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PARASITOLOGY AND SEROLOGY OF FREE-RANGING COYOTES (*CANIS LATRANS*) IN NORTH CAROLINA, USA

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ABSTRACT: Coyotes (*Canis latrans*) have expanded recently into the eastern US and can serve as a source of pathogens to domestic dogs (*Canis lupus familiaris*), livestock, and humans. We examined free-ranging coyotes from central North Carolina, US, for selected parasites and prevalence of antibodies against viral and bacterial agents. We detected ticks on most (81%) coyotes, with *Amblyomma americanum* detected on 83% of those with ticks. Fifteen (47%) coyotes were positive for heartworms (*Dirofilaria immitis*), with a greater detection rate in adults (75%) than juveniles (22%). Serology revealed antibodies against canine adenovirus (71%), canine coronavirus (32%), canine distemper virus (17%), canine parvovirus (96%), and *Leptospira* spp. (7%). We did not detect antibodies against *Brucella abortus/suis* or *Brucella canis*. Our results showed that coyotes harbor many common pathogens that present health risks to humans and domestic animals and suggest that continued monitoring of the coyote's role in pathogen transmission is warranted.

Key words: *Brucella, Canis latrans*, coyote, distemper, heartworm, leptospirosis, parasitology, serology.

INTRODUCTION

Coyotes (*Canis latrans*) have expanded recently into the eastern US and have attracted attention from wildlife management professionals due to their potential effects on prey populations (e.g., white-tailed deer [*Odocoileus virginianus*]; Kilgo et al. 2010; Chitwood et al. 2015). Coyotes are highly adaptable due to dietary and behavioral plasticity and can live in proximity to humans and domestic animals (Gehrt and Riley 2010), so attention to parasites and disease is also warranted. Understanding how coyotes contribute to the transmission or maintenance of pathogens is important because of their possible risk to domestic dogs (*Canis lupus familiaris*; e.g., canine distemper), livestock (e.g., brucellosis), and humans (e.g., leptospirosis). We performed a population survey of coyotes to determine the prevalence of selected parasites and pathogens.

MATERIALS AND METHODS

From February to June 2011, we used foothold traps to capture 37 coyotes at Fort Bragg Military Installation, North Carolina, US (Fort Bragg; 35°08′N, 79°12′W). We deployed GPS collars on 32 coyotes for a habitat selection and diet study (see Elfelt 2014) and obtained ticks, feces, and blood from most individuals. We muzzled and physically restrained all coyotes for attaching GPS collars and obtaining samples, weight, sex, and age (juvenile [≤1 yr], subadult [1–2 yr], adult [≥2 yr]; Gier 1968). We obtained fecal samples from 28 coyotes by collecting fecal material expelled during processing or using a fecal loop to swab the rectum. We obtained blood (~5 mL) from 32 coyotes in serum separator tubes via jugular venipuncture. We stored fecal samples in plastic zip-top bags in a −20 C freezer until processing and stored blood samples on ice until centrifuged (<12 h after collection). We aliquoted serum into vials and froze them at −20 C until processing. We conducted heartworm testing using a small aliquot of serum from all 32 coyotes and submitted the remainder for serology. Two serum samples had insufficient

volumes for serology, so we submitted 30 samples to the Southeastern Cooperative Wildlife Disease Study at the University of Georgia's College of Veterinary Medicine (methods described in the upcoming text; for some tests only 28 samples could be analyzed). Coyote trapping and handling protocols were reviewed and approved by the North Carolina Wildlife Resources Commission and the North Carolina State University (NCSU) Institutional Animal Care and Use Committee (11-005-O).

