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Seroepidemiologic Study of Three Zoonoses (Leptospirosis, Q Fever, and Tularemia) among Trappers in Québec, Canada

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This study was undertaken to evaluate the prevalence of antibodies against *Francisella tularensis*, *Coxiella burnetii*, and certain serovars of *Leptospira interrogans* among trappers in Québec, Canada. Muskrat trapping was identified as a risk factor for *F. tularensis* infection, whereas having a cat at home apparently protected trappers against infection by *L. interrogans*. High percentages of control sera were positive for antibodies against *C. burnetii* (15%) and *L. interrogans* (5%), most frequently serovar bratislava. This is the first report of human infection by serovar bratislava in North America.

Zoonoses are infectious diseases transmitted from animals to humans. This study focused on three of these diseases which are frequently associated with wildlife: tularemia, Q fever, and leptospirosis.

Francisella tularensis is the agent responsible for tularemia. In the United States tularemia is primarily transmitted by ticks (31), but in Canada muskrats and rabbits are the main sources of infection (21).

Q fever is caused by the rickettsia *Coxiella burnetii*. This rickettsia has been found in many animal species, but those most often associated with *C. burnetii* are cattle, sheep, goats (1), and, as shown more recently, cats (10, 18, 19, 27).

Although often associated with sewer workers, leptospirosis has been recently found among rafters (22). It is also an important pathogen in veterinary medicine because of its devastating effects on farm animals. It infects a number of mammals, particularly rats, livestock (pigs and cattle), dogs, and some wild mammals (such as foxes, skunks, and raccoons) (9).

Trappers have been identified as a specific population at risk for zoonoses (12, 16, 25). Because data on zoonotic risks in the province of Québec, Canada, are scarce, we undertook an epidemiologic study to compare the seroprevalences of antibodies against the agents responsible for leptospirosis, Q fever, and tularemia in trappers and controls from the general population. The research also aimed to identify risk factors associated with seropositivity for these agents.

During the fall of 1992 and winter of 1992–1993, we recruited volunteers from five regional trappers' associations in the Québec City area. Volunteers signed a consent form and then provided blood samples and completed a questionnaire that sought information on trapping experience (number of years of trapping, species and quantity of animals trapped during the last year, use of gloves when handling animals, and wildlife consumption habits), medical history (episodes of brucellosis, tularemia, leptospirosis, trichinosis, Q fever, meningitis, or jaundice), occupational history possibly related to zoonoses (farm, butchery, or slaughterhouse work), and presence of pets at home. Each subject seropositive for any of the three bacteria investigated (*F. tularensis*, *C. burnetii*, and *Leptospira interrogans*) was contacted by a physician to investigate symptoms suggestive of these infections. A serum sample from a control subject matched for age, sex, and area of residence was selected for each trapper. These anonymous, unlinked sera were obtained from outpatients who had undergone lipid testing. Controls were assumed to represent a valid sample of the population, since testing for lipids is widely used and recommended not only for patients but also for healthy individuals.

Antibodies against *F. tularensis* were detected by the standard latex agglutination test with BBL antigens. Testing was performed at serum dilutions of 1:20 to 1:2,048. Reactions at dilutions of 1:20 or greater are considered specific and significant.

An immunofluorescent antibody test was performed with the *C. burnetii* phase II antigen prepared from the Nine Mile strain (7). Sera to be tested were diluted in a phosphate-buffered saline solution containing 3% normal yolk sac. The sera were tested at a screening dilution of 1:64. Positive sera were titrated to endpoint dilutions. A serum sample was considered positive if at least 50% of the organisms fluoresced at the test dilution.

Leptospiral antibodies were detected by the microscopic agglutination test (24). On the basis of studies of leptospirosis in farm and domestic animals in Canada (13, 17), the *L. interrogans* serovars tested were bratislava, icterohaemorrhagiae, grippo typhosa, hardjo, and pomona. Only titers of 1:50 and greater were considered positive in order to avoid nonspecific reactions (23).

