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Source: Journal of Wildlife Diseases, 20(2) : 95-99

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-20.2.95

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Journal of Wildlife Diseases, 20(2), 1984, pp. 95–99
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SEROLOGIC STUDIES ON BRUCELLOSIS, LEPTOSPIROSIS AND TULAREMIA IN MOOSE (*ALCES ALCES*) IN QUEBEC

Michel Bourque[1] and Robert Higgins[2]

ABSTRACT: Blood samples were obtained from 208 moose in La Vérendrye and Matane Reserves and in Laurentides Park, Quebec, Canada. Sera were tested for antibodies to *Brucella abortus*, *Leptospira interrogans* serovar *ballum*, *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*, and *Francisella tularensis*. Fifteen sera contained evidence of prior exposure to *F. tularensis*. Only one animal was a seroreactor to *L. interrogans* serovar *grippotyphosa* and none of them had antibodies to *B. abortus*.

INTRODUCTION

Brucellosis, leptospirosis and tularemia can affect humans, domestic and wild animals (Francis, 1937; Fox and Kaufmann, 1977; Bell and Reilly, 1981; Shotts, 1981; Witter, 1981; Hanson, 1982). Brucellosis is a disease which has to be reported to the Canadian Government when diagnosed. The federal Ministry of Agriculture maintains an eradication program, and the Province of Quebec is declared a low prevalence region, i.e., the number of infected bovine herds does not exceed 0.3% of the herds within the region. The importance of wild animals has been recognized in the epidemiology of the disease (Meyer, 1974; Witter, 1981). Many wildlife species can act as reservoirs of leptospirosis for other wild or domestic animals and even for humans. In domestic animals, economic losses resulting from reproductive problems are important. A diagnosis based on clinical signs alone being difficult, the risk of human exposure is increased. Tularemia is endemic in Quebec, and almost every year many hunters and trappers become infected (Gattereau et al., 1970).

The moose is an important big game animal in Quebec, and its territory frequently encroaches upon farming areas. The assessment of its health status is therefore a prerequisite in wildlife management as well as for the protection of domestic livestock and humans. The objective of this survey was to evaluate the presence of the three above mentioned diseases in moose in Quebec.

MATERIALS AND METHODS

This survey was carried out in September and October 1979, in La Vérendrye and Matane Reserves and in Laurentides Park. Although they constitute three distinct ecological environments (Gauthier, 1978), these three areas are representative of southern Quebec zones of high moose population density.

Two 15-ml plastic tissue culture tubes were distributed to each hunting party with instructions to fill them as soon as possible after killing a moose, with blood from the heart or large vessels. The mean time between kill and return of blood samples was 22.6 hr ± 14.1 (SD). Hunters brought back 293 samples, of which 208 sera were retained for this study. Serum was separated by centrifugation, and kept frozen.

Detection of antibodies to *Brucella abortus* was done by the slide agglutination test. Constant antigen was provided by Institut de Recherches vétérinaires (Nepean, Ontario K2H 8P9, Canada). Titers lower than 1:100 obtained with this test were considered nonsignificant. The microscopic agglutination test (Cole et al., 1973) was used in the search for *Leptospira interrogans* antibodies, with the following serovars: *ballum*, *canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae* and *pomona*. With this technique, a titer of 1:100 was considered evidence of previous contact of the animal with the leptospires. In the case of *Francisella tu-*

Received for publication 23 May 1983.

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TABLE 1. Results of serologic analyses for antibodies to *Brucella*, *Leptospira* and *Francisella* in moose in Quebec.