We sampled ticks from the ears of each coyote. We did not attempt to collect all ticks present but focused on sampling ticks of all life stages and species. We stored ticks in vials of 85% ethanol until identification by C. S. Apperson (Department of Entomology, NCSU). We evaluated intestinal parasite presence for 28 coyotes by performing a standard fecal float with centrifugation (Dryden et al. 2005). We used a sodium nitrate solution ($NaNO_3$; specific gravity 1.18–1.20), and J. R. Flowers (Department of Population Health and Pathobiology, NCSU) identified the parasites. Also, we tested serum samples of 32 coyotes for heartworms (*Dirofilaria immitis*) using a microwell enzyme-linked immunosorbent assay kit (DiroChek, Synbiotics Corporation, San Diego, California, USA) shown to have good specificity and sensitivity in coyotes (Sacks et al. 2002).

We focused serologic testing on selected pathogens, some of which previously have been evaluated in coyotes. We tested for *Brucella abortus/suis*, *Brucella canis*, canine adenovirus, canine coronavirus, canine distemper virus, canine parvovirus, and *Leptospira* spp. *Brucella abortus* is a bacterium that causes abortion and infertility in ruminants (Thorne 2001; Davidson 2006). *Brucella suis* is a closely related species that causes similar disease in domestic pigs (*Sus scrofa domesticus*), a major farming commodity in North Carolina. Antibodies against *B. abortus* and *Brucella suis* cannot be distinguished serologically, so they were considered together in this study. The Veterinary Diagnostic Laboratories (VDL; College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA) tested for antibodies to *B. abortus/suis* using a standardized, highly sensitive card test, with follow-up testing on positive samples via the Rivanol test, which is more specific.

Brucella canis circulates in dogs and causes similar clinical signs and zoonotic risk as *B. abortus*. Serologically, it is distinguishable, but it has not been detected in previous coyote surveys (Holzman et al. 1992; Gese et al. 1997; Bischof and Rogers 2005). The VDL conducted initial testing for *B. canis* using a high-sensitivity immunofluorescent antibody assay. Positive samples were sent to the Veterinary Diagnostic Laboratories in Tifton, Georgia, for confirmation using a tube agglutination test.

Canine adenovirus (CAV) has been reported in coyotes with varying prevalence (Holzman et al. 1992; Gese et al. 1997; Cypher et al. 1998; Grinder and Krausman 2001). Canine adenovirus includes two viruses (CAV-Type 1 and CAV-Type 2), and our testing did not distinguish between the two. Our samples were analyzed at the Washington Animal Disease Diagnostic Lab (WADDL; Washington State University, Pullman, Washington, USA) using a virus neutralization method.

Canine coronavirus (CCV) causes disease in domestic dogs, but clinical disease and maintenance of the virus is not well studied in free-ranging canids. Antibodies to CCV were not detected in a survey of coyotes from Georgia (Holzman et al. 1992). Our samples were analyzed by WADDL using an immunofluorescent antibody assay.

Canine distemper virus (CDV) is a *Morbillivirus* that can result in severe or fatal disease in numerous carnivore species. Antibodies to CDV have been detected in free-ranging coyotes (e.g., Trainer and Knowlton 1968; Gese et al. 1997; Cypher et al. 1998; Grinder and Krausman 2001), although at least one study failed to detect antibodies to this virus (Holzman et al. 1992). Our samples were analyzed by the VDL using a serum neutralization technique.

Canine parvovirus (CPV) produces severe, sometimes fatal, disease in numerous canid species but is usually most severe in young animals. High antibody prevalence has been reported in coyotes, with Gese et al. (1997) reporting 100% prevalence at all ages except very young and Grinder and Krausman (2001) reporting 100% prevalence in urban coyotes in Arizona. The VDL analyzed our samples using a serum neutralization assay.

Leptospira spp. are spirochete bacteria that cause leptospirosis, a zoonotic infection that can occur in most mammals (Leighton and Kuiken 2001). The bacteria are transmitted via urine of infected animals, and infection can be asymptomatic or involve fatal septicemia, hepatitis, nephritis, or reproductive disorders. Prevalence can range from 0% to 27% in coyotes (Trainer and Knowlton 1968; Holzman et al. 1992; Gese et al. 1997; Grinder and Krausman 2001; Bischof and Rogers 2005). The VDL analyzed our samples against *Leptospira interrogans* serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona.