The proportion of seropositive trappers was compared with the proportion of seropositive controls. The seropositive trappers were also compared with seronegative trappers with regard to different risk factors. To determine which animals could be involved in transmitting the diseases, we compared the average number of animals of each species captured by trappers who had antibodies with the average number of animals of each species caught by those who were seronegative. Multivariate logistic regression was then used to assess confounding between data for different animal species. As the results were not confounded, only results from univariate analysis are presented.

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TABLE 1. Epidemiologic characteristics of the 165 trappers studied

Parameter	Value[a]
Age (yrs)	40 ± 12
Yrs of trapping experience	15 ± 11
Number of animals trapped during the last yr	
Rodents (beavers, muskrats, and squirrels)	68 ± 118
Mustelids (fishers, martens, mink, otters, and weasels)	13 ± 25
Canids (coyotes, foxes, and wolves)	17 ± 29
Wearing of gloves while handling animals	69 (42)
Wildlife consumption	139 (84)
Occupational history	
Farm	100 (61)
Slaughterhouse	14 (8)
Butchery	15 (9)
Pets at home during the last 5 yr	
Cat	53 (32)
Dog	75 (45)
Other	21 (13)

[a] Trappers' ages, years of trapping, and numbers of animals trapped are given as means ± standard deviations. Other values are numbers of trappers, with percentages of the total number of trappers in the study given in parentheses.

A total of 165 trappers (157 men and 8 women) volunteered for this study (Table 1). The medical histories documented by the questionnaire were negative, except for three trappers who reported a prior tularemia episode but were seronegative in the study. The comparison of antibody prevalences in trappers and controls showed no statistical difference between the two groups for any of the three bacterial species (Table 2).

Among trappers, the proportion positive for antibodies to *F. tularensis* was 2%, and there was no correlation between the presence of antibodies and age, number of years of trapping, use of gloves when skinning animals, or occupational history. The only association found was with trapping of muskrats. Antibodies against *F. tularensis* were found in 27% of trappers (4 of 15) who caught 100 or more muskrats during the last trapping season, whereas no *F. tularensis* antibodies were found in 150 trappers who caught fewer than 100 muskrats ($P < 0.001$).

As for *C. burnetii*, trappers and controls had the same percentage (15%) of seropositivity (Table 2), implying similar risks of infection. No risk factors were associated with seropositivity among trappers.

Among the subjects positive for *L. interrogans*, only three serovars were found: bratislava, icterohaemorrhagiae, and hardjo (Table 2). The proportion of trappers with antibodies against hardjo was higher than the proportion of controls with such antibodies ($P = 0.06$). The only risk factor significantly associated with leptospirae was cat ownership. Owning a cat seems to protect against infection by *L. interrogans*, since none of 53 cat owners were seropositive but 15 of 112 trappers who did not have a cat were seropositive ($P = 0.003$).

TABLE 2. Prevalence of antibodies against *L. interrogans*, *C. burnetii*, and *F. tularensis* among trappers and controls

Bacterium	No. (%) with antibodies to bacterium		P^a
	Trappers ($n = 165$)	Controls ($n = 165$)	
C. burnetii	25 (15.1)	25 (15.1)	1.00
F. tularensis	4 (2.4)	1 (0.6)	0.18
L. interrogans serovars	15 (9.1)	8 (4.8)	0.13
bratislava	8 (4.8)	7 (4.2)	0.79
hardjo	6 (3.6)	1 (0.6)	0.06
icterohaemorrhagiae	1 (0.6)	0 (0.0)	1.00

[a] Statistical significance of differences between results for trappers and controls was assessed by χ^2 or Fisher's exact test.

Only three seropositive trappers reported symptoms compatible with any of the three zoonoses examined in this study during the last 5 years. Two of them had antibodies against *C. burnetii* and had been treated at home for pneumonia of unknown origin, and one described an unusual, severe, flu-like illness and was positive for *L. interrogans* serovar hardjo.

