Epidemiology of *Sarcocystis neurona* infections in domestic cats (*Felis domesticus*) and its association with equine protozoal myeloencephalitis (EPM) case farms and feral cats from a mobile spay and neuter clinic


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Abstract

Equine protozoal myeloencephalitis (EPM) is a serious neurologic disease in the horse most commonly caused by *Sarcocystis neurona*. The domestic cat (*Felis domesticus*) is an intermediate host for *S. neurona*. In the present study, nine farms, known to have prior clinically diagnosed cases of EPM and a resident cat population were identified and sampled accordingly. In addition to the farm cats sampled, samples were also collected from a mobile spay and neuter clinic. Overall, serum samples were collected in 2001 from 310 cats, with samples including barn, feral and inside/outside cats. Of these 310 samples, 35 were from nine horse farms. Horse serum samples were also collected and traps were set for opossums at each of the farms. The *S. neurona* direct agglutination test (SAT) was used for both the horse and cat serum samples (1:25 dilution). Fourteen of 35 (40%) cats sampled from horse farms had circulating *S. neurona* agglutinating antibodies. Twenty-seven of the

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275 (10%) cats from the spay/neuter clinic also had detectable *S. neurona* antibodies. Overall, 115 of 123 (93%) horses tested positive for anti-*S. neurona* antibodies, with each farm having greater than a 75% exposure rate among sampled horses. Twenty-one opossums were trapped on seven of the nine farms. Eleven opossums had *Sarcocystis* sp. sporocysts, six of them were identified as *S. neurona* sporocysts based on bioassays in γ-interferon gene knockout mice with each opossum representing a different farm. Demonstration of *S. neurona* agglutinating antibodies in domestic and feral cats corroborates previous research demonstrating feral cats to be naturally infected, and also suggests that cats can be frequently infected with *S. neurona* and serve as one of several natural intermediate hosts for *S. neurona*.

**1. Introduction**

Equine protozoal myeloencephalitis (EPM), primarily caused by the apicomplexan parasite *Sarcocystis neurona*, is the most commonly diagnosed neurologic disease of the horse in the US (Dubey et al., 2001a). Based on reported surveys from individual horse owners, EPM has been labeled as the most important infectious disease facing the equine industry (NAHMS, 2001). Surveys in Ohio revealed greater than 50% (53.6%) of horses with *S. neurona* antibodies (Saville et al., 1997). Opossums (*Didelphis virginiana*) serve as definitive hosts for *S. neurona* in the US and are responsible for shedding infective *S. neurona* sporocysts (Fenger et al., 1997; Dubey and Lindsay, 1998). Until recently, exactly how the opossum became infected was unknown because hosts harboring sarcocysts had not been identified. Recently, *S. neurona* sarcocysts were identified in muscles of cats, raccoons, armadillos, sea otters and skunks (Cheadle et al., 2001; Dubey et al., 2000, 2001b,d; Turay et al., 2002). Recent studies from Michigan and Florida reported *S. neurona* antibodies in 5% of domestic cats based on Western blot analysis (Gillis et al., 2003; Rossano et al., 2002).

Due to the frequency of cat sightings on horse farms and their potential relationship in this disease, it was of interest to examine the percentage of barn and feral cats that had been exposed to *S. neurona*. Reports indicate a burgeoning feral cat population estimated at 60 million in the US, which could aid in the transmission of the parasite.

The purpose of the present study was to determine the level of *S. neurona* infections in cats on horse farms where EPM has been diagnosed and in a select feral cat population.

**2. Materials and methods**

**2.1. Criteria for farm and horse selection**

Nine farms, known to have had horses diagnosed with EPM were identified, with each farm being selected based on the recommendations of licensed veterinary practitioners. Farms that were included in this study came from Madison, Franklin, Union, Champaign and Logan counties, all of which are located in the Southwest extension district of Ohio.
The four factors used in the identification of sample farms were as follows: (1) farms had to house more than 10 horses, (2) farm and surrounding area was sylvatic enough area to support an opossum population, (3) cats were present on the premises and (4) each farm had to have experienced an episode of EPM, either a current case or previous case of disease. The diagnosis of EPM was based upon clinical signs, serology and response to treatment for all farms. Cerebrospinal fluid (CSF) analysis was also used for diagnosis on Farms B and C. Two equine veterinarians were relied upon for the diagnosis of EPM. Based on previous seroprevalence estimates in Ohio, only 10 samples were needed from each farm to detect infected horses at a 95% confidence level (Saville et al., 1997). Horses were selected for testing according to the owners and veterinarians suggestions. All samples were representative of the farms horse population in regards to age.

