DOI: 10.7589/2014-03-065

Journal of Wildlife Diseases, 50(4), 2014, pp. 946–950

Serologic Survey for Cross-Species Pathogens in Urban Coyotes (*Canis latrans*), Colorado, USA

Ashley Malmlov,[1] Stewart Breck,[2] Tricia Fry,[2] and Colleen Duncan[1,3] [1]Department of Microbiology, Immunology, Pathology, Colorado State University, 1644 Campus Delivery, Fort Collins, Colorado 80523, USA; [2]US Department of Agriculture, Animal and Plant Health Inspection Service, National Wildlife Research Center, 4101 Laporte Ave., Fort Collins, Colorado 80521, USA; [3]Corresponding author (email: colleen.duncan@colostate.edu)

ABSTRACT: As coyotes (*Canis latrans*) adapt to living in urban environments, the opportunity for cross-species transmission of pathogens may increase. We investigated the prevalence of antibodies to pathogens that are either zoonotic or affect multiple animal species in urban coyotes in the Denver metropolitan area, Colorado, USA, in 2012. We assayed for antibodies to canine parvovirus-2, canine distemper virus, rabies virus, *Toxoplasma gondii*, *Yersinia pestis*, and serotypes of *Leptospira interrogans*. Overall, 84% of the animals had antibodies to canine parvovirus-2, 44% for canine distemper virus, 20% for *T. gondii* (IgG), 28% for *Y. pestis*, and 4% for *L. interrogans* serotype Grippotyphosa. No neutralizing antibodies were detected to rabies virus, *T. gondii* (IgM), or *L. interrogans* serotypes other than Grippotyphosa. With 88% of animals exposed to at least one pathogen, our results suggest that coyotes may serve as important reservoirs and sentinels for etiologic agents.

Key words: Coyotes, distemper, *Leptospira*, parvovirus, rabies, *Toxoplasma*, urban, *Yersinia*.

Coyote (*Canis latrans*) populations and ranges have greatly expanded and now include nearly all major cities of the US and Canada (Gehrt and Riley 2010). This population increase creates potential for interactions with humans and domestic animals and for spread of disease. Existing serologic studies investigate a variety of pathogen exposures in coyotes within rural settings; however, few assess urban populations. We measured the prevalence of exposure to multiple pathogens that are either zoonotic or cause disease in coyotes in the Denver metropolitan area (DMA), Colorado, USA.

We collected serum samples from 25 coyotes (16 males, nine females) that we captured, radio-collared, and released between April and November 2012 as part of a larger study (USDA-National Wildlife Research Center's IACUC QA-1972) in the DMA (Fig. 1). We captured and handled coyotes only once. We used locational data collected from animals (via radio-tracking and global positioning system [GPS] collars) to classify each individual as exurban or urban and, through tracking, were able to demonstrate that none ventured far from human settlement—making them distinct from rural coyotes (S.B. unpubl. data). These classifications were developed by collapsing definitions of housing density (Theobald 2005; Poessel et al. 2013) for rural and exurban into "exurban" and suburban and urban into "urban." Labeling a coyote this way is difficult because of 1) large movements, especially by dispersing juveniles and 2) the ability of individuals to use varying degrees of housing density during a single night. We were not able to attain location data on three individuals, which are classified as unknown (Table 1).

At Colorado State University's Veterinary Diagnostic Laboratory, the following assays were performed: hemagglutination inhibition (HI) for antibodies to canine parvovirus-2 (Carmichael et al. 1980; positive titer $\geq$1:8); serum neutralization for detection of canine distemper virus (adapted from Carbrey et al. 1971; positive titer $\geq$1:2); microscopic agglutination test (MAT) for antibodies against *Leptospira* serotypes (Anderson 2009; positive titer $\geq$1:100); and enzyme-linked immunosorbent assay (ELISA) for immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against *Toxoplasma gondii* (adapted from Lappin et al. 1989; positive titer $\geq$serum dilution of 1:64). At Kansas State University, Manhattan, Kansas, USA, a

