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Source: Journal of Parasitology, 88(5):1027-1029. 2002.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/0022-3395(2002)088[1027:POAATS]2.0.CO;2
URL: http://www.bioone.org/doi/full/10.1645/0022-3395%282002%29088%5B1027%3APOAATS%5D2.0.CO%3B2

J. Parasitol., 88(5), 2002, pp. 1027–1029

Prevalence of Agglutinating Antibodies to *Sarcocystis neurona* in Skunks (*Mephitis mephitis*), Raccoons (*Procyon lotor*), and Opossums (*Didelphis virginiana*) From Connecticut

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ABSTRACT: Equine protozoal myeloencephalitis is the most important protozoan disease of horses in North America and is usually caused by *Sarcocystis neurona*. Natural cases of encephalitis caused by *S. neurona* have been reported in skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*). Opossums (*Didelphis* spp.) are the only known definitive host. Sera from 24 striped skunks, 12 raccoons, and 7 opossums (*D. virginiana*) from Connecticut were examined for agglutinating antibodies to *S. neurona* using the *S. neurona* agglutination test (SAT) employing formalin-fixed merozoites as antigen. The SAT was validated for skunk sera using pre- and postinfection serum samples from 2 experimentally infected skunks. Of the 24 (46%) skunks 11 were positive, and all 12 raccoons were positive for *S. neurona* antibodies. None of the 7 opossums was positive for antibodies to *S. neurona*. These results suggest that exposure to sporocysts of *S. neurona* by intermediate hosts is high in Connecticut. The absence of antibodies in opossums collected from the same areas is most likely because of the absence of systemic infection in the definitive host.

Equine protozoal myeloencephalitis is a neurologic disease in horses from the Americas and is usually caused by infection with the apicomplexan parasite *Sarcocystis neurona* (Dubey et al., 1991). It is the most important protozoan disease of horses in North America (reviewed by Dubey, Lindsay, Saville et al., 2001). The Virginia opossum, *Didelphis virginiana*, is the only known definitive host in North America (Dubey and Lindsay, 1998), whereas *D. albiventris* is a host in South America (Dubey, Lindsay, Kerber et al., 2001). Nine-banded armadillos (*Dasypus novemcinctus*), raccoons (*Procyon lotor*), and sea otters (*Enhydra lutris*) are natural intermediate hosts (Cheadle, Tanhauser et al., 2001; Dubey, Rosypal, et al., 2001; Dubey, Saville, et al., 2001; Tanhauser et al., 2001), and domestic cats (*Felis domesticus*) and striped skunks (*Mephitis mephitis*) are known experimental intermediate hosts (Dubey et al., 2000; Cheadle, Yowell et al., 2001).

The present study was conducted to determine the serological prevalence of antibodies to *S. neurona* in the intermediate hosts and in the definitive host in Connecticut using the direct *S. neurona* agglutination test (SAT; Lindsay and Dubey, 2001).

Animals used in this study were collected alive with the assistance of nuisance wildlife control personnel in Connecticut. They were collected from the cities of Branford, Cheshire, Hamden, Madison, and Wallingford in eastern New Haven County, and from Durham, Killingworth, and Middletown in western Middlesex County. Animals were killed humanely. Blood was obtained immediately at death by cardiac puncture and placed into a collection tube. The serum was collected and placed in a microcentrifuge tube and frozen at −70 C. Frozen sera were sent to the Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, Virginia, for agglutination testing. The SAT was used to test sera at dilutions of 1:50, 1:100, and 1:500. The SAT test was validated for skunks using pre- and postinfection samples from 2 skunks fed *S. neurona* sporocysts (Cheadle, Yowell et al., 2001). The SAT has previously been validated using raccoon sera (Dubey, Saville et al., 2001; Lindsay et al., 2001).

Sera from 24 striped skunks collected from 6 cities were examined. Of the 24 skunks, 13 were male and 11 were female. All males were adults, 9 females were adults, and 2 were juveniles. Their collection locations are presented in Table I. Sera from 12 raccoons (8 males and 4 females; all adults) and 7 opossums (*D. virginiana*) (4 males and 3 females; all adults) were also examined (Table II).

In the validation of the SAT in the study of skunk, neither skunk had detectable antibodies (<1:50) in the SAT before infection. Both skunks were seroconverted and had titers ≥1:500 after infection, indicating that the SAT works with skunk sera. Of (46%) the 24 sera collected from skunks, 11 were positive (Table I). Sera from 5 males and 6 females were positive. No significant difference in prevalence because of age was noted when examined using the chi-square test. No juvenile male was examined, but both juvenile females were SAT positive.