2.2. Farm description

Information regarding breed, horse and cat populations, clinical cases of EPM and wildlife seen on the property is given in Table 1. Some farms had more severe problems with EPM than others, but each farm had at least one clinically diagnosed case of EPM. Farm B had two confirmed cases of fatal EPM, and three clinically diagnosed cases that recovered.

Farms B and G were both located in highly wooded areas, whereas the additional farms were located in relative proximity to sylvatic areas. Eight of the nine farms reported wildlife in and around the property. All farms protected their grain using sealed containers, but only one farm was successfully able to preclude wildlife access to the hay according to the owner’s report. Altogether, nine horse breeds were represented from the nine farms, with Quarterhorses comprising the largest percentage of the breeds.

2.3. Sample collection

Antibodies to *S. neurona* were examined in both the cat and horse population on each of the identified farms. A sample of Ohio barn, feral and free ranging house cats admitted to a mobile spay and neuter clinic were also examined for anti-*S. neurona* agglutinating antibodies. Blood (10 ml vacutainer tubes) was collected through jugular venapuncture from horses and sera was stored at \(-80^\circ\text{C}\).

All of the cats were captured by hand using humane methods according to guidelines of The Ohio State University Institutional Laboratory Animal Care and Use Committee protocols with samples collected as follows. One equine veterinarian collected the samples without the aid of any anesthesia, while the other equine veterinarian anesthetized the cats using 1.25 ml of ketamine (Fort Dodge, IA). The spay/neuter cats were anesthetized using inhaled isoflurane (Vedco, St. Joseph, MO). In all cases, 3 ml of blood was collected through jugular venapuncture. Samples were centrifuged and stored at \(-80^\circ\text{C}\). Serum samples from the mobile spay and neuter clinic cats were collected while cats were under general anesthesia for surgery. The inside/outside group of cats was the only group that appeared domesticated, evident through the presence of collars.

Opossums were trapped and collected on each of the identified farms to determine whether *S. neurona* was being excreted on the premises. Opossums were live-trapped (Tomahawk
Table 1
Results from each of the nine sampled horse farms

<table>
<thead>
<tr>
<th>Farms</th>
<th>No. of cats sampled/farm total</th>
<th>SAT positive cats (%)</th>
<th>No. of horses sampled/farm total</th>
<th>SAT positive horses (%)</th>
<th>No. of opossums trapped</th>
<th>S. neurona infected opossums</th>
<th>Horse breeds(^a)</th>
<th>Wildlife seen in barns</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4/4</td>
<td>0 (0)</td>
<td>24/40</td>
<td>24 (100)</td>
<td>5</td>
<td>1</td>
<td>QH</td>
<td>Yes</td>
</tr>
<tr>
<td>B</td>
<td>5/24</td>
<td>4 (80)</td>
<td>15/18</td>
<td>12 (80)</td>
<td>0</td>
<td>XX</td>
<td>QH, SB, TB, D</td>
<td>Yes</td>
</tr>
<tr>
<td>C</td>
<td>2/6</td>
<td>0 (0)</td>
<td>19/21</td>
<td>19 (100)</td>
<td>7</td>
<td>1</td>
<td>QH</td>
<td>Yes</td>
</tr>
<tr>
<td>D</td>
<td>5/10</td>
<td>1 (25)</td>
<td>14/18</td>
<td>11 (79)</td>
<td>1</td>
<td>1</td>
<td>H, SB, STB</td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>5/5</td>
<td>2 (40)</td>
<td>15/32</td>
<td>13 (87)</td>
<td>2</td>
<td>0</td>
<td>TB, QH</td>
<td>Yes</td>
</tr>
<tr>
<td>F</td>
<td>4/5</td>
<td>2 (50)</td>
<td>11/26</td>
<td>11 (100)</td>
<td>2</td>
<td>1</td>
<td>QH, TB, WB</td>
<td>Yes</td>
</tr>
<tr>
<td>G</td>
<td>2/2</td>
<td>2 (100)</td>
<td>5/29</td>
<td>5 (100)</td>
<td>2</td>
<td>1</td>
<td>TB, TK</td>
<td>Yes</td>
</tr>
<tr>
<td>H</td>
<td>6/15</td>
<td>2 (33)</td>
<td>10/100</td>
<td>10 (100)</td>
<td>2</td>
<td>1</td>
<td>QH, A, TB, WB</td>
<td>Yes</td>
</tr>
<tr>
<td>I</td>
<td>2/3</td>
<td>1 (50)</td>
<td>10/25</td>
<td>10 (100)</td>
<td>0</td>
<td>XX</td>
<td>STB, TB, WB</td>
<td>Yes</td>
</tr>
<tr>
<td>Total</td>
<td>35/74</td>
<td>14 (40)</td>
<td>123/309</td>
<td>115 (93)</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) A: Arabian; D: Draft; H: Hackney; QH: Quarterhorse; SB: Saddlebred; STB: Standardbred; TB: Thoroughbred; TK: Trakhener; WB: Warmblood; XX: no opossums were trapped on these farms.
#108 traps, Tomahawk Live Trap Company, Tomahawk, WI). If opossums had previously been observed on the premises, traps were placed in proximity to previous sightings. When opossums had not been seen on the premises, sites were selected based on common opossum habitat. Consequently, traps were set along fencelines, brushpiles and along ditches and creek banks. Traps were baited with canned cat food.