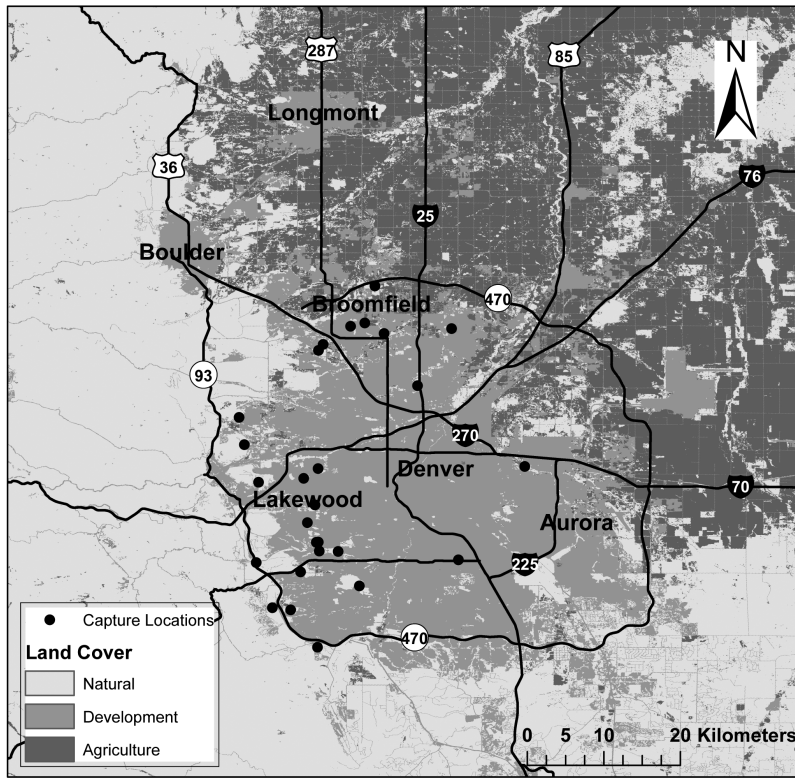

FIGURE 1. Capture locations in relation to land-use categories for coyotes (*Canis latrans*) tested for antibodies to several pathogens in the Denver metropolitan area, Colorado, USA.

rapid fluorescent focus inhibition test (RFFIT) was used to detect antibodies to rabies virus. A positive reportable range was 0.1 to 15.0 IU/mL. A passive hemagglutination/inhibition assay (PHA/HI) for antibodies to *Yersinia pestis* was performed at the Centers for Disease Control and Prevention, Fort Collins, Colorado. A titer ≥1:16 was considered positive. Table 2 summarizes the antibody prevalence as a group.

An 84% antibody prevalence was determined for canine parvovirus-2 (CPV-2). Titers ranged from 1:512 to ≥1:16,384. It is believed that CPV-2 emerged from a mutated strain of feline panleukopenia virus in Europe and spread globally within 2 yr during the late 1970s, first reported in the US in 1978 (Carmichael 2005). Prior to the 1970s, no antibodies to CPV-2 were detected in coyotes from Texas, Utah, or Idaho. Shortly after the introduction of CPV-2 to the US, ≥90% prevalence was found in these states (Thomas et al. 1984). With the development of an effective vaccine, the incidence of disease has dramatically decreased in the domestic dog population (Carmichael 2005). However, high levels of exposure are still seen in coyotes in rural areas of Colorado, with 70% antibody prevalence between 1985 and 1988, and 92% prevalence between 1997 and 2001 (Gese et al. 1991, 2004). The virus is resistant to environmental degradation and may remain infectious in the environment for months to years (Lamm and Rezabek 2008). If CPV-2 is enzootic in coyote populations, they may infect other susceptible species directly, or indirectly by shedding virus and contaminating the environment.

Prevalence of antibody to canine distemper virus (CDV) was 44% (11/25; seven males, four females). Titers ranged from 1:2 to 1:2,048. Our results are

TABLE 1. Individual coyotes (*Canis latrans*) tested, test results (titers), and urban/exurban status. Urban status was determined based on location data collected from coyotes (see Methods for details), Denver metropolitan area, Colorado, USA, 2012.