Bacterial agent	La Vérendrye		Laurentides		Matane		Total	
	No. of sera tested	No. of sero-reactors (%)	No. of sera tested	No. of sero-reactors (%)	No. of sera tested	No. of sero-reactors (%)	No. of sera tested	No. of sero-reactors (%)
Brucella abortus	86	0 (0)	79	0 (0)	42	0 (0)	207	0 (0)
Leptospira interrogans								
ser. *ballum*	84	0 (0)	80	0 (0)	42	0 (0)	206	0 (0)
canicola	84	0 (0)	80	0 (0)	42	0 (0)	206	0 (0)
grippotyphosa	84	0 (0)	80	0 (0)	42	1 (2.38)	206	1 (0.49)
hardjo	84	0 (0)	80	0 (0)	42	0 (0)	206	0 (0)
icterohaemorrhagiae	84	0 (0)	80	0 (0)	42	0 (0)	206	0 (0)
pomona	84	0 (0)	80	0 (0)	42	0 (0)	206	0 (0)
Francisella tularensis	82	9 (10.98)	79	5 (6.33)	42	1 (2.38)	203	15 (7.39)

larensis, analyses were done using the rapid slide test (Bacto-*Francisella tularensis* Antigens and Control Antiserum, Difco Laboratories, Detroit, Michigan 48201, USA). A titer of 1:20 was considered as evidence of previous exposure to *Francisella tularensis*, and all sera meeting or exceeding this titer will referred to as positive.

Age was determined by size for the calves (6-mo-old class), by premolar tooth eruption for 1.5-yr-old class, and by counting the annual growth rings in incisor teeth cement for the others (Ouellet, 1977).

RESULTS

The 208 sera came from 125 males and 83 females. The mean age was 4.1 yr for the sample. For males it was 3.8 yr (6 mo to 13.5 yr) and for females 4.5 yr (6 mo to 14.5 yr).

The results of analyses for moose from each area are shown in Table 1. All sera were negative for antibodies to *Brucella abortus*. One serum sample from a 6.5-yr-old female from Matane Reserve had a titer of 1:400 to *Leptospira interrogans* serovar *grippotyphosa*. Fifteen sera were found positive for antibodies to *Francisella tularensis*. Twelve of these 15 sera were from male moose. Ages ranged from 0.5 to 14.5 yr, with a predominance of younger animals. Nine sera from La Vérendrye Reserve and five from Laurentides Park had titers of 1:20. The single animal (a 0.5-yr-old female) from Matane Reserve had a titer of 1:80. The prevalences of *Francisella* antibodies were not significantly different between the three areas ($\chi^2 = 3.204$, df = 2, $P > 0.20$). Also, there were no significant differences according to sex ($\chi^2 = 2.67$, df = 1, $P > 0.10$), and mean age of animals with positive sera (4.57 ± 3.33 yr, $n = 15$) was comparable to mean age of animals with negative sera (4.02 ± 3.20 yr, $n = 188$) ($F = 1.08$, $t = 0.64$, $P = 0.05$).

DISCUSSION

In 1972, 50 moose blood samples from various parks and reserves in Quebec were found negative for antibodies to *Brucella*, *Leptospira* and *Francisella* (Désilets, 1973). In the present study, all sera were negative for antibodies to *Brucella*. Many other serologic studies in North America also failed to detect *Brucella* antibodies in moose (Fenstermacher and Jellison, 1933; Fenstermacher, 1937; Diesch et al., 1972; Zarnke and Yuill, 1981), even in moose from areas with many positive cattle herds (Hudson, 1978; Hudson et al., 1980). Nevertheless, two clinical cases of brucellosis in moose were described in the United States (Jellison et al., 1953; Fenstermacher and Olsen, 1978) and two other cases in

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Alberta (Corner and Connell, 1958). Jellison et al. (1953) also found antibodies in 11 of 46 sera. According to Witter (1981), the small number of seropositives would indicate that moose are refractory to the disease. Corner and Connell (1958) consider that moose are dead-end hosts for brucellosis, the infection usually resulting in fatal septicemia, leaving few survivors with antibodies.