Trapped opossums were transported to Critter Control (Columbus, OH) where they were euthanized using >70% CO₂ following standards set by The Ohio State University Animal Use and Care Committee.

### 2.4. Collection of sporocysts

Sporocysts were collected from the small intestine of opossums as described by Dubey et al. (2000). Sporocysts were counted and stored in HBSS with antibiotics at 4°C.

### 2.5. Serologic examination for S. neurona antibodies

Antibodies to *S. neurona* were determined in sera of horses and cats using the direct agglutination test (SAT) (1:25 dilution) described by Lindsay and Dubey (2001). Positive controls consisted of cat, horse and mouse sera (Dubey et al., 2000, 2002a; Saville et al., 2001). All positive controls were animals that were experimentally infected with *S. neurona* sporocysts. Pre-infected horse and cat samples served as negative controls.

### 2.6. Detection of S. neurona sporocysts

Sporocysts from intestinal scrapings of opossums collected on individual farms were detected by bioassay in γ-interferon gene knockout (KO) mice as described previously (Dubey and Lindsay, 1998; Dubey et al., 2001b).

### 3. Results

#### 3.1. Serological evidence of S. neurona infections in cats

Fourteen of 35 (40%) cats sampled from the nine horse farms yielded detectable *S. neurona* agglutinating antibodies. Seven of the nine farms had at least one positive cat, with four of the nine farms having two or more positive cats. Four of five cats sampled from Farm B tested positive for anti-*S. neurona* antibodies (Table 1).

Serum samples collected from the mobile spay and neuter clinic revealed 27 of 275 (10%) cats with circulating *S. neurona* agglutinating antibodies. Samples of known barn cats collected from the mobile clinic demonstrated 11 of 91 (12%) cats to have detectable *S. neurona* antibodies, and 7 of 76 (9%) feral cats to be exposed to *S. neurona* (Table 2). Inside/outside cats were also sampled and 6 of 80 (8%) cats tested positive for anti-*S. neurona* antibodies (Table 2). Eight Ohio counties were represented from the samples collected from the mobile clinic (Table 3). There was at least one positive cat reported from each county.
Table 2
Prevalence of *S. neurona* antibodies from three categories of cats collected from the mobile spay and neuter clinic

<table>
<thead>
<tr>
<th>Sampled cats</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feral</td>
<td>7/76 (9)</td>
</tr>
<tr>
<td>Barn</td>
<td>11/91 (12)</td>
</tr>
<tr>
<td>Inside/outside</td>
<td>6/80 (8)</td>
</tr>
<tr>
<td>Unknown habitat</td>
<td>3/28 (11)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>27/275 (10)</strong></td>
</tr>
</tbody>
</table>

One county (Preble), in which 72% of the samples were either feral or barn cats, had a 22% seroprevalence. Results of all eight counties are listed in Table 3.

