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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. Of the 232 feral cats from Virginia (92% *Felis catus* and 9% *Felis mewa*) and 105 domestic cats from Virginia (96% *F. domesticus* and 4% *F. catus*), 62 (27%) were 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*) are the first animals described as an intermediate host for *S. neurona*. Other domestic and wild mammals, including raccoons, skunks, and a variety of other mammals as intermediate hosts, can act as reservoirs for *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, ATCC CCL-70, American Type Culture Collection, Manassas, Virginia) and maintained in 75-mm² 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% CO₂ and 95% air. For collection of merozoites, the infected CV-1 cells were removed with a cell scraper 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 × 10⁶ 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 plasma were diluted 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 IFAT in the sera and plasma of 32% (70 of 232) of the 232 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 1:200, 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, opossums, and sea otters, can act as intermediate hosts and develop sarcocysts in their muscle tissues (Dubey et al., 2000; Cheadle, Tanhauser 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., 2005; 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 corticosteroids were agglutination negative as early as 7 days after inoculation (Dubey, Lindsay, Saville et al., 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.

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