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Prevalence of Antibodies to *Neospora caninum* in White-Tailed Deer, *Odocoileus virginianus*, From the Southeastern United States

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ABSTRACT: Serum samples from 305 white-tailed deer (*Odocoileus virginianus*) from 14 states in the southeastern United States were examined for antibodies to *Neospora caninum* using a direct agglutination test. Positive agglutination titers were found in 145 (48%) of the white-tailed deer examined: 21 (7%) had titers of 1:25, 92 (30%) had titers of 1:50, and 32 (10%) had titers of $\geq$ 1:500. These findings that antibodies to *N. caninum* are common in white-tailed deer support the con-

cept that a sylvatic cycle might exist for this economically important parasite of domestic cattle.

Neospora caninum is recognized as a cause of neonatal neuromuscular disease in dogs and abortion in cattle worldwide (reviewed by Dubey and Lindsay, 1996; Lindsay and Dubey, 2000). Dogs are a de-

definitive host and excrete coccidial oocysts in their feces after ingesting *N. caninum* tissue cysts (McAllister et al., 1998; Lindsay, Dubey, and Duncan, 1999; Lindsay, Upton, and Dubey, 1999). However, little is known about the prevalence of the parasite in wildlife populations. *Neospora caninum* antibodies have been found in coyotes (Lindsay et al., 1996), dingos (Barber et al., 1997), and red foxes (Barber et al., 1997; Buxton et al., 1997; Simpson et al., 1997), suggesting a role for wild canids in the epidemiology of neosporosis. Dubey et al. (1999) found that 162 (41%) of 400 white-tailed deer (*Odocoileus virginianus*) from northeastern Illinois had agglutinating antibodies to *N. caninum*. A fatal case of neosporosis has been found in a 2-mo-old black-tailed deer fawn (*O. hemionus columbianus*) from California (Woods et al., 1994). These reports suggest that a wild canid-wild ruminant cycle might exist for *N. caninum*.

The present study was done to determine the prevalence of antibodies to *N. caninum* in white-tailed deer from the southeastern United States. Samples were collected from 305 white-tailed deer by members of the Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia. Samples were sent to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, for serological testing for agglutinating antibodies to *N. caninum*.

Tachyzoites of the NC-1 strain of *N. caninum* were used as antigen for the *Neospora* agglutination test (NAT). The general procedure described by Packham et al. (1998) was used with slight modifications. Briefly, tachyzoites were collected from infected African green monkey (*Cercopithecus aethiops*) kidney cells (CV-1 cells, ATTC CCL-70, American Type Culture Collection, Manassas, Virginia) and purified from host cells by passage through a 27-ga needle attached to a 10-ml syringe and filtration through a 3- μ m filter. The purified tachyzoites were fixed in 2 ml of 37% formaldehyde solution, which was then diluted to 6% with phosphate-buffered saline (PBS) and stored at 4 C. Prior to use in the NAT, the tachyzoites were washed twice in PBS and resuspended in alkaline buffer containing eosin (Packham et al., 1998) at 4×10^7 tachyzoites/ml and 0.2 M 2-mercaptoethanol. The eosin aided in the visualization of the agglutination reaction, and the mercaptoethanol was added to destroy IgM antibodies and prevent nonspecific agglutination caused by IgM molecules. The direct agglutination test was conducted in 96-well, round-bottom plates. Test sera were diluted with PBS, and 25 μ l of serial test dilutions was added to 75 μ l antigen solution and mixed thoroughly by pipetting up and down several times. Positive serum from *N. caninum*-infected mice and negative control sera from mice not infected were run on each plate. Sera from mice infected with *Toxoplasma gondii* and *Sarcocystis neurona* were also used on each plate as controls. The plates were covered and incubated

overnight at 37 C in a CO₂ incubator. The reaction was read the next morning. Diffuse white opacity across the entire diameter of the well was considered a positive agglutination reaction. A central discrete opaque dot or button was considered a negative reaction.

Serum samples from 145 (48%) of the 305 white-tailed deer examined were positive (Table I). Positive white-tailed deer samples were present in all 14 states. Of the positive white-tailed deer, 21 (7%) had titers of 1:25, 92 (30%) had titers of 1:50, and 32 (10%) had titers of $\geq 1:500$.

The results of the present study indicate that 48% of white-tailed deer from the southeastern United States have antibodies to *N. caninum*, similar to the 41% of white-tailed deer from northeastern Illinois reported by Dubey et al. (1999). These studies indicate that exposure to *N. caninum* by white-tailed deer is common. Because white-tailed deer are ruminants, it is likely that they become infected by ingesting *N. caninum* oocysts while feeding or drinking. The effect of *N. caninum* on white-tailed deer populations is not known. The finding of neonatal neosporosis in a black-tailed deer (Woods et al., 1994) indicates the potential for disease in white-tailed deer as well.

Dogs are the only known definitive host for *N. caninum* (McAllister et al., 1998; Lindsay, Dubey, and Duncan, 1999; Lindsay, Upton, and Dubey, 1999). Antibodies to *N. caninum* have been found in wild canids, but patent infections or clinical disease has not been observed (Lindsay and Dubey, 2000). Domestic dogs excrete relatively few *N. caninum* oocysts in their feces (Lindsay, Dubey, and Duncan, 1999; Lindsay et al., 2001), indicating that they are a poor definitive host. The role of wild canids needs to be studied further to determine their importance in the transmission of *N. caninum*.

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TABLE I. Prevalence of agglutinating antibodies to *Neospora caninum* in white-tailed deer from the southeastern United States.

State	Total*	Positive agglutination titer†			
		<1:25	1:25	1:50	1:500
Alabama	10/5 (50)	5 (50)	1 (10)	4 (40)	0
Arkansas	14/10 (71)	4 (29)	4 (29)	5 (36)	1 (7)
Florida	21/6 (29)	15 (71)	2 (10)	4 (19)	0
Georgia	61/20 (33)	41 (67)	1 (2)	17 (28)	2 (3)
Kentucky	24/6 (25)	18 (75)	1 (4)	3 (13)	2 (8)
Louisiana	20/6 (30)	14 (70)	0	3 (15)	3 (15)
Maryland	10/10 (100)	0	4 (40)	4 (40)	2 (20)
Mississippi	15/6 (40)	9 (60)	0	6 (40)	0
Missouri	22/12 (55)	10 (45)	3 (14)	7 (32)	2 (9)
North Carolina	15/5 (33)	10 (67)	0	4 (27)	1 (7)
South Carolina	27/15 (56)	12 (44)	2 (7)	12 (44)	1 (4)
Tennessee	20/11 (55)	9 (45)	2 (10)	5 (25)	4 (20)
Virginia	15/7 (47)	8 (53)	1 (7)	4 (27)	2 (13)
West Virginia	31/26 (84)	5 (16)	0	14 (45)	12 (39)
Total, all animals	305/145 (48)	160 (52)	21 (7)	92 (30)	32 (10)

* Total number sampled/number positive (percent positive from that state).

† Number positive at that titer (percent of total for that state).

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