3.2. Serologic evidence of *S. neurona* infections in horses

Horses with detectable *S. neurona* agglutinating antibodies were reported from each farm. Six farms had a 100% seroprevalence of anti-*S. neurona* antibodies in the horses sampled. Horses sampled at all farms had >75% exposure to *S. neurona* (Table 4). It did not appear that season of sample collection affected the prevalence of antibodies as one farm sampled in January had a 100% seroprevalence, while several farms sampled in the summer also had a 100% seroprevalence.

3.3. Demonstration of *Sarcocystis* sporocysts in opossums

*Sarcocystis* sporocysts were seen in 11 of the 21 trapped opossums. These results closely match those found in a previous study (Dubey et al., 2001b). Opossums were successfully trapped on seven of nine farms. Individual farm results are seen in Table 1. Eleven opossums were trapped during the winter between 6 February and 2 March 2001, with only one of these opossums shedding sporocysts. Of the 10 opossums trapped in the spring and summer, 9 contained sporocysts in the small intestine. Overall, nine opossums were trapped in February, four in March, four in April, two in May, and one in June and August 2001. The opossum

Table 3
County totals for positive *S. neurona* infected cats collected from mobile spay and neuter clinic

<table>
<thead>
<tr>
<th>Sampled Ohio counties</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franklin</td>
<td>8/86 (9)</td>
</tr>
<tr>
<td>Delaware</td>
<td>2/21 (9)</td>
</tr>
<tr>
<td>Preble</td>
<td>4/18 (22)</td>
</tr>
<tr>
<td>Medina</td>
<td>2/28 (7)</td>
</tr>
<tr>
<td>Licking</td>
<td>2/25 (8)</td>
</tr>
<tr>
<td>Muskingum</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>Montgomery</td>
<td>5/65 (8)</td>
</tr>
<tr>
<td>Miami</td>
<td>3/25 (12)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>27/275 (10)</strong></td>
</tr>
</tbody>
</table>
Table 4
Identification of *Sarcocystis neurona* sporocysts in intestines of opossums trapped on horse farms

<table>
<thead>
<tr>
<th>Opossum No.</th>
<th>Farm No.</th>
<th>Bioassay in KO mice</th>
<th>KO mouse No.</th>
<th>Died/killed&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tissues parasitized&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SAT titer&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 D</td>
<td>8769</td>
<td>DK34</td>
<td>8769</td>
<td>DK34</td>
<td>B&lt;sup&gt;d&lt;/sup&gt;, H, L</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>8910</td>
<td>DK34</td>
<td>8910</td>
<td>DK34</td>
<td>B, L</td>
<td>≥500</td>
</tr>
<tr>
<td>11 C</td>
<td>8803</td>
<td>D26</td>
<td>8803</td>
<td>D26</td>
<td>L</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>8798</td>
<td>DK26</td>
<td>8798</td>
<td>DK26</td>
<td>B, E, H, L</td>
<td>100</td>
</tr>
<tr>
<td>13 F</td>
<td>4629</td>
<td>DK21</td>
<td>4629</td>
<td>DK21</td>
<td>B, St</td>
<td>≥500</td>
</tr>
<tr>
<td></td>
<td>4630</td>
<td>DK24</td>
<td>4630</td>
<td>DK24</td>
<td>B, St</td>
<td>≥500</td>
</tr>
<tr>
<td>14 A</td>
<td>4625</td>
<td>DK30</td>
<td>4625</td>
<td>DK30</td>
<td>B, L</td>
<td>≥500</td>
</tr>
<tr>
<td></td>
<td>4635</td>
<td>K48</td>
<td>4635</td>
<td>K48</td>
<td>None</td>
<td>&lt;50</td>
</tr>
<tr>
<td>17 H</td>
<td>4631</td>
<td>DK21</td>
<td>4631</td>
<td>DK21</td>
<td>B, L, St</td>
<td>≥500</td>
</tr>
<tr>
<td></td>
<td>4632</td>
<td>DK24</td>
<td>4632</td>
<td>DK24</td>
<td>B, L</td>
<td>≥500</td>
</tr>
<tr>
<td>19 G</td>
<td>4633</td>
<td>DK21</td>
<td>4633</td>
<td>DK21</td>
<td>B, L</td>
<td>≥500</td>
</tr>
<tr>
<td></td>
<td>4634</td>
<td>DK24</td>
<td>4634</td>
<td>DK24</td>
<td>B, L</td>
<td>≥500</td>
</tr>
</tbody>
</table>

<sup>a</sup> D: died, K: killed, DK: killed when ill.