Of the agents investigated, *F. tularensis* is the one most often linked with wildlife in the medical literature. In this study, even though the rate of seropositivity for *F. tularensis* among trappers was fourfold higher than the rate among controls, the difference was not statistically significant. Many studies conducted among trappers or North American natives have shown higher seroprevalences ranging from 6 to 17% (11, 12, 21, 25, 26, 33). The lower seroprevalence found in this study may be due to a low prevalence of tularemia in the wildlife of our region or due to limited exposure, since most of the participants trapped only as a hobby. In previous studies, when investigators sought information on symptoms, they found that most subjects were asymptomatic. As there are two types of *F. tularensis*, this apparent absence of clinical signs could be related to infections mostly due to type B, the less virulent one (15). The association found between the number of muskrats trapped and tularemia supports findings of another study in Canada (21). An epidemic among trappers in Vermont, near the U.S.-Canada border, was also related to muskrats (34).

Tularemia may be more common than is currently thought, but it may be restricted to populations in close contact with wildlife. Physicians should be aware of the disease, particularly with patients from native or rural communities who are exposed to wildlife (21). Moreover, groups at risk (e.g., hunters and trappers) should also be made aware of this disease, its clinical features, its association with muskrats, and safe procedures for handling dead animals.

In this study, the 15% rate of positivity for antibodies to the agent of Q fever for both trappers and controls is of concern. In Nova Scotia, *C. burnetii* was incriminated as the cause of 21.8% of 110 cases of acute pneumonia among patients admitted to hospitals during a 1-year period (20).

In the province of Québec, Q fever is a reportable disease, but only 14 cases were reported from 1989 to 1993 (10). The very small number of cases reported is probably due to a lack of diagnosis and to the limited availability of testing procedures. An effort to inform physicians about the high prevalence of the infection and to reinforce the importance of reporting the disease to public health authorities should thus be made.

As for leptospiral antibodies, the proportions of seropositive trappers and seropositive controls were not statistically different except for serovar hardjo. The apparently increased risk of infection by this serovar could be accounted for by contact with wildlife. However, as cat ownership is shown to protect against leptospiral infections, this relationship should be questioned. Similar protection related to cats was also demonstrated by Childs et al. (4). These authors suggest that cats probably reduce human contact with rodents. It may also be the case that carcasses or pelts of fur-bearing animals can attract rodents, which would be the probable causal factor.

L. interrogans, the causal agent of leptospirosis in humans, is known to have more than 200 serologic varieties (8). Serovar icterohaemorrhagiae is the variety discussed most often because of the prevalence of leptospirosis among sewer workers.

However, as a result of contamination of cattle, hardjo became the major infecting serovar in Australia and in Great Britain, and farmers have been identified as the main group at risk (32). Moreover, it seems that leptospirae of the Australis serogroup, which includes serovars bratislava, australis, and lora, are now the main agents of human leptospirosis in Italy (2). In 1984, members of this serogroup, particularly serovar bratislava, were incriminated in a waterborne outbreak of leptospirosis with a fatality rate of 8.6% (3).

In our study, antibodies against serovars hardjo and bratislava were found in 21 of the 22 positive subjects. To our knowledge, the 4 to 5% rate of positivity for serovar bratislava found among both trappers and controls is the first finding of this serovar in humans in North America. In Italy, the prevalence of leptospiral antibodies against at least 1 of 14 serovars of *L. interrogans* was found to vary between 8 and 10% in healthy people (2, 6). In those studies, one-third of the positive subjects had antibodies against serovar bratislava. Serovars hardjo and bratislava are not yet included in the serologic tests used to confirm leptospirosis in humans in Canada (28). Data on animal and human leptospirosis clearly justify the inclusion of these two serovars in the diagnostic tests, since both bratislava (3) and hardjo (32) can cause severe diseases.

In Canada, leptospirosis is not a reportable disease. From 1966 to 1989, between zero and seven cases per year were reported across the country (28). Even though only one seropositive trapper reported symptoms suggestive of leptospirosis, our data show that the prevalence of leptospiral antibodies (4.8%) in our control population is probably in the same range as in countries where the number of leptospirosis cases is larger (5, 14, 29, 30, 32). In agreement with Mumford (22), we believe that leptospirosis is underestimated and that this is probably partly due to a misperception of the disease that limits the degree of clinical suspicion of leptospirosis. The classical clinical presentation with liver and renal failure in sewer workers caused by serovar icterohaemorrhagiae is now an outdated concept (22). Flu-like illness, pyrexia of unknown origin, and aseptic meningitis are now the most frequent clinical manifestations (22). Readily available laboratory tests are also necessary to help clinicians deal with these nonspecific symptoms.

