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## ***Neospora caninum* Exposure in Overlapping Populations of Coyotes (*Canis latrans*) and Feral Swine (*Sus scrofa*)**

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**ABSTRACT:** Limited information exists on *Neospora caninum* transmission dynamics in wildlife. This coccidian parasite, whose presence can lead to substantial economic losses in cattle operations, requires a canid definitive host for reproduction. We examined exposure in a definitive host, coyotes (*Canis latrans*), and in overlapping populations of feral swine (*Sus scrofa*) to determine if spatial proximity between a definitive and incidental host influences the likelihood of parasite exposure. Eighteen percent of coyotes (95% confidence interval [CI]=14.2–21.8) and 15.8% of feral swine (95% CI=12.5–19.2) had been exposed to *N. caninum*, and this is the first report of exposure in US feral swine populations. Analyses suggest that the parasite is present throughout the environment and that exposure is not temporally or spatially linked to antibody-positive coyotes. Antibody-positive feral swine were found in an area where the only definitive host is domestic dogs (*Canis familiaris*), indicating that wild canids are not required to maintain the parasite in the environment.

**Key words:** Canine, coccidian, feral swine, *Neospora*, surveillance.

*Neospora caninum* is a coccidian parasite that produces environmentally resistant, infective oocysts that are passed through the digestive system of definitive hosts as part of a complex reproductive cycle. The only definitive hosts for *N. caninum* identified to date are canids, including coyotes (*Canis latrans*; Gondim et al., 2004), domestic dogs (*Canis familiaris*; Dubey et al., 1988b), and wolves (*Canis lupus*; Dubey et al., 2011). *Neospora caninum* was only first recognized as a separate parasite from *Toxoplasma gondii* in 1988 (Dubey et al., 1988a) and the list of canid definitive hosts will likely increase.

Serologic surveys suggest that a large number of domestic and wild mammals

are exposed to *N. caninum* (Dubey and Schares, 2011), but the parasite has been successfully isolated from only a few species. The primary interest in this organism stems from it being a primary cause of abortion in dairy and beef cattle (Anderson et al., 1991). Transmission in cattle is primarily vertical, with infected females passing the infection to calves. Epidemic outbreaks have been documented where >50% of dairy cows in a herd abort within several weeks of each other, but recrudescence is unpredictable in infected adult cows, which generally exhibit no clinical signs. Economic impacts associated with *N. caninum* are substantial, with annual losses in individual US states approaching tens of millions of dollars (Trees et al., 1999; Larson et al., 2004). In addition to cattle, *N. caninum* can cause clinical disease in dogs, sheep, and goats (Dubey and Schares, 2011). To understand *N. caninum* presence in the environment better, blood samples from coyotes, a definitive host, and feral swine, an invasive species that is expanding its range and abundance, were screened for *N. caninum* antibodies.

A majority of samples were opportunistically collected in conjunction with work conducted by United State Department of Agriculture (USDA)/Animal Plant Health Inspection Agency (APHIS)/Wildlife Services (WS). Samples were collected from 2009 through 2011, in cooperation with state and other federal agencies, from the southwestern and south central United States (Fig. 1). This region was preferentially targeted because of overlapping populations of both species. Sample selection was refined by focusing on

