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# SEROEPIDEMIOLOGY OF LEPTOSPIROSIS IN MINNESOTA WOLVES

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ABSTRACT: Serum samples ($n$ = 457) from wolves (*Canis lupus*) in northern Minnesota were collected from 1972 through 1986 and were tested for antibodies against *Leptospira interrogans* using a microtiter agglutination test. Twelve serovars included in the study were: *australis, autumnalis, ballum, bataviae, bratislava, canicola, copenhageni, grippotyphosa, hardjo, pomona, pyrogenes,* and *tarassovi*. Fifty-two (11%) sera had antibody titers of ≥1:50 against one or more serovars of *L. interrogans*. The seroprevalence of different serovars in decreasing order was: *grippotyphosa, bratislava, autumnalis, canicola, pomona, ballum, pyrogenes, hardjo,* and *copenhageni*. No antibodies were found against *australis, bataviae,* and *tarassovi*. These results indicate that *L. interrogans* infection may occur in wolves of Minnesota.

*Key words:* Leptospirosis, *Leptospira interrogans*, serovars, wolves, *Canis lupus*, seroepidemiology, zoonosis, survey.

## INTRODUCTION

Leptospirosis is a widely distributed zoonosis which affects most mammals throughout the world and is caused by a pathogenic spirochete of the genus *Leptospira* (Thiermann, 1984). All pathogenic leptospires are classified under one species, *L. interrogans*, which contains more than 170 serovars organized into 19 serogroups (Nielsen et al., 1989). *Leptospira interrogans* usually occurs in the kidneys of the infected host and is excreted into the environment through their urine (Diesch and McCulloch, 1966). Susceptible hosts can become infected directly by contact with urine or indirectly by contact with urine-contaminated water, moist soil, stagnant ponds and slow-moving streams where *L. interrogans* can survive for weeks (Roth, 1970; Hathaway et al., 1983a). The disease caused by *L. interrogans* is characterized by abortion and stillbirth in cattle and pigs; periodic ophthalmia (moon blindness) in horses; and acute febrile illness with myalgia, headache, meningitis and occasional kidney failure in humans (Hanson and Tripathy, 1981; Faine, 1986). In humans, leptospirosis was first reported in 1886 by Adolf Weil in Germany (Khan and Diesch, 1987).

Many wild species can act as reservoirs of *L. interrogans* for other wild or domestic animals and even for humans (Hathaway et al., 1981; Hathaway and Blackmore, 1981). Leptospirosis has been reported in foxes (*Vulpes sp.*) (Clark et al., 1961), coyotes (*Canis latrans*) (Cirone et al., 1978; Drewek et al., 1981), and jackals (*Canis sp.*) (van der Hoeden, 1955). Serological evidence of leptospirosis has recently been reported in white-tailed deer (*Odocoileous virginianus*) of Minnesota (Ingebrigtsen et al., 1986). The objective of the present study was to determine the seroprevalence of 12 selected serovars of *L. interrogans* in wolves (*Canis lupus*) from Minnesota (USA).

## MATERIALS AND METHODS

Four hundred fifty seven serum samples were collected from wolves in northern Minnesota that were live-trapped and anesthetized. Blood was collected in glass tubes by saphenous or cephalic venipuncture. Serum was separated by centrifugation and stored at −20 C until tested. Most samples were collected during June through October 1972 to 1986 and were tested in 1987. These sera have been used in previous surveys for canine pathogens (Mech et al., 1986; Goyal et al., 1986).

The following *L. interrogans* serovars were included in the study: *australis, autumnalis, ballum, bataviae, bratislava, canicola, copenhageni, grippotyphosa, hardjo, pomona, pyrogenes,* and *tarassovi*. These serovars have been incriminated as a cause of disease in different

TABLE 1. Titers of *Leptospira interrogans* antibodies in sera of wolves in Minnesota.

| Serovar[a] | Number positive (%) at ≥1:50 | Number of sera showing titer[b] of | | | | | |
|---|---|---|---|---|---|---|---|
| | | 50 | 100 | 200 | 400 | 800 | >1,600 |
| Autumnalis | 15 (3.3) | 5 | 1 | 4 | 1 | 2 | 2[c] |
| Ballum | 3 (0.7) | 2 | 1 | 0 | 0 | 0 | 0 |
| Bratislava | 18 (3.9) | 14 | 1 | 2 | 1 | 0 | 0 |
| Canicola | 13 (2.8) | 5 | 3 | 3 | 2 | 0 | 0 |
| Copenhageni | 3 (0.7) | 3 | 0 | 0 | 0 | 0 | 0 |
| Grippotyphosa | 24 (5.3) | 9 | 4 | 6 | 3 | 1 | 1[d] |
| Hardjo | 2 (0.4) | 1 | 1 | 0 | 0 | 0 | 0 |
| Pomona | 7 (1.5) | 2 | 1 | 3 | 1 | 0 | 0 |
| Pyrogenes | 7 (1.5) | 1 | 1 | 3 | 1 | 1 | 0 |
| | 92 (20)[e] | 42 | 13 | 21 | 9 | 4 | 3 |