Leptospirosis does not seem to pose a problem in moose in Quebec, only one serum being found positive, with a titer of 1:400, which can be regarded as evidence of previous infection (Friend and Halterman, 1967; Diesch, 1980). In the bovine species, antibodies to *Leptospira* can persist for months and even years (Higgins et al., 1975); however, experimental work of Trainer et al. (1961) in white-tailed deer (*Odocoileus virginianus*) showed that in this species antibody titers drop rapidly after 75 to 100 days and can disappear completely. In experimentally infected moose, urinary excretion of *Leptospira* and immune-response would be similar to those of white-tailed deer (McGowan et al., 1963). Therefore, the serologic results are perhaps not a real indicator of the true prevalence of the disease in moose. In fact, moose should be a species at risk. *Leptospira* are usually propagated by mud and water contaminated with urine, and moose are commonly found in such habitat. The serovar *grippotyphosa*, found in our study, had the highest serological prevalence in moose in Minnesota (89/328, 27.1%), while prevalence of serovar *pomona* was the lowest (18/328, 5.5%) (Diesch et al., 1972). In Ontario, however, *pomona* was the serovar found in moose by McGowan et al. (1963) (4/90, 4.4%), and also predominated in white-tailed deer (56/310, 18.1%). Raccoons (*Procyon lotor*) and skunks (*Mephitis mephitis*) would be maintenance hosts for serovar *pomona* infections (McGowan et al., 1963), and raccoons, opossums (*Didelphis virginiana*), and many species of foxes may be the main sources of infection by serovar *grippotyphosa* (Shotts, 1981). In Quebec, leptospirosis is endemic in bovine and porcine populations, and serologic surveys have shown serovar *pomona* to be the most widespread in these domestic species (Higgins et al., 1975; Higgins and Cayouette, 1978; Higgins et al., 1980a, b).

This appears to be the first report of antibodies to *Francisella tularensis* in moose. Moose are likely to be susceptible to tularemia, as more than 100 species of mammals, including many cervine species, are known to be susceptible (Bell and Reilly, 1981). In North America, antibodies to *Francisella tularensis* were demonstrated in white-tailed deer (Thorpe et al., 1965; Friend and Halterman, 1967), mule deer (*Odocoileus hemionus*) (Shaw, 1964; Thorpe et al., 1965; Vest et al., 1965), and Rocky Mountain elk (*Cervus elaphus nelsoni*) (Merrell and Wright, 1978).

Studies in Cervidae (Shaw, 1964; Merrell and Wright, 1978) and humans (Greenberg and Blake, 1957; Philip et al., 1967) have considered titers of 1:8 and 1:20 as significant. On the other hand, titers of 1:20 and 1:40 obtained in red deer (*Cervus elaphus*) in Norway were suspected to be cross reactions (Omland et al., 1977). *Francisella tularensis* is known to cross react with *Brucella abortus*. In the present study, all sera were negative to *Brucella abortus*, so this possibility can be eliminated.

Since antibodies to *Francisella tularensis* persist for life, the results can be regarded as representative of the disease prevalence in the moose population. Because they are less likely to succumb to tularemia infection than small mammals, cervids can be good serologic indicators of the presence of tularemia in a given area (Omland et al., 1977). Positive sera originated from all three areas, and prevalence was comparable between territories. This suggests that the geographic distribution of the disease in Quebec is extensive, as

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already indicated by location of epidemics in small mammals and humans. Mean age of negative and positive animals did not differ significantly, so the prevalence is probably the same from year to year.

Moose are exposed naturally to water contaminated by beaver (*Castor canadensis*) and muskrat (*Ondatra zibethicus*), two species in which tularemia is endemic in Quebec. Many species of Tabanidae can serve as vectors of the disease in moose. Many human infections were ascribed to tabanid bites (Ootmar, 1931; Francis, 1937; Tartakow, 1946; Rand, 1949). Human contamination is also theoretically possible from handling or eating moose meat. Human infections have been suspected to be related to deer (Gilbert and Coleman, 1932; Francis, 1937; Brown, 1944; Anonymous, 1966), and this relation has been confirmed by the isolation of *Francisella tularensis* from the bone marrow of a mule deer, from which a hunter was infected (Centers for Disease Control, 1975; Emmons et al., 1976).

ACKNOWLEDGMENTS

We wish to thank the personnel of the Ministère des Loisirs, de la Chasse et de la Pêche du Québec, for facilitating the collection of blood samples and for age determination of moose.

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