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Leptospira interrogans Exposure in Free-ranging Elk in Washington

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ABSTRACT: Exposure to one or more serovars of *Leptospira interrogans* was observed in five of six sampled elk (*Cervus elaphus roosevelti*) killed in November 1993, from an isolated herd in southwest Washington, USA (46°45′N, 123°6′W). In April 1994, exposure to *L. interrogans* serovars was documented in nine of 11 captured cow elk from the same herd. Leptospires were not isolated from any of the exposed elk, and 10 of the 11 cows were pregnant. The high seroprevalence is evidence that exposure is widespread in the herd. Local productivity of elk was high, however, and the surrounding topography was not conducive for transmission to other elk populations.

Key words: *Leptospira interrogans*, serologic survey, elk, *Cervus elaphus*.

Serologic surveys are widely used to determine prevalence of exposure to various diseases in wildlife populations. Personnel from the Washington (USA) Department of Fish and Wildlife (WDFW) routinely evaluate wild animals captured or killed in special hunts for the presence of wildlife diseases. High antibody titers for *Leptospira interrogans* serovar *pomona* (range 1:200 to 1:800) and lesser titers for *L. grippotyphosa* (range 1:000 to 1:100) occurred in five of six hunter killed elk (*Cervus elaphus roosevelti*) from an isolated herd located on Centralia Mining Company (CMC) property near Nulls Crossing, Washington (46°45′N, 123°6′W). Prior to this, there were few seropositive elk (titers ≥ 1:100) among over 300 elk throughout western Washington tested for *L. interrogans* (P. B. Hall, unpubl.).

Leptospirosis is a zoonosis caused by any of more than 180 known serovars of the spirochete *L. interrogans* (Davidson and Nettles, 1988); 27 serovars have been identified in the United States (Kistner, 1982). Most of these serovars have coevolved with one or more wildlife hosts, with good host-parasite adaptations and little disease within adapted hosts (Thorne, 1982). Two serovars, *L. pomona* and *L. hardjo*, are primarily pathogens of domestic livestock (Kistner, 1982). Exposure to these latter two serovars is found occasionally in cervids in North America, but the source often can be traced back to domestic animals (Kistner, 1982).

Serologic surveys commonly are used to test for exposure to leptospires (Thorne, 1982). Cross reactions between *L. interrogans* serovars are common, making identification of infecting serovars difficult by serologic testing alone (Thorne, 1982). Serovar specificity is more reliable at titers ≥1:100 (Heath and Johnson, 1994), however, and the serovar with the highest titer often is taken as the source of infection (Modric and Huber, 1993).

Clinical disease due to *L. interrogans* infection is rare in wild animals, and leptospirosis generally is considered unimportant to the health of wild populations (Davidson and Nettles, 1988). Exposure has been documented in white-tailed deer (*Odocoileus virgianus*), mule deer (*O. hemionus*), pronghorns (*Antilocapra americana*), moose (*Alces alces*), and red deer (*Cervus elaphus*) (Thorne, 1982). Exposure is common in white-tailed deer, with seroprevalences of up to 40% reported in the southeastern (Davidson and Nettles, 1988) and midwestern United States. Leptospires also have been isolated from deer throughout their range. Although *L. interrogans* has never been isolated from elk, positive sera are evidence of limited exposure in Idaho, Oregon, and California (USA), as well as Canada. (Kistner, 1982).

No detailed accounts of *L. interrogans* exposure in free-ranging elk have been re-

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ported (Kistner, 1982). Additionally, nothing is known of the epizootiology or significance of leptospirosis in free-ranging elk. To address the high seroprevalences identified in the CMC herd, we initiated a study in April 1994 to assess *L. interrogans* in the CMC herd. Our objectives were to determine the level of exposure and if individuals in the CMC herd were either actively infected or passive carriers.

Since isolation and identification of *L. interrogans* from infected animals provides the only proof of infection, we captured 11 cow elk from the CMC herd. We tried to capture yearling cows, which we felt would be more likely to show active infections than older cows. Elk were captured by aerial darting from a helicopter using 3.75 mg carfentanil citrate (Wildlife Pharmaceuticals, Inc., Ft. Collins, Colorado, USA) and 25 mg xylazine HCl (Miles Inc., Shawnee Mission, Kansas, USA). Captured elk were marked individually with color-coded collars and ear tags to aid in future identification. Blood and urine samples were collected from each elk. Urine samples were collected by catheterization using a size 18 FR × 41 cm Sovereign feeding tube and urethral catheter (Sherwood Medical, St. Louis, Missouri, USA). Following sample collection, elk were administered 10 ml penicillin (150,000 units/ml penicillin G benzathine; 150,000 units/ml penicillin G procaine) (Pen BP-48; Pfizer Animal Health, New York, New York, USA), 12 ml of Vitamin B Complex (Phoenix Pharmaceuticals, Inc., St. Joseph, Missouri), 3 ml of MU-SE (a vitamin E and selenium complex) (Schering-Plough Animal Health, Kenilworth, New Jersey, USA), and 5 ml of Clostri Shield 8 (Clostridium-Septicum-Haemolyticum-Novyi-Sordelli-Perfringens types C and D bacterin/toxoid) (Grand Laboratories, Inc., Larchwood, Iowa, USA). The carfentanil was reversed with 300 mg of naltrexone (75 mg intravenous; 225 mg subcutaneous) (Wildlife Pharmaceuticals, Inc., Ft. Collins, Colorado). All cows appeared in good condition.