Coyote ID and sex[a]	Parvovirus HI titer[b]	CDV SN titer[c]	*Y. pestis* PHA/HI[d]	*T. gondii* IgG ELISA titer[e]	*L. interrogans* Grippotyphosa MAT titer[f]	Urban status
13 M	≥1:16,384	1:512	1:1,024	1:64	Neg	Unknown
14 F	≥1:16,384	1:256	Neg	Neg	Neg	Exurban
24 M	≥1:16,384	1:2	1:64	Neg	Neg	Exurban/urban
12 F	≥1:16,384	Neg	1:16	1:128	Neg	Unknown
23 M	≥1:16,384	Neg	Neg	1:64	Neg	Exurban/urban
27 F	1:8,192	Neg	Neg	Neg	Neg	Unknown
08 F	1:8,192	Neg	Neg	Neg	Neg	Urban
11 M	1:4,096	Neg	Neg	Neg	Neg	Exurban/urban
07 M	1:4,096	Neg	1:1,024	Neg	Neg	Urban
01 M	1:2,048	1:256	1:512	Neg	Neg	Urban
10 F	1:2,048	1:64	Neg	Neg	Neg	Urban
06 M	1:2,048	Neg	Neg	Neg	Neg	Urban
19 M	1:2,048	Neg	Neg	Neg	Neg	Urban
21 M	1:2,048	Neg	Neg	Neg	Neg	Urban
04 M	1:1,024	1:1,024	Neg	Neg	Neg	Urban
16 M	1:1,024	1:1,024	1:16	Neg	Neg	Urban
02 F	1:1,024	1:2	1:16	Neg	1:200	Urban
03 F	1:1,024	Neg	Neg	1:64	Neg	Urban
15 M	1:1,024	Neg	Neg	Neg	Neg	Urban
26 M	1:512	1:256	Neg	Neg	Neg	Exurban
07 M	1:512	1:16	Neg	1:128	Neg	Urban
05 F	Neg	1:2,048	Neg	Neg	Neg	Urban
17 M	Neg	Neg	Neg	Neg	Neg	Urban
18 F	Neg	Neg	Neg	Neg	Neg	Exurban/Urban
20 M	Neg	Neg	Neg	Neg	Neg	Urban

[a] ID = identification number; M = Male; F = female.

[b] HI = hemagglutination inhibition; Neg = negative.

[c] CDV = canine distemper virus; SN = serum neutralization; Neg = negative.

[d] *Y. pestis* = *Yersinia pestis*; PHA/HI = passive hemagglutination/inhibition assay.

[e] *T. gondii* = *Toxoplasma gondii*; IgG = immunoglobulin G; ELISA = enzyme-linked immunosorbent assay.

[f] *L. interrogans* = *Leptospira interrogans*; MAT = microscopic agglutination test.

TABLE 2. Prevalence of antibodies to specific pathogens in coyotes (*Canis latrans*) in the Denver metropolitan area, Colorado, USA, 2012.

Pathogen	Prevalence, % (no. positive) $n=25$
Parvovirus-2	84 (21)
Canine distemper virus	44 (11)
Yersinia pestis	28 (7)
Toxoplasma gondii IgG	20 (5)
Leptospira interrogans Grippotyphosa	4 (1)
With detectable antibody to at least one pathogen	88 (22)

consistent with those of investigators in southern Colorado who found 57% and 40% prevalences (Gese et al. 1991, 2004). It has been proposed that, like CPV-2, CDV is enzootic in coyotes. Many species are susceptible to both CPV-2 and CDV. In the multifaceted territorial overlap of urban environments, outbreaks are most likely multifactorial with complicated disease ecologies.

We measured a 20% (5/20; three males, two females) prevalence of *T. gondii* IgG antibody. No coyotes had detectable IgM. Our results are lower when compared to studies in other states: 28–62% in California,

Indiana, Missouri, Kansas, Kentucky, Ohio, and Texas (Lindsay et al. 1996; Dubey et al. 1999). Unlike previous studies, we used an ELISA that allowed us to differentiate between acute and historical exposure by measuring IgG (historical) and IgM (acute). No coyotes had detectable IgM, suggesting that all exposure was historical.