<sup>b</sup> By immunohistochemical staining with anti-*S. neurona* antibody.

<sup>c</sup> *Sarcocystis neurona* agglutination test.

<sup>d</sup> B: brain; E: eye; H: heart; L: lung; St: stomach muscle.

Captured in February contained the lowest number of sporocysts (1.4 × 10<sup>5</sup>). All additional opossums had at least 1.6 × 10<sup>6</sup> sporocysts.

### 3.4. Infectivity of collected sporocysts from trapped opossums

Sporocysts from 6 of the 11 opossums were infective for interferon gamma gene knock-out mice (Table 4). The KO mice fed sporocysts developed neurologic signs and *S. neurona* was identified in tissues of mice examined 21–34 days post-infection (Table 4). Agglutinating antibodies to *S. neurona* were detected in sera of mice fed sporocysts (Table 4). The localization of *S. neurona* in tissues of KO mice fed sporocysts was the same as previously reported (Dubey, 2001).

### 4. Discussion

Serum antibodies to *S. neurona* were detected in 40% of the cats sampled from EPM case farms, whereas only 10% of the cats sampled from the spay/neuter clinic samples were positive for *S. neurona* agglutinating antibodies. To our knowledge, this is the first report demonstrating the level of *S. neurona* infection in cat populations on horse farms where EPM has been diagnosed. The demonstration of *S. neurona* antibodies in both the horse and cat serum samples, as well as identifying *S. neurona* sporocysts from the opossums, suggests that cats are commonly infected, and further corroborates research demonstrating feral cats as natural intermediate hosts (Rossano et al., 2002; Turay et al., 2002).
Many factors must be present for the manifestation of clinical EPM. Previous diagnosis of EPM on the premises, wooded terrain around the premises and wildlife access to hay have all been identified as risk factors for the development of EPM (Saville et al., 2000). Eighty percent of the sampled cats from Farm B tested positive to anti-\textit{S. neurona} agglutinating antibodies. This farm had severe problems with EPM. The farm is located in a highly wooded area, which provides an excellent habitat with both opossums and cats. Farm G, located in a heavily wooded area, had 2 cats test positive for \textit{S. neurona} antibodies. Although only five horses were sampled from Farm G and did not meet the criteria we established as each farm having at least 10 horse samples collected, we included this farm in our results due to the detection of \textit{S. neurona} agglutinating antibodies in the horses that were sampled. All horses sampled at Farm G had been exposed to \textit{S. neurona}.

The disparity between the spay/neuter cat samples and the EPM farm cat samples (10% versus 40%) may be explained by the sampling location of these two different populations. The EPM farm cats were sampled from rural areas whereas the spay/neuter samples came from more urban areas. Even the barn cat samples obtained from the spay/neuter clinic came from farms that were in much closer proximity to urban areas compared to the cat samples from farms with EPM. Recently, \textit{S. neurona} antibodies were reported in 5% of cats from Michigan and Florida (Rossano et al., 2002; Gillis et al., 2003). Sera from cats from Michigan were those sent to a diagnostic laboratory from referring veterinarians (Rossano et al., 2002). The sera from cats from Florida were those obtained from pound cats (Gillis et al., 2003). These cats from Michigan were more likely to be domestic house cats and had a similar seroprevalence to domestic cats from the mobile spay and neuter clinic (8%) in our study. The urban versus rural factor may account for the different infection estimates, as cats in rural areas have a greater propensity of interacting with a larger wildlife population and potentially more \textit{S. neurona} sporocysts. Another possible explanation for the differences in seroprevalence may be explained by the differences in the two different diagnostic tests used. In our study, the positive results were based on SAT, whereas, results from Michigan and Florida were based on Western blots.

The specificity of this SAT test is unknown for \textit{S. neurona}, and therefore some of the positive cat samples may have been false positives leading to misclassification bias. However, this amount is believed to be minimal based on previous work demonstrating accurate differentiation between \textit{S. neurona} and \textit{S. falcata} (Dubey et al., 2002a). Additionally, when testing serum from over 500 city cats from Sao Paulo, Brazil at a dilution factor of 1:25, none of the samples were positive for \textit{S. neurona} agglutinating antibody (Dubey et al., 2002b). The dilution factor (1:25) for the cat samples may have led to some antibody cross-reactivity, however, the most recent published work may suggest otherwise.

