASCHNER, L. E., JOHNSON, C. E. and WOKLEB, J. The epizootiology of bovine leptospirosis in Washington. J. A. V. M. Ass. **129**: 251-259, 1956.

7. Von WENDT, J. A study of leptospirosis in Sweden. J. Nord. Vet. Med. **8**: 711-714, 1956.