Toxoplasma gondii is a public health concern because humans can be aberrant intermediate hosts. The most common routes of human infection are through consumption of contaminated foods or undercooked meat, but infection can also occur when exposed to environmental oocysts. *Toxoplasma gondii* has a complex life cycle involving cats as the definitive host (Dubey 1998). The role of coyotes in the zoonotic component of *T. gondii* is probably small; canids do not support the cycling of oocysts, and coyotes are not consumed by humans. However, dogs given sporulated oocysts orally can pass oocysts in their feces, serving as mechanical vectors (Lindsay et al. 1997). Thus, coyotes may ingest oocysts and spread them to other areas.

Prevalence of antibody to *Y. pestis* was 28% (7/25; five males, two females). Titers ranged from 1:16 to 1:1,024. These results are within ranges previously reported. Gese et al. (2004) reported a prevalence of 82% in northern Colorado in 1997–2001. Salkeld and Stapp (2006) found an average of 15% prevalence across the western US in 1973–2004, while Brinkerhoff et al. (2009) detected no antibodies in coyotes in Boulder County, Colorado, in 2009.

Coyotes have been used as sentinel species for *Y. pestis* because they are resistant to disease yet seroconvert (Salkeld and Stapp 2006). While low antibody prevalence is generally maintained in coyote populations, an increase in prevalence may indicate an epizootic. Coyotes become infected with *Y. pestis* by consuming diseased prey or from the bite of an infected flea. The role of coyotes in the direct transmission of *Y. pestis* is probably small because they may not efficiently transmit the bacterium to the flea vector; however, they may play a significant role in distributing plague laden fleas to different areas and therefor are important to the disease ecology (Salkeld and Stapp 2006).

One female coyote (ID 02F; Table 1) had a titer of 1:200 for *L. interrogans* serovar Grippotyphosa using MAT. No antibodies were detected to the other serovars: Canicola, Hardjo, Icterhaeorrhagiae, Pomona, or Bratislava. Investigators in states surrounding Colorado reported a range in antibody prevalences to *L. interrogans* of 0–27%, and our findings are consistent with those from Nebraska, Wyoming, and Arizona (Gese et al. 1997; Grinder and Krausman 2001; Bischof and Rogers 2005). Because *L. interrogans* may infect a wide range of species by direct or indirect means, multiple urban wildlife species may play a role in disease ecology.

In Colorado, rabies virus is most commonly diagnosed in skunks and bats, but spillover can occur to other species. Because the disease is highly fatal, it was expected that none of the sampled coyotes had rabies virus antibodies using the RFFIT (Smith 1996).

Serologic surveys are useful for understanding the prevalence of pathogens in a population and to guide further investigation. Our prevalences were similar to those reported in the literature in rural settings, even though the environmental pressures between urban and rural settings are different. Urban environments provide an interesting landscape in which to study disease transmission because of the interfaces among wildlife, humans, and domestic animals. Concerns about disease transmission will continue to remain near the forefront as urban areas continue to grow and wildlife adapt, increasing the importance of understanding these interactions. With 84% of the animals in our study having detectable antibody to at least one pathogen, our results reinforce the importance of better understanding this ecologic niche.

We thank J. Brinker, S. Koyle, and F. Quarterone for assistance in trapping and handling coyotes and S. Poessel for help tracking and classifying animals and assistance with geographic information systems. We also appreciate the help of Kansas State University's Rabies Laboratory, and J. Young and the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado, for performing the RFFIT and HA and *Y. pestis* analyses, respectively.

LITERATURE CITED

Anderson T. 2009. *Microscopic agglutination test (MAT) for detection of* Leptospira *antibodies in animal serum*. National Veterinary Services Laboratories, Ames, Iowa, 12 pp.

Bischof R, Rogers DG. 2005. Serologic survey of select infectious diseases in coyotes and raccoons in Nebraska. *J Wildl Dis* 41:787–791.

Brinkerhoff RJ, Collinge SK, Bai Y, Ray C. 2009. Are carnivores universally good sentinals of plague? *Vector-Borne Zoonotic Dis* 9:491–497.

Carbrey EA, Brown LN, Chow TL, Kahrs RF, McKercher DG, Smithies LK, Tamoglia TW. 1971. Recommended standard laboratory techniques for diagnosing infectious bovine rhinotracheitis, bovine viral diarrhea and shipping fever (parainfluenza-3). *Proc US Anim Health Assoc* 75:629–648.

