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Authors: Bischof, Richard, and Rogers, Douglas G.

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Serologic Survey of Select Infectious Diseases in Coyotes and Raccoons in Nebraska

Richard Bischof[1,3] **and Douglas G. Rogers**[2] [1]Nebraska Game and Parks Commission, 2200 North 33rd Street, Lincoln, Nebraska 68503-0370, USA; [2]Department of Veterinary and Biomedical Sciences, Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0907, USA; [3]Corresponding author (email: richard_bischof@usgs.gov)

ABSTRACT: To obtain data about select zoonotic and other infectious diseases in free-ranging predators in five ecoregions in Nebraska, sera were collected from 67 coyotes (*Canis latrans*) and 63 raccoons (*Procyon lotor*) from November 2002 through January 2003. For coyotes, antibodies were detected against canine distemper virus (CDV, 61%), *Francisella tularensis* (32%), *Rickettsia rickettsi* (13%), and flaviviruses (48%). None of the coyote sera had antibodies to *Borrelia burgdorferi, Brucella canis,* or six serovars of *Leptospira interrogans.* Because serologic cross-reactivity exists among flaviviruses, 14 sera from flavivirus-positive coyotes were also tested for St. Louis encephalitis virus (SLE) antibodies and two (14%) were positive, suggesting that up to 48% of coyotes tested had antibodies against West Nile virus (WNV). For raccoons, antibodies were detected against CDV (33%), *F. tularensis* (38%), and three serovars of *L. interrogans* (11%).

Key words: Canine distemper virus, coyote, *Francisella tularensis, Leptospira interrogans,* Nebraska, raccoon, *Rickettsia rickettsi,* serology, West Nile virus.

Wild mammalian predators are hosts to several diseases that may have an impact on wildlife populations, domestic animals, and occasionally humans. Because of this, mammalian predators have been the focus of numerous disease investigations and have been recommended as sentinels for wildlife and zoonotic diseases (Thomas and Hughes, 1992; Mitchell et al., 1999; Olson et al., 2000). Coyotes (*Canis latrans*) and raccoons (*Procyon lotor*) are ideal candidates for surveillance studies because both species are common and widespread in North America (Bekoff, 1982; Kaufmann, 1982). Both species are also highly mobile and readily adapt to areas inhabited by humans where they may serve as reservoirs for zoonoses and other diseases. Coyotes and raccoons have habitats throughout Nebraska, although raccoon numbers tend to be higher near water and in agricultural areas (Jones et al., 1983). Large numbers of both species are killed each year for fur harvest, sport, and damage control in Nebraska (Landholt and Genoways, 2000), and these activities present opportunities for sample collection and disease surveillance.

The objective of this study was to collect baseline serologic data for select zoonotic and other infectious diseases in free-ranging coyotes and raccoons in Nebraska. The infectious diseases surveyed in this study were selected based on relevance to wildlife populations, humans, or domestic animals.

From November 2002 through January 2003, sera were collected from 67 coyotes and 63 raccoons killed in five ecoregions (Chapman et al., 2001) by trappers, hunters, and damage control specialists during fur harvest and depredation control activities. Because of spatial differences in sample collection opportunities, coyote sampling was more likely to occur in the western two-thirds of the state (eight different river basins, five main ecoregions; Fig. 1), and all raccoon samples were collected in the eastern one-half of the state (six different river basins, two main ecoregions; Fig 1).

Blood samples (5–10 ml) were collected postmortem from each animal via cardiac puncture and then centrifuged. Sera were stored at −70 C until testing. The location for each animal was recorded with a global positioning system unit or solicited from the harvester in the form of distance and bearing from a nearby town.