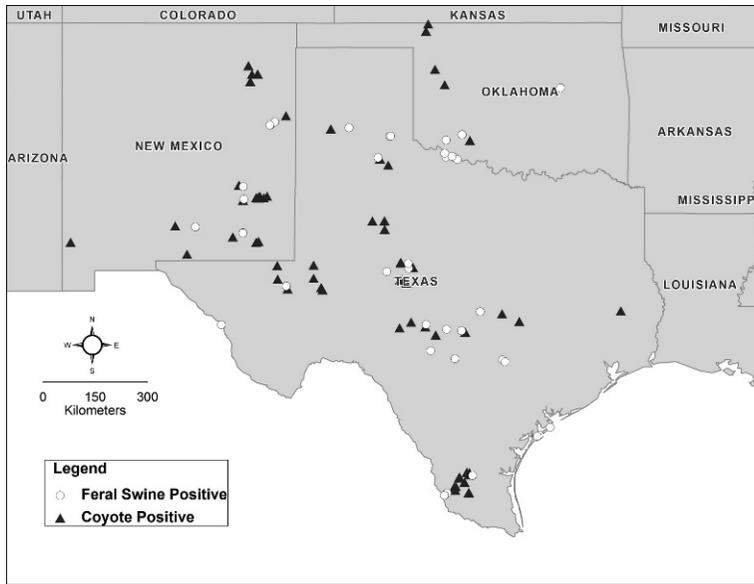


FIGURE 1. Sample locations for 71 *Neospora caninum*-positive coyotes and 74 *N. caninum*-positive feral swine in Oklahoma, New Mexico, and Texas, 2009–2011.

counties where coyotes and feral swine had been concurrently sampled. For coyotes, blood was collected on Nobuto (Advantec MFS, Dublin, California, USA) filter paper and stored at  $-20\text{ C}$  in the National Wildlife Disease Program Nobuto Sample Archive, at the USDA/APHIS/WS/National Wildlife Research Center in Fort Collins, Colorado, USA. For feral swine, blood was collected in ethylenediaminetetraacetic acid or serum separating tubes, processed according to protocol (Pedersen, 2012), and stored at  $-80\text{ C}$  in the Feral Swine Serum Archive at the NWDP.

Blood samples collected from coyotes on filter paper were eluted with phosphate-buffered saline with the use of previously reported protocols, resulting in a 1:10 sample dilution (Dusek et al., 2011). Samples were screened with the use of a commercially available *N. caninum* enzyme-linked immunosorbent assay (ELISA, Biovet®, Saint-Hyacinthe, Quebec, Canada), validated for use in multiple canine species, including coyotes (Wapenaar et al., 2007), at the manufacturer's suggested 1:10 sample dilution. Feral swine sera were tested

with the use of a commercially available competitive ELISA for *N. caninum* (VMRD, Inc., Pullman, Washington, USA) that has been extensively used in multiple species, including *S. scrofa*, because of high specificity and sensitivity, as well as the low cross-reactivity to closely related parasites (Haddad et al., 2005; Almería et al., 2007). All samples, positive controls, and negative controls were run in duplicate according to manufacturer's instructions. For both assays, the optical density sample-to-control ratio positive cutoff was  $\geq 0.30$ .

Data were mapped using ArcMap, v.10 (ESRI, Redlands, California, USA). Mean antibody prevalence and 95% confidence limits were determined with the use of a binomial distribution for prevalence in both species. Separate logistic regression analyses were also run (SAS, v.9.2, Cary, North Carolina, USA) for each species in order to determine pathogen associated risk in relation to animal sex, age (adult, subadult), and their interaction. Both variables are known to impact parasite exposure risk (Wouda et al., 1999). In addition, we ran a bivariate K-function analysis in program R with the use of

TABLE 1. Mean *Neospora caninum* antibody prevalence and 95% confidence intervals (CI) in coyotes and feral swine from the southwest and south central United States, 2009–2011.

Species	Variable	Category	Prevalence (%)	95% CI
Coyote ( <i>Canis latrans</i> )	Age	Adult	17.4	13.30–21.43
		Subadult	22	11.13–33.31
	Sex	Female	18.7	13.23–24.27
		Male	17.4	11.98–22.76
Feral swine ( <i>Sus scrofa</i> )	Age	Adult	19.3	14.81–23.72
		Subadult	9.6	5.15–14.13
	Sex	Female	19.3	14.4–24.14
		Male	11.7	7.40–16.06

spatstat (Baddeley and Turner, 2005) to determine the degree of clustering between positive definitive hosts and positive swine.

