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Prevalence of Agglutinating Antibodies to *Neospora caninum* in Raccoons, *Procyon lotor*

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ABSTRACT: *Neospora caninum* is an apicomplexan parasite that causes neonatal neuromuscular disease in dogs and abortions in cattle. Dogs are the only proven definitive host. Little is known about the prevalence of antibodies to this parasite in wildlife. Sera from 99 raccoons (*Procyon lotor*) were examined for agglutinating antibodies to *N. caninum* using the modified agglutination test employing formalin-fixed tachyzoites as antigen. Raccoons originated in Florida (n = 24, collected in 1996), New Jersey (n = 25, collected in 1993), Pennsylvania (n = 25, collected in 1999), and Massachusetts (n = 25, collected in 1993 and 1994). Ten (10%) had antibodies to *N. caninum*; 9 had titers of 1:50, and 1 (1%) had a titer of 1:100. The present study indicates that raccoons have minimal exposure to *N. caninum.* The sera were also tested for agglutinating antibodies to *Toxoplasma gondii* and 46 (46%) were positive; 16 had titers of 1:50, 8 had titers of 1:100, and 22 had titers of ≥1:500.

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 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 virgianus*) 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 sylvatic cycle may exist for *N. caninum.*

Little is known about the prevalence of *N. caninum* in wild omnivores. Omnivores would have exposure to both the tissue cyst and oo-

TABLE I. Prevalence of agglutinating antibodies to *Neospora caninum* (Nc) and *Toxoplasma gondii* (Tg) in raccoons from the United States.

State	Parasite	Total*	Raccoon titer†		
			1:50	1:100	≥1:500
Florida	Nc	24 (4)	1	0	0
	Tg	24 (29)	4	0	3
New Jersey	Nc	25 (12)	2	1	0
	Tg	25 (40)	2	1	7
Pennsylvania	Nc	25 (12)	3	0	0
	Tg	25 (24)	1	0	5
Massachusetts	Nc	25 (12)	3	0	0
	Tg	25 (92)	9	7	7
Total study	Nc	99 (10)	9	1	0
	Tg	99 (46)	16	8	22

* Total number of samples from that state (% positive).
† Number positive at that titer.

cyst stages of *N. caninum*. The present study was done to determine the prevalence of antibodies to *N. caninum* in raccoons (*Procyon lotor*) using a formalin-fixed whole tachyzoite agglutination test (Packham et al., 1998).

Sera samples from 99 raccoons were collected as part of part of a rabies surveillance program. Raccoons originated in Florida (n = 24, collected in 1996), New Jersey (n = 25, collected in 1993), Pennsylvania (n = 25, collected in 1999), and Massachusetts (n = 25, collected in 1993 and 1994). No information on age or sex of the raccoons is available. Frozen sera were sent to the Center for Molecular Medicine and Infectious Diseases, Virginia Tech, Blacksburg, Virginia, for agglutination testing. A modification of the *N. caninum* tachyzoite agglutination described by Packham et al. (1998) was used to test sera at dilutions of 1:50, 1:100, and 1:500 (Walsh et al., 2000). Sera were also tested for antibodies to *Toxoplasma gondii* using RH strain tachyzoites as antigen in the agglutination test.

Agglutinating antibodies to *N. caninum* were found in 10 (10%) of the 99 raccoons (Table I). Titers of 9 raccoons were 1:50, and 1 raccoon had a titer of 1:100.

Agglutinating antibodies to *T. gondii* were found in 46 (46%) of the 99 raccoons (Table I). Sixteen (16%) had titers of 1:50, 8 (8%) had titers of 1:100, and 22 (22%) had titers of ≥1:500. Seven of the 10 *N. caninum*-positive raccoons also had antibodies to *T. gondii*. The *T. gondii* titers of these 7 raccoons were 1:100 (1 raccoon) and ≥1:500 (6 raccoons).

The results of this study suggest raccoons have little exposure to *N. caninum* oocysts or tissue cysts in prey because of the low (10%) seroprevalence. If raccoons with serological titers to both *N. caninum* and *T. gondii* are omitted from the results, then the prevalence decreases to 3 (3%) out of 99 raccoons. The prevalence of *N. caninum* in white-tailed deer is much higher, at 41% (Dubey et al., 1999).

Dubey et al. (1993) fed 2 raccoons tissues from mice infected with the NC-1 strain of *N. caninum*. These raccoons did not develop clinical signs and did not seroconvert to *T. gondii* using the tachyzoite agglutination test, latex agglutination test, or indirect hemagglutination test, but 1 did develop low Sabin–Feldman dye test titers. No serological testing for *N. caninum* was reported in these animals (Dubey et al., 1993).

The 46% prevalence of *T. gondii* observed in raccoons in the present study is similar to reports where large numbers of raccoons have been examined. Mitchell et al. (1999) found that 184 (49%) of 379 raccoons from Illinois had agglutinating antibody titers to *T. gondii*. This was similar to that found by Dubey et al. (1992), who found that 215 (50%) of 427 raccoons collected from several states (93 from Pennsylvania, 45 from New Jersey, 72 from South Carolina, 68 from Virginia, 30 from Iowa, and 119 from Ohio) were seropositive in the agglutination test. However, Hill et al. (1998) reported that 134 (15%) of 885 raccoons from Iowa had antibodies to *T. gondii* in the agglutination test.

Additional studies need to be done in wildlife to determine the prevalence of *N. caninum* in nondomestic hosts. This may lead to a better understanding of the epidemiology of neosporosis on farms.

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