In conclusion, infections with *F. tularensis* seem to be related to muskrat trapping, and ownership of cats appears to protect against infection by *L. interrogans*. Human antibodies for *L. interrogans* serovar bratislava, not previously found in North America, were detected in this study. Furthermore, our data suggest that infections by the agents of leptospirosis and Q fever are probably more frequent than usually thought in North America and that these bacteria should not be considered exotic or rare pathogens. Greater availability of diagnostic tests, inclusion of these infections as reportable diseases, and active surveillance are necessary to better evaluate and control these two infections among the general population.

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REFERENCES

1. **Babudieri, B.** 1959. Q-fever: a zoonosis. Adv. Vet. Sci. **5:**81–181.
2. **Banfi, E., L. Castagnari, and M. Cinco.** 1989. Serosurvey of leptospira agglutinins among railway employees in the national railway compartments of Bologna and Venice. Eur. J. Epidemiol. **5:**468–473.
3. **Cacciapuoti, B., L. Ciceroni, C. Maffei, F. Distanislao, P. Strusi, L. Calegari, R. Lupidi, G. Scalise, G. Gagnoni, and G. Renga.** 1987. A waterborne outbreak of leptospirosis. Am. J. Epidemiol. **126:**535–545.
4. **Childs, J. E., B. S. Schwartz, T. G. Ksiazek, R. Ross Graham, J. W. LeDuc, and G. E. Glass.** 1992. Risk factors associated with antibodies to leptospires in inner-city residents of Baltimore: a protective role for cats. Am. J. Public Health **82:**597–599.
5. **Ciceroni, L., A. Pinto, and B. Cacciapuoti.** 1988. Recent trends in human leptospirosis in Italy. Eur. J. Epidemiol. **4:**49–54.
6. **Crevatin, D., E. Banfi, D. Crotti, E. Ruaro, and M. Cinco.** 1986. Serosurvey on the presence of leptospiral agglutinins in humans in northern Italy. Eur. J. Epidemiol. **2:**44–47.
7. **Department of Health and Human Services, Public Health Service.** 1987. Indirect fluorescent antibody technique for the detection of *Coxiella burnetii* antibodies. Centers for Disease Control, Atlanta.
8. **Faine, S.** 1991. Leptospirosis, p. 367–393. *In* A. S. Evans and P. S. Brachman (ed.), Bacterial infections of humans, 2nd ed. Plenum Medical Books, New York.
9. **Feigin, R. D., and D. C. Anderson.** 1975. Human leptospirosis. Crit. Rev. Clin. Lab. Sci. **5:**413–467.
10. **Goyette, M., A. Poirier, J. Bouchard, and E. Morrier.** 1994. Q-fever in Quebec (1989–93): report of 14 cases. Can. J. Infect. Dis. **5:**113–118.
11. **Greenberg, L., J. D. Blake, and M. F. Connell.** 1957. A immunological study of the Canadian Indian. Can. Med. Assoc. J. **77:**211–216.
12. **Heidt, G. A., C. Harger, H. Harger, and T. C. McChesney.** 1985. Serological study of selected disease antibodies in Arkansas furbearer trappers, a high risk group. J. Arkansas Med. Soc. **82:**265–269.
13. **Higgins, R., P. Cayouette, F. Hoquet, and F. De LaSalle.** 1980. Serological studies on leptospirosis in domestic animals in Quebec. Can. J. Comp. Med. **44:**229–231.
14. **Hodgen, A. N., and M. S. Stuckey.** 1986. Leptospirosis in western Australia, 1983–1984. Med. J. Aust. **144:**567–569.
15. **Hornick, R. B.** 1991. Tularemia, p. 787–802. *In* A. S. Evans and P. S. Brachman (ed.), Bacterial infections of humans, 2nd ed. Plenum Medical Books, New York.
