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Prevalence of Antibodies to *Sarcocystis neurona* in Cats From Virginia and Pennsylvania

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ABSTRACT: *Sarcocystis neurona* is best known as the causative agent of equine protozoal myeloencephalitis of horses in the Americas. Domestic cats (*Felis domesticus*) were the first animals described as an intermediate host for *S. neurona*. However, *S. neurona*-associated encephalitis has also been reported in naturally infected cats in the United States. Thus, cats can be implicated in the life cycle of *S. neurona* as natural intermediate hosts. The present study examined the seroprevalence of IgG antibodies to merozoites of *S. neurona* in populations of domestic cats from Virginia and Pennsylvania. Overall, sera or plasma from 441 cats (Virginia = 232, Pennsylvania = 209) were tested by an indirect immunofluorescent assay at a 1:50 dilution. Antibodies to *S. neurona* were found in 32 (7%) of 441 cats. Of these, 22 (9%) of the 232 cats from Virginia and 10 (5%) of the 209 cats from Pennsylvania were seropositive for *S. neurona*.

Sarcocystis neurona, an apicomplexan parasite, is the primary etiologic agent of a frequently diagnosed neurological disease in equids known as equine protozoal myeloencephalitis (EPM) (Dubey, Lindsay, Saville et al., 2001). Its life cycle alternates between the established definitive host, the Virginia opossum (*Didelphis virginiana*), and a wide range of mammalian intermediate hosts. Domestic cats (*Felis domesticus*), along with sea otters (*Enhydra lutris*), raccoons (*Procyon lotor*), nine-banded armadillos (*Dasypus novemcinctus*), striped skunks (*Mephitis mephitis*), and fishers (*Martes pennanti*), have been described as intermediate hosts (Dubey et al., 2000; Cheadle, Tanhauser et al., 2001; Cheadle, Yowell et al., 2001; Dubey, Lindsay, Saville et al., 2001; Dubey, Rosypal et al., 2001; Dubey, Saville et al., 2001; Tanhauser et al., 2001; Butcher et al., 2002; Turay et al., 2002; Gerhold et al., 2005). The horse (*Equus caballus*) is involved as an aberrant host (Dubey et al., 2000).

Immunocompetent cats fed *S. neurona* sporocysts seroconvert by day 20 post-inoculation in an agglutination test, while some cats treated with cortisone seroconvert as early as 7 days after inoculation (Dubey, Lindsay, and Saville, 2002). Few studies have examined the seroprevalence of *S. neurona* in cats; therefore, the present study was conducted to investigate the seroprevalence of antibodies to *S. neurona* in cats from Virginia and Pennsylvania.

For the present study, 441 feline serum or plasma samples were collected. Sera or plasma was obtained from 232 cats from Virginia in an ongoing study of kidney disease conducted in the Department of Small Animal Clinical Science at the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia. An additional 209 serum samples were acquired from an animal shelter in Philadelphia, Pennsylvania. These samples were initially used in a study of the seroprevalence of *Toxoplasma gondii* (Dubey et al., 2009). Serum and plasma samples were stored at −20 C until used.

Merozoites of the SN-37R isolate (Sofaly et al., 2002) of *S. neurona* were cultivated in African green monkey (*Cercopithecus aethiops*) kidney cells, (CV-1, ATTC CCL-70, American Type Culture Collection, Manassas, Virginia) and maintained in 75-mm^2 tissue culture flasks to confluence. Growth media used consisted of 10% (v/v) fetal bovine serum (FBS) in RPMI 1640 medium supplemented with 10,000 U penicillin and 10,000 mg/ml streptomycin per ml. When the monolayer reached confluence, the growth medium was removed and replaced by a maintenance medium of 2% (v/v) FBS with an otherwise identical formula. Flasks were incubated at 37 C in a humidified incubator containing 5% CO_2 and 95% air. For collection of merozoites, the infected CV-1 cells were removed with a cell scrapper and the media filtered through 3-μm polycarbonate filters (GE Water and Process Technologies, Minnetonka, Minnesota) and pelleted by centrifugation. After 3 washes in phosphate-buffered saline (PBS), the cell suspension, containing approximately 0.5 to 1×10^4 merozoites in 25 μl PBS, were dispensed onto each well of 12-well, Teflon-coated indirect fluorescent antibody test (IFAT) slides (Fisher Scientific, Pittsburgh, Pennsylvania). Antigen-containing slides were then left to dry at room temperature for 4 to 12 hr and stored at −20 C until use.