Sera were examined for neutralizing antibodies to canine distemper virus (CDV)

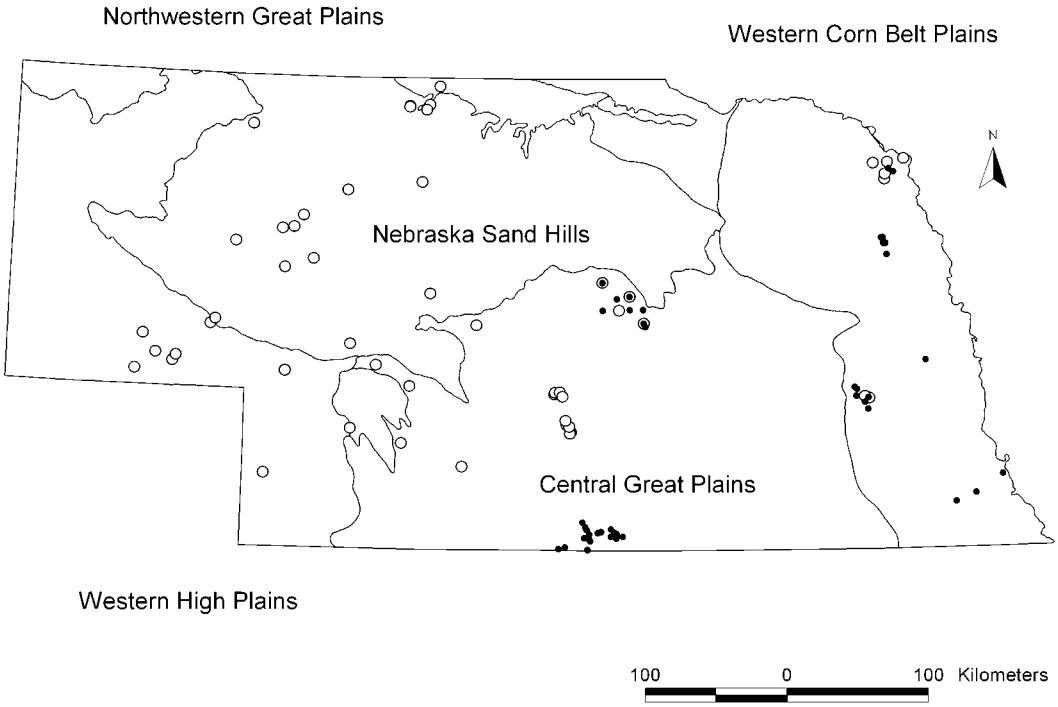

FIGURE 1. Map of Nebraska showing ecoregions and sampling locations for coyote (*Canis latrans*) sera (open circles) and raccoon (*Procyon lotor*) sera (black dots).

by using the Onderstepoort strain of CDV adapted to Vero cells (Kansas State University Veterinary Diagnostic Laboratory, Manhattan, Kansas). The starting dilution was 1:2, and neutralization at this dilution or higher was considered positive for antibodies to CDV.

Immunoglobulin G (IgG) antibodies to flaviviruses were detected as previously described (Davidson et al., 2005), but a 1:400 dilution of goat anticanine IgG served as the capture antibody and 3,3′,5,5′-tetramethylbenzidine served as the enzyme substrate. Because of possible cross-reactivity between West Nile Virus (WNV) and St. Louis encephalitis (SLE) virus antibodies, 14 coyote sera that were positive for flavivirus antibodies were tested at the New York State Animal Health Diagnostic Laboratory (Cornell University, Ithaca, New York) for SLE antibodies using a plaque reduction neutralization test (PRNT) (De Madrid and Porterfield, 1974; Calisher et al., 1989).

Test kits (VMRD Inc., Pullman, Washington) using indirect fluorescent antibody methodology were used to detect antibodies to *Rickettsia rickettsi* and *Borrelia burgdorferi.* Each procedure incorporated positive and negative control sera and was done according to the manufacturer's instructions.

Test kits were also used to detect antibodies to *Brucella canis* (Synbiotics, San Diego, California) and *Francisella tularensis* (Difco, Detroit, Michigan). Both of these rapid slide agglutination procedures were done according to the manufacturers' instructions.

Antibodies to *Leptospira interrogans* were detected by microagglutination methodology adopted by the National Veterinary Services Laboratories (NVSL), Ames, Iowa. Live serovars of *L. interrogans* (bratislava, canicola, grippotyphosa, icterohemorrhagiae, hardjo, pomona) were mixed with serially diluted test sera, serovar-specific positive control sera, and neg-

ative control serum (fetal calf serum) in 96-well, flat-bottom microtiter plates and incubated at room temperature for 1.5 hr. Samples were then examined by darkfield microscopy (10×) for agglutination. Sera that caused agglutination at dilutions of 1:200 or higher were considered to be positive for exposure to a respective serovar. The 95% binomial confidence interval was calculated for all antibody prevalence estimates.

