

Leptospirosis in Wild Animals in Eastern Canada with Particular Attention to the Disease in Rats

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While rats have been known for many years to be important carriers of leptospires, only two limited studies on the occurrence in Canada of leptospira-infected rats have been reported. The first study was carried out in Toronto by Cameron and Irwin (3) in 1926 and 1927. A microorganism identified as *Leptospira icterohemorrhagiae* on the basis of its appearance and its pathogenicity for guinea pigs, was obtained from one of 78 rats tested. Dark-field examination of macerated tissue, histological study of stained sections, and appearance of symptoms of leptospirosis in inoculated guinea pigs enabled the authors to designate an additional 28 rats as infected, giving an incidence of 37%. The second study was carried out by Humphreys, Campbell and Smith (4) on rats collected in the coastal region of British Columbia during a three year period beginning in 1948. They found that 4.8% of 313 rats were carriers of leptospirae. Four of the strains were identified as *L. ballum*. None of the rat sera reacted with *L. icterohemorrhagiae* in the macroscopic tube agglutination test.

Only five human cases of leptospirosis in Canada have been described in the literature. Three of these were caused by *L. icterohemorrhagiae*, one was due to *L. canicola*, and one was attributed to *L. sejroe* (2). To this number may be added the two cases in Toronto mentioned by Cameron and Irwin as having recovered from Weil's disease. This number probably would increase rapidly if this disease were considered more frequently when diagnoses are made and if diagnostic services were more readily available. The use of plate antigens for screen testing should enable such a service to be provided without adding unduly to the work load.

Just as many more data are required to evaluate the importance of human leptospirosis in Canada, so too is needed much more information on the incidence of leptospires in wild animals. The present study was begun in 1958 for the purpose of increasing our limited knowledge of the disease in nature in Canada.

Method

Most of the animals tested were collected by live-trapping. The trap which we have found most suitable for collecting rats is a collapsible type measuring 6 x 6 x 16 inches and is sold by the National Live Traps Company, Tomahawk, Wisconsin. Havahart traps 11 x 11 x 36 inches were used to collect skunks and ground hogs. A small number of animals was obtained by shooting. In each study area, attempts were made to procure specimens from several widely separated points. Sites for trapping rats included garbage dumps, abattoirs, piggeries, chicken ranches, stables, feed mills, fish processing plants, restaurants and private homes.

Trapped specimens were anesthetized and exsanguinated. A few drops of blood were inoculated into tubes containing 5 to 7 cc. of Korthof's medium and the remainder was allowed to clot for serologic studies. Each animal was dissected and a small slice of kidney was placed in Korthof's medium. When specimens were collected outside the Ottawa area, the second kidney was frozen in a dry ice chest. Inoculated media, blood serum and frozen tissues were shipped to the Laboratory of Hygiene for further study. Tissues of specimens collected in the Ottawa area were tested without prior freezing.

Inoculated media were incubated at 29-30°C. Transfers to fresh media were made at 3 to 5 day intervals. All cultures were

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TABLE I

Incidence of leptospirosis (*L. icterohemorrhagiae*) in rats in Eastern Canada

Area (1)	Numbers of specimens examined and per cent positive			
	(2) By culture	(3) By serology of test animal	(4) By serology of specimen	(5) By either (2), (3) or (4)
Ottawa, Ont.....	79—22%	52—40%	68—10%	79—39%
Kingston, Ont.....	44—11%	34—6%	36—25%	44—30%
Brockville, Ont.....	49—8%	49—6%	28—25%	49—22%
Cornwall, Ont.....	74—3%	74—0%	46—9%	74—7%
Montreal, P.Q.....	84—11%	71—1%	47—6%	84—11%
Saint John, N.B.....	24—0%	18—0%	13—0%	24—0%
Halifax, N.S.....	72—21%	68—3%	52—40%	72—39%
Total number examined and per cent positive.....	426—12%	366—10%	280—18%	426—23%

transferred at least three times. They were examined by darkfield microscopy prior to each transfer and thereafter at weekly intervals for at least four weeks.

Sera were tested for antibodies by tube agglutination employing as antigens well-grown, 5 to 7 day-old cultures killed with 0.5% formalin. The serotypes used included *L. icterohemorrhagiae*, *L. canicola*, *L. pomona*, *L. ballum*, *L. autumnalis*, *L. gripotyphosa*, and *L. sejroe*. Reactions were recorded as negative or 1+ to 4+. The highest dilution showing at least a 3+ reaction was designated as the titre. Identification of *L. icterohemorrhagiae* cultures was made on the basis of cross-agglutination tests.

Kidneys were held at -20°C from the time they were received at the Laboratory of Hygiene until inoculated into guinea pigs. Two guinea pigs each weighing 200 to 250 gm. were injected intraperitoneally with 1 cc. of a 10% suspension of kidney tissue in saline. Four to 7 days later, 2 cc. of heart blood were withdrawn from one of these guinea pigs and passed to two additional guinea pigs and to one or two tubes of medium. Rectal temperatures of inoculated guinea pigs were taken three times weekly for three weeks post-inoculation. If elevated temperatures were found, passage of blood to other guinea pigs and to artificial medium was carried out.