Cat sera or plasmas were diluted at 1:50 in PBS, and 25 μl was pipetted into each well of the antigen-containing slides. The slides were incubated for 30 min at room temperature in a humidified chamber. Subsequent to 3 consecutive washes in PBS, fluorescence-labeled goat anti-cat (Kirkegaard and Perry Labs Inc., Gaithersburg, Maryland) was diluted 1:5 in PBS, and 25 μl were added to each well of the slides. The slides were incubated for 30 min at room temperature in a humidified chamber. Following 3 consecutive washes in PBS, slides were mounted in Fluoromount-G (Southern Biotechnology Associates Inc., Birmingham, Alabama) and assessed using an Olympus BX60 epifluorescent microscope equipped with differential contrast optics (Olympus America Inc., Center Valley, Pennsylvania). Samples that exhibited fluorescence of the entire parasitic surface were considered to be positive. All positive samples were also examined at dilutions of 1:100 and 1:200.

Antibodies to *S. neurona* were detected by IFA in the sera and plasma of 32 (7%) of the 441 cats at a 1:50 dilution. Twenty-two (9%) were of the 232 cats from Virginia, and the other 10 (5%) were of the 209 cats from Pennsylvania. The titers of the 32 positive cats were 14 at 1:40, 2 at 1:00; and 16 at ≥ 1:200.

Equine protozoal myeloencephalitis is the most-commonly diagnosed neurological disease of horses from the Americas caused by a protozoan parasite (Dubey, Lindsay, Saville et al., 2001). As part of its life cycle, a variety of mammals, namely cats, raccoons, armadillos, skunks, and sea otters, can act as intermediate hosts and develop sarcocysts in their muscle tissues (Dubey et al., 2000; Cheadle, Tanhauser et al., 2001; Cheadle, Yowell et al., 2001; Dubey, Lindsay, Saville et al., 2001; Dubey, Rosypal et al., 2001; Dubey, Saville et al., 2001; Tanhauser et al., 2001). Opossums in the genus *Didelphis* act as the definitive hosts that are capable of excreting sporocysts in the feces as a result of ingesting tissues of intermediate hosts that harbor the sarcocysts (Dubey, Lindsay, Kerber et al., 2001). Horses are identified as aberrant or dead-end hosts in the parasite life cycle (Dubey et al., 2000).

A previous study reported the seroprevalence of antibodies to *S. neurona* to be 27% of 196 domestic pet cats (Rossano et al., 2002) from Michigan evaluated by IFAT. Using the direct agglutination test (SAT), 13% of 310 feral cats from Ohio were found to be positive (Stanek et al., 2003). None of 502 cats from Brazil was positive via the SAT (Dubey, Lindsay, Hill et al., 2002). The present study demonstrated that the seroprevalence of *S. neurona* in cats was relatively low in the 2 geographic locations examined. High seroprevalences have been found in raccoons from Connecticut (100% of 12; Mitchell et al., 2002) and from Fairfax County, Virginia (92% of 469; Hancock et al., 2004) using the SAT. Eleven (46%) of 24 skunks from Connecticut were positive by the SAT (Mitchell et al., 2002). These data suggest that raccoons and skunks are more susceptible to *S. neurona* infection than are cats, or that they have a higher risk of exposure to sporocysts. The role of domestic cats as intermediate hosts in perpetuating the life cycle of *S. neurona* is probably minimal compared to that of raccoons, skunks, and armadillos.

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