RESULTS

We detected ticks on 30 of 37 (81%) coyotes. Of those with ticks, 50% ($n=15$) had >1 species; specifically, 83% ($n=25$) had *Amblyomma americanum*, 13% ($n=4$) *Amblyomma maculatum*, 47% ($n=14$) *Ixodes scapularis*, and 17% ($n=5$) *Dermacentor variabilis*. Of those with ticks, 20% ($n=6$) were parasitized by *A. americanum* nymphs; we did not detect nymphs of other tick species. From the fecal float, we detected *Ancylostoma caninum* in 26 (93%) of the tested coyotes and *Trichuris vulpis* in eight (29%); two of the 28 (7%) coyotes did not present intestinal parasites. Fifteen (47%) coyotes were positive for heartworms. Heartworms were more prevalent in adult coyotes (9/12=75%) than subadults (4/11=36%) or juveniles (2/9=22%).

All 28 coyotes tested for *B. abortus/suis* antibodies and all 30 tested for *B. canis* were negative. Serology for viral antibodies yielded the following prevalences: CAV=71% (20/28); CCV=32% (9/28); CDV=17% (5/30); and CPV=96% (27/28; the only negative coyote was a juvenile). Antibody prevalence to *Leptospira* spp. was 7% (2/30); one adult male had antibodies against serovar Canicola, and another adult male had antibodies against serovar Grippotyphosa.

DISCUSSION

The 47% heartworm prevalence we detected is similar to values reported in other coyote populations from the region (Holzman et al. 1992; Miller et al. 2009; Gates et al. 2014). However, the antigen test we used could have biased prevalence low if coyotes had male-only heartworm infections or low numbers of female worms. Coyote expansion into urban areas places them into proximity with domestic dogs, which increases the potential for mosquito transmission of heartworms (Gates et al. 2014). Likewise, proximity of coyotes and domestic dogs facilitates the potential to share endo- and ectoparasites, although our study identified ticks and intestinal parasites similar to previous reports from the southeastern US (Gates et al. 2014).

Negative results for *Brucella* spp. are encouraging given the risk brucellosis poses for domestic animals, particularly swine in North Carolina. Our results are consistent with previous surveys of coyotes that failed to detect antibodies to *B. abortus* (Trainer and Knowlton 1968; Gese et al. 1997) and *B. canis* (Holzman et al. 1992; Gese et al. 1997; Bischof and Rogers 2005). However, the North Carolina swine industry expanded rapidly in the 1990s (Furuseth 1997), commensurate with the expansion and establishment of coyotes in most parts of the state (Parker 1995; DeBow et al. 1998). *Brucella suis* has not been investigated in coyotes, and the simultaneous expansion of coyote populations, swine production, and feral swine (*Sus scrofa*) presence represented a novel disease risk with economic implications. Sandfoss et al. (2012) recently detected *B. suis* antibodies for the first time in feral swine in North Carolina. Coyotes are ubiquitous in North Carolina and occur together with feral swine in many areas. Coupled with the likelihood that coyotes consume feral swine at least as carrion (if not also through direct predation; Schrecengost et al. 2008), *Brucella* transmission from feral swine to coyotes is possible. Although our testing could not distinguish between *B. abortus* and *B. suis*, it appears likely the *Brucella* risk posed by coyotes is low currently. However, future research should evaluate coyote exposure to *Brucella* in areas where they are sympatric with feral swine because Fort Bragg does not have a feral swine population.

The prevalence of antibody to CPV (96%) at Fort Bragg was similar to other studies of coyotes (Holzman et al. 1992; Gese et al. 1997; Cypher et al. 1998; Grinder and Krausman 2001; Gates et al. 2014). Our study and several others did

not evaluate virus shedding, but Miller et al. (2009) reported that one of 36 coyotes in their South Carolina population was shedding based on electron microscopic examination of feces. Notably, Gates et al. (2014) reported 32% of Georgia coyotes were positive for CPV DNA via real-time PCR testing of feces, which suggested coyotes were shedding virus. Because sequencing results revealed CPV type 2 subtypes have been detected in domestic dogs in Georgia (Hong et al. 2007; Kapil et al. 2007), Gates et al. (2014) noted the potential for coyotes to serve as a reservoir or spillover host for CPV. Thus, the lack of understanding about the coyote's role in the epidemiology of CPV warrants continued monitoring and research.