Results

All of the rats collected were *Rattus norvegicus*. The areas in which collections were made are given in Table I and, in addition, the numbers of rats tested and the percentages positive for *L. icterohemorrhagiae* by culture and serologic methods. Columns

2 and 3 of this table present respectively the percentages of rats which yielded cultures or whose tissue emulsions stimulated production of leptospiral antibodies in guinea pigs. Column 4 shows the incidence of positive serologic reactions found in sera of the rats examined. The results of the three methods (culture, serology of test animal, serology of specimen) were combined to show the incidence of positivity by one or more of these methods and these figures are given in column 5.

Accordingly, the incidence of leptospirosis in rats caused by *L. icterohemorrhagiae* is about 39% in Ottawa, 30% in Kingston, 22% in Brockville, 7% in Cornwall, 11% in Montreal, and 39% in Halifax. None of the rats collected in Saint John was positive for leptospirosis by any of these methods. Inadequate sampling of the rat population is believed responsible for this negative result. The average infection rate for all areas is 23%.

TABLE II

Relationship of agglutination titres to presence of *L. icterohemorrhagiae* in rats

Agglutination titre	Number of sera	Number positive by culture	Number negative by culture
Negative*	239	30	209
1:25	8	3	5
1:100	11	4	7
1:200	15	8	7
1:400	8	3	5
1:800	4	2	2
1:1600	4	2	2
1:3200	1	0	1
Total	290	52	238

*Negative means less than 1:25

TABLE III
Results of tests for leptospirosis in mammals other than rats

Species	Number tested	Number positive	Remarks
<i>Felis domesticus</i> (domestic cat)	5	0	
<i>Lepus americanus</i> (Snowshoe hare)	2	0	
<i>Marmota monax</i> (ground hog)	27	2	1—Serum titre 1:400 <i>L. pomona</i> ; <i>L. pomona</i> culture isolated.
<i>Mephitis mephitis</i> (striped skunk)	10	2	1—Serum titre 1:25 <i>L. pomona</i> . 1—Serum titre 1:100 <i>L. pomona</i> . Culture isolated, lost; serotype not known.
<i>Microtus pennsylvanicus</i> (meadow vole)	6	0	1—Serum titre 1:200 <i>L. pomona</i> .
<i>Sciurus carolinensis</i> (gray squirrel)	7	0	
<i>Tamias striatus</i> (chipmunk)	4	0	

The total number of rat sera which was tested for agglutinins to *L. icterohemorrhagiae* was 290 as shown in column 4 of Table I. Table II gives the frequencies of the various agglutination titres of rat sera and, as well, a breakdown of these figures to show the numbers of specimens positive or negative by culture. Although the figures are too small to draw a firm conclusion, it appears that the chances of isolating a culture from seropositive rats do not decrease as the antibody titre increases.

Of the 290 rats tested, 209 (72%) were negative for leptospirosis by both serology and culture, 30 (10%) were negative by serology and positive by culture, 29 (10%) were positive by serology and negative by culture, and 22 (8%) were positive by both serology and culture.

A comparison of infectivity rates in 290 male and female, medium-to-large size rats was made to ascertain whether one sex might be more frequently infected than the other. Forty-two (31%) of the 136 males and 54 (35%) of the 154 females in this group were classified as positive. The difference between these two percentages is not significant, indicating that male and female rats are equally liable to infection with *L. icterohemorrhagiae*.

As noted previously, rectal temperatures of guinea pigs inoculated with test tissues were recorded three times a week as an aid in detecting infection. This criterion proved to be of limited value in this study inasmuch as less than one-quarter of the specimens subsequently classified as positive produced a rise in body temperatures of guinea pigs.

L. icterohemorrhagiae was the only serotype isolated from rats in this study. How-

ever, a guinea pig inoculated with tissues from a rat trapped in a feed mill in Montreal revealed an antibody titre of 1:100 against *L. pomona*. The serum of a rat trapped in a piggery in the Ottawa area had a titre of 1:3200 to *L. pomona* antigen but failed to react to the other serotypes tested. It is of interest that swine in the neighborhood of this piggery were shown to be carriers of *L. pomona* by Boulanger et al (1) in 1959.

Tests Carried Out on Other Mammals

A list of other mammals tested for leptospirosis is shown in Table III. Positive results were obtained only in ground hogs (*Marmota monax*) and in striped skunks (*Mephitis mephitis*).