Blood samples were tested for antibodies to *L. interrogans* serovars *pomona, grippotyphosa, bratislava, hardjo, canicola,* and *icterohemorrhagiae* using the microscopic agglutination test (Gochenour et al., 1958). Microagglutination titers of ≥1:100 were considered evidence of previous exposure. Pregnancy was determined by radioimmunoassay for pregnancy specific placental protein B (Sassar et al., 1986). Urine samples were tested for the presence of *L. interrogans* by fluorescent antibody tests (Bolin et al., 1989) and by culture (Bolin et al., 1989). Leptospiral Transport Medium (Thiermann et al., 1984) was inoculated with urine in the field ≤2 hr after collection, and shipped to the United States Department of Agriculture National Animal Disease Center (Ames, Iowa, USA) to be cultured for *L. interrogans* (Bolin et al., 1989).

Nine of 11 cows had antibodies to one or more *L. interrogans* serovars. Three cows tested positive for *L. bratislava* alone (titers of 1:100, 1:400, and 1:400). Four cows were positive for both *L. bratislava* and *pomona* (paired titers of 1:200 and 1:100, 1:200 and 1:400, 1:200 and 1:400, and 1:400 and 1:400 for *L. bratislava* and *pomona,* respectively). One cow was positive for both *L. bratislava* (1:100) and *grippotyphosa* (1:400), and one cow was positive for *L. bratislava* (1:1600), *pomona* (1:400), and *grippotyphosa* (1:200). *Leptospira* spp. were not isolated from any of the seropositive elk, and all fluorescent antibody tests were negative. Ten of 11 elk, including eight of nine seropositive cows, were pregnant based on the presence of pregnancy specific protein B (Sassar et al., 1986).

The high seroprevalence of *L. interrogans* antibodies in both captured elk and the elk killed in November 1993 is evidence that exposure in the CMC herd was widespread. Exposure to *L. interrogans* usually occurs through direct contact with urine from carrier animals or indirectly by contact with a urine-contaminated environment (Thorne, 1982). *Leptospira inter-*

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rogans excreted in urine may survive for several weeks in stagnant ponds, moist alkaline soils, and slow-moving alkaline streams (Thorne, 1982). *Leptospira interrogans* is very sensitive to drying; survival is poor in well drained soils, even slightly acidic (pH < 7) environments, and under conditions of slow freezing and thawing of substrates. On the CMC lands, abundant shallow standing water and the presence of marshy drainage ditches on the adjacent agricultural lands provided an ideal environment for *L. interrogans* to avoid desiccation and persist locally. Additionally, a herd of unvaccinated cattle occurred sympatrically with the CMC elk herd. Previously, we had tested >300 elk from throughout western Washington and found little or no exposure to *L. interrogans* (P. B. Hall, unpubl.). Although we did not specifically test either the local cattle or small mammal populations for *L. interrogans,* the presence of the unvaccinated cattle was the most apparent difference between the CMC elk herd and other elk populations which had no exposure. Local elk thus were likely to be continually exposed to *L. interrogans.*

Leptospirosis causes abortion in ungulates (Fraser and Mays, 1986). Elk calf recruitment was high in the CMC herd (fall calf : cow ratios of >40:100), however, despite the high seroprevalence to *L. interrogans.* Thus leptospirosis, if present, may be unimportant in the productivity of elk, in contrast to the effects seen in livestock (Heath and Johnson, 1994). Additionally, the nature of the hilly, heavily logged industrial forest surrounding the CMC lands makes the spread of *L. interrogans* to other elk populations unlikely. The surrounding topography minimizes the occurrence of slow moving or shallow stagnant water, limiting the chances of spread to other elk populations. The short bacterial shedding periods documented in other cervids, <15 days in white-tailed deer (Trainer et al., 1961) and <6 wk in moose (McGowan et al., 1963), along with the lack of transfer to susceptible individuals in contact with shedding individuals (Trainer et al., 1961), are further evidence that leptospirosis is self-limiting in wild cervids (Thorne, 1982).

Lastly, we observed serologic reaction to three serovars (*L. grippotyphosa, bratislava,* and *pomona*) in the CMC herd. Although serovar cross-reactions are likely (Thorne, 1982), the high titer levels are evidence for wide exposure to different sources of *L. interrogans.* However, it also is possible that a previously unidentified serovar exists and is causing false positive tests among the other identified *L. interrogans* serovars. In the future, we hope to test this hypothesis by capturing and testing calves from the CMC herd.

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