Samples from 394 coyotes and 467 feral swine were screened for *N. caninum* antibodies (Table 1). In total, 334 of the coyotes sampled were adults and 54 were subadults. Feral swine samples consisted of 301 adults and 166 subadults. Sampling of males and females was roughly equal, with 192 female and 190 male coyotes sampled, and 254 female and 213 male feral swine sampled. Both species showed exposure to *N. caninum* across Oklahoma, New Mexico, and Texas (Fig. 1). Overall, 18% of coyotes (95% CI=14.2–21.8) and 15.8% of feral swine (95% CI = 12.5–19.2) had been exposed to the parasite (Table 1). The mean inhibition for positive feral swine was 41.8% (SD=14.0); the mean optical density sample ratio for positive coyotes 44.6 (SD=16.9). Positive control means were 56.4 (SD=7.6) and 1.0 (SD=0.0), respectively, for feral swine and coyotes.

Logistic regression revealed different parasite exposure patterns for the two species. For coyotes, neither sex, age, nor their interaction were significant predictors of *N. caninum* exposure; however, age ( $F=6.26$ ,  $P=0.01$ ) and sex ( $F=3.8$ ,  $P=0.04$ ) were significant in feral swine. The sex-by-age interaction was not significant. Female feral swine were more likely to be infected than males ( $\chi^2=3.86$ ,  $P=0.49$ ; odds ratio [OR]=1.69, 95%

CI=1.001–2.865) and adults were more likely to be infected than younger animals ( $\chi^2=6.25$ ,  $P=0.01$ ; OR=2.1, 95% CI=1.17–3.85). Results from the bivariate-K spatial analyses revealed that positive feral swine did not tend to cluster with positive coyotes.

We examined *N. caninum* exposure in a definitive host, coyotes, and in overlapping populations of feral swine. The absence of differences in *N. caninum* antibody prevalence between coyote age classes or sexes suggests that exposure to the parasite is ubiquitous. As a definitive host (Gondim et al., 2004), coyotes are likely exposed through multiple routes of infection, including environmental contamination and ingestion of infected prey. This is supported by our finding of exposure in both adult animals and younger animals, with the latter possibly being exposed in denning sites.

Conversely, feral swine showed significant differences in antibody prevalence in relation to both age and sex. The higher prevalence in adult animals may be indicative of an increased chance for exposure over time through rooting behaviors. It is also possible that adult animals are more likely to eat an intermediate host (Barrett, 1978). A higher *N. caninum* antibody prevalence in female feral swine may reflect different behaviors that impact exposure risk; female domestic dogs have been found to have higher prevalence than males, possibly because of higher recrudescence rates associated with reproduction (Wouda et al., 1999).

Positive feral swine did not cluster spatially or temporally with positive coyotes. This is not surprising, because *N. caninum* is a coccidian parasite, which typically has infectious oocysts that persist in the environment for long periods of time once shed from the definitive host. There are multiple canid species in New Mexico, Texas, and Oklahoma, and they move across large spatial scales, so oocysts are likely widespread in the environment, leading to exposure in a wide range of species. Coyotes, domestic dogs, and other canids could all contribute to the parasite load in the environment (Dubey and Schares, 2011); however, previous research has not definitively linked exposure in cattle to either domestic dogs or coyotes (Barling et al., 2000, 2001). As a corollary to this study, feral swine samples collected in Hawaii revealed *N. caninum* exposure on all three islands tested—Hawaii, Oahu, and Kauai—with antibody prevalences of 24.1% (95% CI=13.9–37.2), 33.3% (95% CI=18–51.8), and 45.4% (95% CI=28.1–63.6), respectively (Bevins, unpublished). There are no native canids on Hawaii, with the only known source of infection being domestic dogs.

We have presented evidence of widespread *N. caninum* exposure in a definitive host species across a large spatial scale and provided the first reports of *N. caninum* exposure in US populations of feral swine, whose exposure may be indicative of cattle infection risk. This rapidly expanding invasive species is a potential sentinel for many infectious diseases, because of their presence in a range of habitats and their generalist diet. The analysis revealed different levels of exposure in each species, and spatial clustering analyses suggest that the parasite is present across the landscape. Much is still unknown about this disease system, and additional studies are required to understand exposure risk in livestock better.