Sera from all 12 raccoons were positive (100%) in the SAT, and no serum from 7 opossums (0%) was positive in the SAT. Locations of the 8 cities from which animals were collected are given in Table II.

A case of granulomatous encephalitis has been reported in a juvenile striped skunk (Dubey et al., 1996), and natural cases of encephalitis and myocarditis caused by *S. neurona* or a *S. neurona*-like parasite have frequently been reported in raccoons (*P. lotor*) in the United States (Dubey et al., 1990; Stoffregen and Dubey, 1991; Thulin et al., 1992; Dubey and Hamir, 2000; Hamir and Dubey, 2001). This indicates that

TABLE I. Prevalence of agglutinating antibodies to *Sarcocystis neurona* in striped skunks from 6 cities in Connecticut.

City	Sex	Age	Titer
Branford	Male	Adult	≥1:500
Cheshire	Female	Juvenile	1:100
Durham	Male	Adult	≥1:500
Madison	Female	Adult	≥1:500
Middletown	Male	Adult	<1:50
	Female	Adult	1:100
	Male	Adult	<1:50
	Male	Adult	<1:50
	Female	Adult	≥1:500
	Male	Adult	<1:50
	Female	Adult	<1:50
	Male	Adult	<1:50
	Female	Adult	<1:50
	Male	Adult	<1:50
	Female	Adult	<1:50
	Male	Adult	≥1:500
	Male	Adult	<1:50
	Female	Adult	≥1:500
	Male	Adult	≥1:500
	Male	Adult	<1:50
Wallingford	Female	Adult	<1:50
	Male	Adult	≥1:500
	Female	Adult	<1:50
	Female	Juvenile	≥1:500

TABLE II. Prevalence of agglutinating antibodies to *Sarcocystis neurona* in raccoons and opossums in 8 cities from Connecticut.

City	Number of raccoons*	Number of opossums†
Branford	0	1
Cheshire	4	2
Durham	3	0
Hamden	1	0
Killingworth	0	1
Madison	2	1
Middletown	1	1
Wallingford	1	1

* All raccoons were positive in the *Sarcocystis neurona* agglutination test.
† All opossums were negative in the *Sarcocystis neurona* agglutination test.

in addition to being intermediate hosts skunks and raccoons are susceptible to clinical disease caused by *S. neurona*.

Skunks are definitive host for *S. rileyi* (Cawthorn et al., 1981; Wicht, 1981). Little is known about the prevalence or identity of sarcocysts in skunks. Erdman (1978) first reported the presence of a *Sarcocystis* species in striped skunks but did not fully describe or name the parasite. Odening (1997) named the species found by Erdman (1978), *S. erdmanae*. Dubey et al. (2002) described *S. mephiitisi* from skunks and reported natural *S. neurona* infections in the muscles of skunks.

A high (100%) seroprevalence of *S. neurona* in raccoons from Connecticut was seen in the present study. We have previously demonstrated that 33% of raccoons (N = 24) from Florida, 72% of raccoons (N = 25) from New Jersey, 52% of raccoons (N = 25) from Pennsylvania, and 96% of raccoons (N = 25) from Massachusetts were SAT positive (Lindsay et al., 2001). *Sarcocystis kirkpatricki* is the only named species of *Sarcocystis* infecting the muscles of raccoons (Snyder et al., 1990). Kirkpatrick et al. (1987) found sarcocysts in 26 (50%) of the 52 raccoon from Ohio, Pennsylvania, Florida, and Maryland. Snyder et al. (1990) found *S. kirkpatricki* sarcocysts in 66 of the 100 raccoon examined from Illinois.

Serological surveys using the ''gold standard'' Western blot test indicate that 33–60% of horses have antibodies to *S. neurona* (Bentz et al., 1997; Blythe et al., 1997; Saville et al., 1997; Tillotson et al., 1999; Rossano et al., 2001; Vardeleon et al., 2001), indicating that exposure of horses to *S. neurona* is high. When comparative data are available, the seroprevalence of *S. neurona* raccoons appears to accurately reflect the seroprevalence in horses; therefore, skunks and raccoons may be good indicators of environmental contamination with *S. neurona* sporocysts (Lindsay et al., 2001).

We thank Peter Aubrey for his assistance in the collection of animals. This work was supported in part by Animal Health and Disease grant 137180 to D.S.L. and a grant from the Faculty Research Committee, Quinnipiac University to D.J.R.

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