[a] No serum was positive for serovars *australis*, *bataviae*, and *tarassovi*.
[b] Reciprocal of highest serum dilution showing 50% agglutination.
[c] One serum was positive at 1:1600 and the other at 1:6400.
[d] This serum had a titer of 1:1600.
[e] Of 457 sera examined, 52 were positive at ≥1:50. Because some sera had antibodies to more than one serovar, the number of positive sera appears to be 92.

species of hosts in various parts of the world (Faine, 1982). Stock cultures of these bacteria were obtained in semi-solid media from the National Veterinary Services Laboratory (United States Department of Agriculture, Ames, Iowa 50010, USA). These organisms were sub-cultured in both semi-solid and liquid (bovine albumin-polysorbate 80, Intergen Company, Purchase, New York 10577, USA) media in our laboratory. The stock cultures were maintained in semi-solid media and liquid cultures were used as sources of antigen for the microagglutination test.

The microscopic agglutination test (Cole et al., 1979) was performed in 96-well, flat-bottom microtiter plates (Linbro; Flow Laboratories, McLean, Virginia 22102, USA). Briefly, a 1:25 dilution of test serum was made in phosphate buffered saline, pH 7.2. To 50 μl of this diluted serum was added 50 μl of live antigen at 25 nephelometeric turbidity units (NTU) making a final serum dilution of 1:50. The plates were incubated at 29 C for 120 ± 30 min and examined for agglutination under a dark-field microscope. The samples found positive on screening at a 1:50 dilution were further titrated using serial two-fold dilutions of up to 1:6,400. We considered microagglutination titers of ≥1:50 as positive.

Data were analyzed via a Chi square test with Statistix software (N.H. Analytical Software, St. Paul, Minnesota 55104, USA, 1985) and an IBM personal computer (IBM, PC, International Business Machines, Rochester, Minnesota 55901, USA).

## RESULTS

Of 457 sera examined, 52 (11.4%) were positive at titers of ≥1:50, and 31 (6.8%) were positive at titers of ≥1:100 for antibodies against one or more serovar of *L. interrogans*. Because some sera were positive for more than one serovar, the number of positive samples is shown to be 92 in Table 1. Of the 52 positive sera, 25 were positive to more than one serovar. Twelve, 11, and 2 serum samples contained antibodies against two, three, and four serovars, respectively. At titers of ≥1:50, the prevalence of antibodies to serovars in decreasing order was: *grippotyphosa* (5.3%), *bratislava* (3.9%), *autumnalis* (3.3%), *canicola* (2.8%), *pomona* (1.5%), *pyrogenes* (1.5%), *ballum* (0.7%), *copenhageni* (0.7%), and *hardjo* (0.4%). None of the samples was positive for serovars *australis*, *bataviae*, and *tarassovi*. The range in titers of antibodies to different serovars was: ≥1:50–6,400 for *autumnalis*; ≥1:50–1,600 for *grippotyphosa*; ≥1:50–800 for *pyrogenes*; ≥1:50–400 for *bratislava*, *canicola* and *pomona*; and ≥1:50–100 for *ballum* and *hardjo*. Antibody titers against serovar *copenhageni* were positive at 1:50 only.

Of wolves whose sex was known, 212

TABLE 2. Distribution of *Leptospira interrogans* antibodies in wolves from Minnesota according to counties.

| County | Number tested | Number positive (%) | Number showing a titer of >1:50 against[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Autumnalis | Ballum | Bratislava | Canicola | Grippo | Hardjo | Pomona | Pyrogenes |
| Lake | 181 | 15 (8.3) | 3 | 0 | 4 | 8 | 2 | 0 | 0 | 1 |
| Beltrami | 9 | 1 (11.1) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Koochiching | 22 | 5 (22.7) | 1 | 0 | 2 | 1 | 4 | 0 | 1 | 1 |
| St. Louis | 20 | 7 (35.0) | 1 | 2 | 4 | 0 | 7 | 0 | 1 | 0 |
| Kittson | 62 | 3 (4.8) | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 |
| Itasca | 11 | 3 (27.3) | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| Roseau | 2 | 0 (0.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 307[b] | 34 (11.2) | 8 | 2 | 11 | 10 | 17 | 1 | 4 | 2 |

[a] Some of the animals showed titers to two or more serovars.
[b] Excludes samples for which county of origin was unknown.

were males and 198 were females. The rate of seropositivity for males was 11.6% and for females it was 12.3%. There was no statistically significant difference ($P > 0.05$) in the rates of seropositivity between males and females.

Wolves were collected from seven counties in northern Minnesota. Prevalence of antibody positive wolves from these counties is shown in Table 2. The prevalence of seropositive wolves near farming areas (20.1%) was 2.6 times greater than that of wolves living in wilderness away from farms (7.7%) (Table 3).