16. **Lantis, M.** 1981. Zoonotic diseases in the Canadian and Alaskan north. Inuit Stud. **5:**83–107.
17. **Lees, V. W., and S. P. Gale.** 1994. Titers to leptospira species in horses in Alberta. Can. Vet. J. **35:**636–640.
18. **Marrie, T. J., J. Van Buren, J. Fraser, E. V. Haldane, R. S. Faulkner, J. C. Williams, and C. Kwan.** 1985. Seroepidemiology of Q-fever among domestic animals in Nova Scotia. Am. J. Public Health **75:**763–776.
19. **Marrie, T. J., H. Durant, J. C. Williams, E. Mintz, and D. M. Waag.** 1988. Exposure to parturient cats: a risk factor for acquisition of Q-fever in maritime Canada. J. Infect. Dis. **158:**101–108.
20. **Marrie, T. J., E. V. Haldane, R. S. Faulkner, C. Kwan, B. Grant, and F. Cook.** 1985. The importance of *Coxiella burnetii* as a cause of pneumonia in Nova Scotia. Can. J. Public Health **76:**233–236.
21. **Martin, T., I. H. Holmes, G. A. Wobeser, R. F. Anthony, and I. Greefkes.** 1982. Tularemia in Canada with a focus on Saskatchewan. Can. Med. Assoc. J. **127:**279–282.
22. **Mumford, C. F.** 1989. Leptospirosis and water sports. Br. J. Hosp. Med. **41:**519.
23. **Nuti, M., D. Amaddeo, G. L. Autorino, M. Crovatto, C. Crucil, A. Ghionni, M. Giommi, F. Salvati, and G. F. Santini.** 1992. Seroprevalence of antibodies to hantaviruses and leptospires in selected Italian population groups. Eur. J. Epidemiol. **8:**98–102.
24. **Palmer, M. F.** 1988. Laboratory diagnosis of leptospirosis. Med. Lab. Sci. **45:**174–178.
25. **Philip, R. N., E. A. Casper, and D. B. Lackman.** 1967. The skin test in an epidemiologic study of tularemia in Montana trappers. J. Infect. Dis. **117:** 393–402.
26. **Philip, R. N., B. Huntley, D. B. Lackman, and G. W. Comstock.** 1962. Serologic and skin test evidence of tularemia infection among Alaskan Eskimos, Indians and Aleuts. J. Infect. Dis. **110:**220–230.
27. **Pinsky, R. L., D. B. Fishbein, C. R. Greene, and K. F. Gensheimer.** 1991. An outbreak of cat-associated Q-fever in the United States. J. Infect. Dis. **164:** 202–204.
28. **Rivest, N., J. Jobin, and C. Gaudreau.** 1989. Leptospirosis: a diagnosis to be kept in mind. Can. Dis. Weekly Rep. **15:**13–16.
29. **Skilbeck, N. W., and G. T. Miller.** 1986. A serological survey of leptospirosis in Gippsland dairy farmers. Med. J. Aust. **144:**565–567.
30. **Swart, K. S., C. R. Wilks, K. B. Jackson, and J. A. Hayman.** 1983. Human leptospirosis in Victoria. Med. J. Aust. **1:**460–463.
31. **Taylor, J. P., G. R. Istre, T. C. McChesney, F. T. Satalowich, R. L. Parker, and L. M. McFarland.** 1991. Epidemiologic characteristics of human tularemia in the Southwest-central states, 1981–1987. Am. J. Epidemiol. **133:**1032–1038.
32. **Waitkins, S. A.** 1986. Leptospirosis as an occupational disease. Br. J. Ind. Med. **43:**721–725.
33. **Wood, W. J.** 1951. A study based on the incidence of positive agglutination tests against *P. tularensis* in the Indian population of Manitoba and North-Western Ontario. Manit. Med. Rev. **31:**641–644.
34. **Young, L. S., D. S. Bicknell, B. G. Archer, J. M. Clinton, L. J. Leavens, J. C. Feeley, and P. S. Brachman.** 1969. Tularemia epidemic: Vermont, 1968. Forty-seven cases linked to contact with muskrats. N. Engl. J. Med. **280:** 1253–1260.