The elevated seroprevalence in the sampled horse population was expected, due to the fact that sampling was biased towards populations with previous problems of EPM. Also, each of the sampled farms were located in the Southwestern Extension district. An Ohio seroprevalence study revealed this district to have the highest seroprevalence in Ohio (Saville et al., 1997). The elevated seroprevalence could also be partially attributed to false positive results from cross-reaction to \textit{S. fayeri} as the specificity between these two species has not been investigated with the \textit{S. neurona} agglutination test. Previous work performed in central Ohio and in Kentucky has demonstrated high seroprevalence on some farms (Saville, 1998; Fenger et al., 1997). A confirmatory Western blot test may have increased the specificity, but
the major focus of the equine seroprevalence work was to determine if farms had infected horses and not specific prevalence of infection.

Altogether, 6 of the 21 (28%) trapped opossums were infected with \textit{S. neurona}. All of the six infected opossums were trapped from separate farms. \textit{S. neurona} infected opossums were collected from six of seven farms (86%); we were unable to collect opossums from two farms. The demonstration of \textit{S. neurona} sporocysts in the local opossum population suggests that \textit{S. neurona} is being maintained on the premises, and that a suitable intermediate host was available in the surrounding area.

The domestic cat was the first intermediate host identified for \textit{S. neurona} (Dubey et al., 2000). Cats were examined as possible definitive hosts in the early stages of this disease investigation due to their ubiquitous presence on horse farms (Fenger et al., 1995). \textit{S. neurona}-like disease has been reported in the domestic cat (Dubey et al., 1994, 2003). Recent research has demonstrated that a feral cat was naturally infected and served as a viable intermediate host (Turay et al., 2002). \textit{Sarcocystis} sp. sarcocysts have been previously reported in the domestic cat, but the prevalence of \textit{S. neurona} sarcocysts in cats is unknown (Edwards et al., 1988; Eisenstein and Innes, 1956; Everitt et al., 1987; Fiori and Lowndes, 1988; Gillis et al., 2003; Hill et al., 1988; Kirkpatrick et al., 1986). Sarcocyst-infected tissue of the intermediate host must be consumed by the definitive host in order to complete the lifecycle. Therefore, predatory species do not often serve as intermediate hosts for many of the parasitic life cycles. However, the opossum is a scavenger by nature and is omnivorous (Burton and Burton, 1994; Gardner, 1982; Stieglitz and Klimstra, 1962). It will readily prey upon small rodents or abandoned carcasses. One study examining the dietary intake of 68 opossums found domestic cats to comprise 2.4% of the total volume (Reynolds, 1945).

How cats are exposed to \textit{S. neurona} sporocysts has yet to be determined, but environmental contamination with \textit{S. neurona} appears to be high with over 53% of Ohio horses exposed to this parasite, which may also help explain the cat exposure levels (Saville et al., 1997).

Results of this investigation suggest that cats may serve an important role in the natural maintenance of this parasite. However, it must be noted that cats are not the only intermediate host. Recent reports indicate several other species (i.e. skunks, raccoons and nine-banded armadillos) serve as natural intermediate hosts for \textit{S. neurona} (Cheadle et al., 2001; Dubey et al., 2000a,b,c,d; Tanhauser et al., 2001). This confirms previous research suggesting that unlike most \textit{Sarcocystis} sp. that exist through a single intermediate host, \textit{S. neurona} appears to maintain its existence through multiple intermediate hosts (Dubey et al., 1989).

The exact role of cats in this lifecycle is still uncertain. Perhaps the close ecological niche shared by opossums, skunks, and raccoons allow opossums to prey mainly upon these two intermediate host species. This predator–prey relationship may be the significant cause of environmental \textit{S. neurona} contamination and the cat may be an aberrant intermediate host that is not preyed upon as readily by opossums. Thus, more research is needed to elucidate this ecological relationship.

In conclusion, results of this study provide evidence that suggest that feral cats are commonly exposed to \textit{S. neurona} sporocysts and have circulating \textit{S. neurona} antibodies. Demonstration of \textit{S. neurona} agglutinating antibodies in both the horse and cat populations on these farms as well as isolation of \textit{S. neurona} sporocysts from opossums trapped on these farms suggests that cats are naturally infected under conditions of high prevalence.
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