Five of the six ground hogs tested during 1958 were trapped on a farm near the Ottawa city limits during the period July 3 to August 11, and from one of these five, a culture of leptospirae was obtained. The third passage of the strain in Korthof's medium resulted in sufficient growth to serve as an antigen in agglutination tests. This strain, designated as LGI, agglutinated *L. pomona* antiserum prepared in rabbits to its full titre (1:3200). Absorption tests employing LGI antiserum, also prepared in rabbits, confirmed the identity of the strain as *L. pomona*. The serum from the leptospira-positive ground hog had a titre of 1:400 with *L. pomona* antigen but failed to react with *L. ballum*, *L. canicola*, *L. icterohemorrhagiae*, *L. autumnalis*, *L. grippotyphosa* or *L. sejroe*. The serum from another of the above-mentioned five ground hogs was positive at a dilution of 1:25 against *L. pomona* but leptospirae were not isolated. The following year, 12 ground

hogs were collected on this farm and all were negative for leptospirosis. The isolation of *L. pomona* from the ground hog constitutes a new host record for this serotype.

All of the 10 striped skunks referred to in Table III were collected in 1959 and 9 of these were trapped alive on the farm from which *L. pomona* had been obtained the previous year. One skunk obtained on June 24 had a serum titre of 1:200 against *L. pomona*. Attempts to isolate leptospire from the blood and kidneys of this animal yielded negative results. A second seropositive skunk was trapped on this farm on July 14. A leptospiral culture was obtained from the blood of this specimen but not from kidney tissues. Growth was poor in the two tubes of medium inoculated initially. The organism failed to survive in subsequent transfers and was lost without being identified.

Summary

A survey of leptospirosis in wild mammals has been in progress for two years in eastern Canada. Most of the animals tested were rats (*Rattus norvegicus*). The areas surveyed included Ottawa, Kingston, Brockville and Cornwall, Ontario; Montreal, Province of Quebec; Saint John, New Brunswick; and Halifax, Nova Scotia.

The incidence of infection caused by *L. icterohemorrhagiae* in rats for all areas was 23% with two areas having infection rates of 39%. While *L. icterohemorrhagiae* was the only serotype isolated from rats, two rat sera reacted with *L. pomona* but not with other serotype antigens tested. The difference in infection rates between male and female rats was not significant. Likewise there was no significant difference between the percentages of rats classified as (a) positive by serology as well as by

culture, (b) positive by serology but negative by culture, or (c) negative by serology but positive by culture.

Of the mammals tested other than rats, two ground hogs (*Marmota monax*) and two striped skunks (*Mephitis mephitis*) had antibodies to *L. pomona*. A culture of *L. pomona* was isolated from the kidneys of one of the ground hogs. A *Leptospira* culture, serotype unknown, was isolated from the blood of one of the skunks and subsequently lost.

Abstract

A survey for leptospirosis was carried out in animals collected in Ontario, Quebec, New Brunswick and Nova Scotia. Most of the animals tested were rats (*Rattus norvegicus*). The incidence of infection due to *L. icterohemorrhagiae* in rats was 23% for all areas. While *L. icterohemorrhagiae* was the only serotype isolated from these rodents, agglutinins in two rat sera were demonstrated to *L. pomona* but not to six other serotypes.

Of the mammals tested other than rats, two ground hogs (*Marmota monax*) and two striped skunks (*Mephitis mephitis*) had antibodies to *L. pomona*. A culture of this serotype was isolated from the kidneys of one of the ground hogs. A *Leptospira* culture, serotype unknown, was isolated from the blood of one of the skunks and subsequently lost.

References

1. BOULANGER, P., MITCHELL, D., CORNER, A. and BOURASSA, M. Observations on leptospirosis in swine. *Canad. J. Comp. Med.* **23**, 354-359, 1959.
2. BOURNE, F. M., Leptospirosis sejeoe, with report of a case. *Canad. M. A. J.* **73**, 886-890, 1955.
3. CAMERON, G. C. and IRWIN, D. A. 1929. *Leptospira icterohemorrhagiae* occurrence in wild rats in Toronto. *Canad. J. Pub. Hlth.* **20**, 386-392, 1929.
4. HUMPHREYS, F. A., CAMPBELL, A. G., and SMITH, E. S. Studies on leptospira infection in rodents and dogs in British Columbia. *Canad. J. Comp. Med.* **17**, 206-212, 1953.

In Steaks and Stews, We're Seventh

Canada ranked seventh among the world's leading meat eating countries in 1959, with each Canadian eating an average of 143 pounds of red meat. The biggest meat consumers, it is reported, were Australia and Uruguay, with a per capita consumption of over 225 pounds of meat each. Other leading countries, advises the Meat Packers' Council of Canada, with per capita consumption in brackets were: New Zealand (222 lbs.), Ar-

gentina (166 lbs.), United States (160 lbs.), Denmark (147 lbs.), Canada (143 lbs.), Paraguay (134 lbs.), United Kingdom (131 lbs.), and France (124 lbs.). Per capita red meat consumption rose in at least 21 countries. Countries with a lower per capita consumption were Japan (9 lbs.), Phillipines (22 lbs.), and Spain (36 lbs.). In the USSR, consumption was 65 pounds.