Similar attention is warranted for CDV and CAV because they have the potential to affect domestic dogs and wildlife. Though Holzman et al. (1992) did not detect antibodies to CDV, we reported similar antibody prevalence as two other coyote studies from the southeastern US (Miller et al. 2009; Gates et al. 2014). Gates et al. (2014) speculated that it was possible these results could represent a recent spillover of CDV into coyotes in the region (from domestic dogs, which are considered the primary reservoir). Holzman et al. (1992) did not report results for CAV, but again, we detected similar antibody prevalence compared to recent studies from the southeastern US (Miller et al. 2009; Gates et al. 2014). Further, antibodies to CAV are reported with similar prevalence from other parts of the coyote's geographic range (Wyoming [Gese et al. 1997]; California [Cypher et al. 1998]; Arizona [Grinder and Krausman 2001]), suggesting consistent exposure to CAV in wild populations and potential transmission to domestic canids where they occur together.

Canine coronavirus is not known to be zoonotic, and the risk of transmission between wild and domestic canids is not well understood. The only coyote-related results we found from the southeastern US were by Holzman et al. (1992), who reported no antibodies to CCV. Davidson et al. (1992) reported an antibody prevalence of 23% in coyotes confiscated in South Carolina from an illegal translocation; however, the coyotes were most likely obtained in or near Ohio. Foreyt and Evermann (1985) reported 5% antibody prevalence in coyotes from the western US, and Zarnke et al. (2001) reported prevalences ranging from 0% to 70% (depending on time of year) in gray wolves (*Canis lupus*) in Alaska. Thus, the high prevalence (32%) at Fort Bragg indicates frequent exposure, at least for the southeastern US where antibodies to CCV have not been reliably detected in free-ranging coyotes. Although the implications are difficult to interpret due to a paucity of information on CCV, future research should explore the transmission and maintenance of this pathogen in free-ranging canids and the possible risk to domestic animals.

Leptospirosis is zoonotic, and although risk of exposure is not specific to coyotes, increasing proximity of coyotes, domestic canids, and humans makes the disease important. Although reported prevalence of antibodies to *Leptospria* has been low (i.e., no higher than 27%), a variety of serovars has been detected. Miller et al. (2009) detected antibodies to five serovars, and only Grippotyphosa overlapped with our results. Similarly, Gese et al. (1997) detected antibodies to Grippotyphosa but not Canicola (our only other detection). Grinder and Krausman (2001) detected antibodies to five serovars, including both of those that we detected. Variation in detection of these serovars may reflect geographic or temporal differences (Miller et al. 2009); only future research will elucidate the pattern and potential risk.

As coyote populations increase, interaction and risk of pathogen transmission with domestic animals and humans also increases. We showed that coyotes are exposed to many common pathogens that

present health risks to domestic dogs. However, results must be interpreted cautiously because our primary research goal (deploying GPS collars) precluded the use of potentially invasive sampling. Thus, our methods did not include gross or histologic testing to assess lesions or clinical signs of disease. Though the coyote's role in transmission and maintenance has not been conclusively established, future research should investigate the epidemiologic role of coyotes and the interface with domestic dogs (Gates et al. 2014), as well as potential effects on domestic stock. The recent expansion of coyote populations across the southeastern US has created the potential for new ecological interactions (e.g., behavioral, epidemiologic). Understanding disease dynamics among coyotes and the species with which they interact is important because their relationships are novel in many areas. Continued surveillance of coyote pathogens will give researchers a better understanding of coyote mortality and morbidity sources, while elucidating their role in disease ecology in the region (Miller et al. 2009).

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