## DISCUSSION

The 11.4% seroprevalence in wolves in our study is lower than that observed by Everard et al. (1983) in 31 species of wild animals from Trinidad and Grenada. They examined 894 animals and found 198 (22.1%) positive at titers of ≥1:100. Our results, however, are in sharp contrast to those of Zarnke and Ballard (1987) who found only 1 of 80 wolf samples from Alaska positive for *L. interrogans* antibodies. Presence of antibodies in wolves may indicate previous or current infection which may have resulted either from direct contact with urine of other wolves or from eating infected prey. Reilly et al. (1970) have indicated that oral infection of Canidae and other carnivores with *L. interrogans* is possible. They observed that *L. interrogans* may be protected from gastric acidity because the gulped bolus passes partially digested into the alkaline duodenum. Once infected, wolves may act as maintenance hosts for *L. interrogans* on account of their highly social behavior.

TABLE 3. Relative prevalence of antibodies against *Leptospira interrogans* in wolves depending upon their proximity to dairy and beef cattle farms.

| Wolf population | Seropositive at ≥1:50 | Seronegative at 1:50 | Total | Prevalence (%) |
|---|---|---|---|---|
| Farm related | 27 | 107 | 134 | 20.1 |
| Non-farm related | 25 | 298 | 323 | 7.7 |
| Total | 52 | 405 | 457 | |

Leptospirosis is endemic in bovine, porcine and equine populations of Minnesota. Of 17,014 animal sera examined from 1984 to 1986 at the Minnesota Veterinary Diagnostic Laboratory (St. Paul, Minnesota 55108, USA), 3,040 (17.9%) had antibodies against one or more serovars of *L. interrogans* at titers of ≥1:50. The prevalence of antibodies against different serovars in decreasing order was: *icterohaemorrhagiae* (24.9%), *hardjo* (21.4%), *canicola* (20.1%), *pomona* (18.5%), and *grippotyphosa* (15.1%). It is possible for a particular serovar to be shared by many maintenance hosts or to be specific to a single host (Hathaway et al., 1983b). In our study, the highest seroprevalence (5.3%) in wolves was for serovar *grippotyphosa*. In a previous study of moose in Minnesota, Diesch et al.

(1972) found that the prevalence of serovar *grippotyphosa* was the highest, 89 of 328 (27.1%), while antibodies against serovar *pomona* were the least prevalent (18 of 328) (5.5%).

Serovar *grippotyphosa* is prevalent in many countries including the U.S.S.R. and China (Tonkonozhenko et al., 1965; Armitsu et al., 1987) and has been isolated from dog, cattle, swine, muskrat (*Ondatra zibethicus*), squirrels (*Sciurus niger, S. carolinensis*), bobcat (*Lynx rufus*), cottontail rabbit (*Sylvilagus floridanus*), swamp rabbit (*S. aquaricus*), raccoon (*Procyon lotor*), striped skunk (*Mephitis mephitis*), red fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), hare (*Lepus americanus*) and opossum (*Diadelphus virginianus*) (Hanson, 1982; Shotts et al., 1971). Many species of foxes may be infected with *L. interrogans* organisms (Shotts, 1981). Amundson and Yuill (1981) examined the prevalence of antibodies to five different serovars of *L. interrogans* in red and gray foxes (*U. cinereoargenteus*) of southwestern Wisconsin. Antibodies against serovar *grippotyphosa* were the most prevalent with 25 of 53 (47%) red foxes and 11 of 36 (31%) gray foxes having titers. Juvenile foxes had significantly higher geometric mean titers than adults.

Two animals were positive to serovar *hardjo* albeit at low titers e.g., 1:50 and 1:100. Collins et al. (1981) have reported the presence of antibodies against *L. hardjo* in pronghorns (*Antilocapra americana*) of Colorado. Antibodies against serovar *pomona*, which is a common serovar in domestic animals, were also low in our study. The low antibody titers in wolves may indicate that the wolves were infected sometime earlier and that their titers have subsequently decreased. This is common in *L. interrogans* infections where high titers may mean active infection or vaccination and a fall in titer may indicate residual infection. Titers to serovar *pomona* can persist in deer for 3 mo after infection or longer (Ferris et al. 1960). The existence of antibodies to multiple serotypes of *L. interrogans*, especially with low titers, may indicate cross-reactivity among various serovars.

The prevalence of antibodies in wolves inhabitating farming areas was 20.1% compared to 7.7% prevalence in wilderness. This is not surprising because *L. interrogans* (especially serovar *pomona*) has been isolated from the environment such as in recreational waters, ponds, and farm waters (Diesch and McCulloch, 1966) where it has been found to survive for a long time. For example, leptospires survived for 61 days in experimentally contaminated oxidation ditch manure which led Diesch (1971) to believe that manure in the open farm area was a potential public health problem and could also be a source of infection to wild animals. It was suggested that disinfection of livestock wastes may help break the chain of infection from farms to wild animals, domestic animals, and rodent populations (Will and Diesch, 1972). Contrary to this opinion, it has been postulated that wild animals may act as sources of *L. interrogans* infection for deer and cattle. However, conclusive proof in this area of *L. interrogans* epidemiology is not available indicating the need for more detailed studies to determine the actual role of wolves and other wildlife in spreading and maintaining leptospirosis in domestic animals in Minnesota and elsewhere.

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