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#### LITERATURE CITED

- Almería S, Vidal D, Ferrer D, Pabón M, Fernández-de-Mera MIG, Ruiz-Fons F, Alzaga V, Marco I, Calvete C, Lavin S, et al. 2007. Seroprevalence of *Neospora caninum* in non-carnivorous wildlife from Spain. *Vet Parasitol* 143:21–28.
- Anderson ML, Blanchard PC, Barr BC, Dubey JP, Hoffman RL, Conrad PA. 1991. Neospora-like protozoan infection as a major cause of abortion in California dairy cattle. *J Am Vet Med Assoc* 198:241–244.
- Baddeley A, Turner R. 2005. Spatstat: An R package for analyzing spatial point patterns.
- Barling KS, Sherman M, Peterson MJ, Thompson JA, McNeill JW, Craig TM, Adams LG. 2000. Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves. *J Am Vet Med Assoc* 217:1361–1365.
- Barling KS, McNeill JW, Paschal JC, McCollum FT, III, Craig TM, Adams LG, Thompson JA. 2001. Ranch-management factors associated with antibody seropositivity for *Neospora caninum* in consignments of beef calves in Texas, USA. *Prev Vet Med* 52:53–61.
- Barrett RH. 1978. The feral hog on the Rye Creek Ranch, California. *Hilgardia* 46:283–355.
- Dubey JP, Schares G. 2011. Neosporosis in animals—The last five years. *Vet Parasitol* 180:90–108.
- Dubey JP, Carpenter JL, Speer CA, Topper MJ, Uggla A. 1988a. Newly recognized fatal protozoan disease of dogs. *J Am Vet Med Assoc* 192:1269–1285.
- Dubey JP, Hattel AL, Lindsay DS, Topper MJ. 1988b. Neonatal *Neospora caninum* infection in dogs—Isolation of the causative agent and experimental transmission *J Am Vet Med Assoc*, 193:1259–1263.
- Dubey JP, Jenkins MC, Rajendran C, Miska K, Ferreira LR, Martins J, Kwok OCH, Choudhary S. 2011. Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. *Vet Parasitol* 181:382–387.
- Dusek RJ, Hall JS, Nashold SW, TeSlaa JL, Ip HS. 2011. Evaluation of nobuto filter paper strips for the detection of avian influenza virus antibody in waterfowl. *Avian Dis* 55:674–676.
- Gondim LFP, McAllister MM, Pitt WC, Zemlicka DE. 2004. Coyotes (*Canis latrans*) are definitive

- hosts of *Neospora caninum*. *Int J Parasitol* 34:159–161.
- Haddad JPA, Dohoo IR, VanLeewen JA. 2005. A review of *Neospora caninum* in dairy and beef cattle—A Canadian perspective. *Can Vet J* 46:230.
- Larson RL, Hardin DK, Pierce VL. 2004. Economic considerations for diagnostic and control options for *Neospora caninum*-induced abortions in endemically infected herds of beef cattle. *J Am Vet Med Assoc* 224:1597–1604.
- Pedersen K, Bevin SN, Schmit BS, Lutman MW, Milleson MP, Turnage CT, Bigelow TT, Deliberto TJ. 2012. Apparent prevalence of swine brucellosis in feral swine in the United States. *Hum Wildl Interact* 61:38–47.
- Trees AJ, Davison HC, Innes EA, Wastling JM. 1999. Towards evaluating the economic impact of bovine neosporosis. *Int J Parasitol* 29:1195–1200.
- Wapenaar W, Barkema HW, Schares G, Rouvinen-Watt K, Zeijlemaker L, Poorter B, O'Handley RM, Kwok OCH, Dubey JP. 2007. Evaluation of four serological techniques to determine the seroprevalence of *Neospora caninum* in foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) on Prince Edward Island, Canada. *Vet Parasitol* 145:51–58.
- Wouda W, Dijkstra T, Kramer AMH, van Maanen C, Brinkhof JMA. 1999. Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *Int J Parasitol* 29:1677–1682.

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