CDV serology was conducted on 66 coyotes and 63 raccoons. Titers of 2 or greater were detected in sera from 40 coyotes (61%, 95% confidence interval[CI]: 47.8–72.4) and 21 raccoons (33%, 95% CI: 22.0–46.3). Prevalence of CDV antibodies in coyotes was within the range of values reported elsewhere: 7% in Utah (Arjo et al., 2003), 57% in Colorado (Gese et al., 1991), and 76% in Wyoming (Gese et al., 1997). Prevalence of CDV antibodies in raccoon sera was also within the range of values reported for raccoons in Illinois (23%; Mitchell et al., 1999) and Florida (55%; Hoff et al., 1974).

F. tularensis serology was conducted on 60 coyotes and 60 raccoons. Sera from 19 coyotes (32%, 95% CI: 20.3–45.0) and 23 raccoons (38%, 95% CI: 26.1–51.8) were positive. Prevalence of *F. tularensis* antibodies has also been reported for coyotes in Wyoming (0–30%; Gese et al., 1997) and Idaho (88%; Gier, 1978), and for raccoons in Tennessee (45.3%; Burgdorfer et al., 1974) and in Georgia and Florida (24.9%; McKeever et al., 1958).

Antibodies to *L. interrogans* were not detected in sera from the 67 coyotes tested (95% CI: 0.0–4.4). Sera from seven of the 63 raccoons were positive for *L. interrogans* (11%, 95% CI: 4.6–21.6) and antibodies to the following serovars were detected: *L. interrogans* serovar bratislava in two raccoons (3%, 95% CI: 0.4–11.0), *L. interrogans* serovar grippotyphosa in six raccoons (10%, 95% CI: 3.6–19.6), and *L. interrogans* servoar pomona in one raccoon (2%, 95% CI: 0.04–8.5). Exposure to these serovars has been reported in raccoons (Shotts, 1981; Mikaelian et al., 1997), and raccoons have been identified as a major reservoir of *L. interrogans,* especially *L. interrogans* serovar grippotyphosa (Shotts, 1981; Mitchell et al., 1999). The prevalence of *L. interrogans* antibodies in raccoons in this study was lower than that seen in Louisiana (Roth, 1964) and Illinois (Mitchell at al., 1999) where prevalence of 22% and 48%, respectively, was reported.

Ricketsia rickettsi serology was conducted on 64 coyotes, and eight (13%, 95% CI: 5.6–23.2) were positive. Antibodies to *B. canis* and *B. burgdorferi* were not detected in sera from 64 and 63 coyotes, respectively.

Flavivirus antibodies were detected in sera from 32 of 67 coyotes (48%, 95% CI: 35.4–60.3). Sera from two (14%) of 14 flavivirus-positive coyotes tested positive for SLE. The remaining 18 flavivirus-positive sera could not be examined for SLE antibodies because of insufficient amounts. Our limited testing for SLE and the fact that the majority of coyote sera were collected 6 months after WNV was first reported in Nebraska (W. Kramer, Nebraska Health and Human Services System, pers. comm.) suggest that the flavivirus antibodies found in this study are probably WNV antibodies.

Despite the relatively limited number of samples in this study, these data represent the most detailed serologic investigation for select diseases in wild mammalian predators in Nebraska. The results indicate that coyotes and raccoons in Nebraska are exposed to several diseases, and seroprevalence is comparable to that seen in other investigations in North America. An exception to this was the high prevalence of flavivirus antibodies in coyotes. The high prevalence and widespread occurrence of what are likely WNV antibodies within 6 months of the first reported case of WNV in Nebraska suggests a potential role for coyotes as sentinels for WNV exposure. Coyotes are readily available for serologic testing as they occur throughout

the United States and portions of Alaska, Canada, and Mexico where they are harvested for fur, sport, and damage control purposes. Dogs have also been suggested as sentinels for WNV (Komar et al., 2001).

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