Carmichael L. 2005. An annotated historical account of canine parvovirus. *J Vet Med* 52:303–311.

Carmichael L, Joubert J, Pollock R. 1980. Hemagglutination by canine parvovirus: Serology studies and diagnostic applications. *Am J Vet Res* 41:784–791.

Dubey J. 1998. Advances in the life cycle of *Toxoplasma gondii*. *Int J Parasitol* 28:1019–1024.

Dubey JP, Storandt ST, Kwok OCH, Thulliez P, Kazacos KR. 1999. *Toxoplasma gondii* antibodies in naturally exposed wild coyotes, red foxes, and gray foxes and serological diagnosis of toxoplasmosis in red foxes fed *T. gondii* oocysts and tissue cysts. *J Parasitol* 85:240–243.

Gehrt SD, Riley SPD. 2010. Coyotes (*Canis latrans*). In: *Urban carnivores: Ecology, conflict and conservation*, Gehrt SD, Riley SPD, Cypher BL, editors. The John Hopkins University Press, Baltimore, Maryland, pp. 79–95.

Gese EM, Schultz RD, Rongstad OJ, Andersen DE. 1991. Prevalence of antibodies against canine parvovirus and canine distemper virus in wild coyotes in southeastern Colorado. *J Wildl Dis* 27:320–323.

Gese EM, Schultz RD, Johnson MR, Williams ES, Crabtree RL, Ruff RL. 1997. Serological survey for diseases in free-ranging coyotes (*Canis latrans*) in Yellowstone National Park, Wyoming. *J Wildl Dis* 33:47–56.

Gese EM, Karki SM, Klavetter ML, Schauster ER, Kitchen AM. 2004. Serological survey for canine infectious diseases among sympatric swift foxes (*Vulpes velox*) and coyotes (*Canis latrans*) in southeastern Colorado. *J Wildl Dis* 40:741–748.

Grinder M, Krausman PR. 2001. Morbidity-mortality factors and survival of an urban coyote population in Arizona. *J Wildl Dis* 37:312–317.

Lamm CG, Rezabek G. 2008. Parvovirus infection in domestic companion animals. *Vet Clin Sm Anim* 38:837–850.

Lappin M, Greene CE, Prestwood A, Dawe DL, Tarleton RL. 1989. Diagnosis of recent *Toxoplasma gondii* infection in cats by use of an enzyme-linked immunosorbent assay for immunoglobulin M. *Amer J Vet Res* 50:1580–1585.

Lindsay DS, Kelly EJ, McKown RD, Stein FJ, Plozer J, Herman J, Blagburn BL, Dubey JP. 1996. Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in coyotes (*Canis latrans*) and experimental infections of coyotes with *Neospora caninum*. *J Parasitol* 82:657–659.

Lindsay DS, Dubey J, Butler J, Blagburn B. 1997. Mechanical transmission of *Toxoplasma gondii* oocysts by dogs. *Vet Parasitol* 73:27–33.

Poessel SA, Breck SW, Teel TL, Shwiff S, Crooks KR, Angeloni L. 2013. Patterns of human coyote conflicts in the Denver metropolitan area. *J Wildl Manage* 77:297–305.

Salkeld DJ, Stapp P. 2006. Seroprevalence rates and transmission of plague (*Yersinia pestis*) in mammalian carnivores. *Vector-Borne Zoonot Dis* 6:231–239.

Smith JS. 1996. New aspects of rabies with emphasis on epidemiology, diagnosis, and prevention in the United States. *Clin Microbiol Rev* 9:166–176.

Theobald DM. 2005. Landscape patterns of exurban growth in the USA from 1980 to 2020. *Ecol Soc* 10:32.

Thomas NJ, Foreyt WJ, Evermann JF, Windberg LA, Knowlton FF. 1984. Seroprevalence of canine parvovirus in wild coyotes from Texas, Utah and Idaho (1972 to 1983). *J Am Vet Med Assoc* 185:1283–1287.

Submitted for publication 8 March 2014.
Accepted 12 June 2014.