Lack of *Sarcocystis neurona* Antibody Response in Virginia Opossums (*Didelphis virginiana*) Fed *Sarcocystis neurona*–Infected Muscle Tissue

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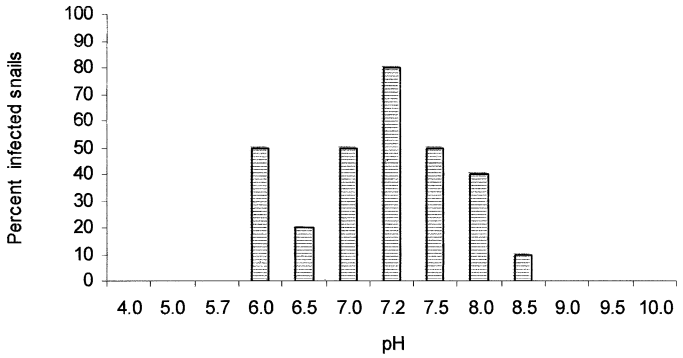

FIGURE 2. Infection of *L.* (*Fossaria*) *humilis* with *F. hepatica* at different pHs. Every 24 hr, dead snails were dissected and examined to determine infection by *F. hepatica*. The experiment was stopped at day 18, when the remaining molluscs were examined.

2005). Humidity and temperature have been observed to correlate with peaks of infection in snails. Changing pH of a water body also could modify the infectivity of this parasite for its intermediate host, but this hypothesis has not yet been addressed under natural conditions.

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Lack of *Sarcocystis neurona* Antibody Response in Virginia Opossums (*Didelphis virginiana*) Fed *Sarcocystis neurona*–Infected Muscle Tissue

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ABSTRACT: Serum was collected from laboratory-reared Virginia opossums (*Didelphis virginiana*) to determine whether experimentally infected opossums shedding *Sarcocystis neurona* sporocysts develop serum antibodies to *S. neurona* merozoite antigens. Three opossums were fed muscles from nine-banded armadillos (*Dasypus novemcinctus*), and 5 were fed muscles from striped skunks (*Mephitis mephitis*). Serum was also collected from 26 automobile-killed opossums to determine whether antibodies to *S. neurona* were present in these opossums. Serum was analyzed using the *S. neurona* direct agglutination test (SAT). The SAT was modified for use with a filter paper collection system. Antibodies to *S. neurona* were not detected in any of the serum samples from opossums, indicating that infection in the opossum is localized in the small intestine. Antibodies to *S. neurona* were detected in filter-paper–processed serum samples from 2 armadillos naturally infected with *S. neurona*.

Sarcocystis neurona is a causative agent of the neuromuscular disease equine protozoal myeloencephalitis (EPM) (Dubey et al., 1991; 2001) and has been isolated from horses in North and South America (Dubey

TABLE I. Results of the *Sarcocystis neurona* direct agglutination test using serum and blood from Virginia opossums (*Didelphis virginiana*).

Sample number	Animal	*S. neurona* agglutination test	Collection location	Muscles fed	Sporocysts in intestinal scrapings
4211	Opossum	N	Gainesville, FL	N/A*	N†
4218	Opossum	N	Gainesville, FL	N/A	N
4223	Opossum	N	Gainesville, FL	N/A	N
4224	Opossum	N	Gainesville, FL	N/A	Y
4225	Opossum	N	N/A	N/A	N
4236	Opossum	N	Gainesville, FL	N/A	N
4239	Opossum	N	Ocala, FL	N/A	Y
4240	Opossum	N	Ocala, FL	N/A	N
4246	Opossum	N	Melrose, FL	N/A	N
4247	Opossum	N	Gainesville, FL	N/A	Y
4248	Opossum	N	Gainesville, FL	N/A	N
4261	Opossum	N	N/A	N/A	N
4263	Opossum	N	Gainesville, FL	N/A	Y
4264	Opossum	N	Gainesville, FL	N/A	Y
4272	Opossum	N	Gainesville, FL	N/A	N
4273	Opossum	N	Gainesville, FL	N/A	N
5000	Opossum	N	Gainesville, FL	N/A	N
5005	Opossum	N	Gainesville, FL	N/A	N
5006	Opossum	N	Gainesville, FL	N/A	N
5014	Opossum	N	Alabama	N/A	Y
5015	Opossum	N	Gainesville, FL	N/A	Y
5023	Opossum	N	Gainesville, FL	N/A	N
5029	Opossum	N	Gainesville, FL	N/A	N
DV09	Opossum	N	Gainesville, FL	Armadillo DN58, DN59, DN63	Y
DV10	Opossum	N	Gainesville, FL	Armadillo DN58, DN59, DN64	Y
DV14	Opossum	N	Gainesville, FL	Armadillo DN60, DN64	Y
DV34	Opossum	N	Gainesville, FL	Skunk SK03	Y
DV36	Opossum	N	Gainesville, FL	Skunk SK02	Y
DV37	Opossum	N	Gainesville, FL	Skunk SK03	Y
DV38	Opossum	N	Gainesville, FL	Skunk SK01	Y
DV39	Opossum	N	Gainesville, FL	Skunk SK01	Y
DN65	Armadillo	1:1000	Gainesville, FL	N/A	N/A
DN69	Armadillo	1:10,000	Gainesville, FL	N/A	N/A

* N/A = not available.
† N = negative; Y = positive.

et al., 1991, 2001; Granstrom et al., 1992). The Virginia opossum (*Didelphis virginiana*) has been identified as a definitive host of *S. neurona* (Fenger et al., 1997; Dubey and Lindsay, 1998; Tanhauser et al., 1999). Two intermediate hosts for *S. neurona* are the nine-banded armadillo (*Dasypus novemcinctus*) and striped skunk (*Mephitis mephitis*) (Cheadle, Tanhauser et al., 2001; Cheadle, Yowell, et al., 2001). Cheadle, Yowell et al. (2001) and Tanhauser et al. (2001) have shown that skunks seroconvert to *S. neurona* after experimental infection and that naturally infected, wild-caught armadillos have plasma antibodies to *S. neurona*. No previous studies have documented whether Virginia opossums develop antibodies to *S. neurona* when stages of *S. neurona* are present in their intestines. Mitchell et al. (2002) did not find antibodies to *S. neurona* in 7 opossums from Connecticut; however, they did not examine the intestines of their skunks for sporocysts, so it is unknown whether these potential hosts were infected at the time of sampling. The presence of antibodies to *S. neurona* would allow investigators to determine whether an opossum was infected with *S. neurona* gut stages without scraping the intestine and looking for sporocyst/oocyst stages. In the present study, we examined serum samples from experimentally inoculated Virginia opossums to determine whether opossums experimentally exposed to *S. neurona* develop antibodies to *S. neurona* using a direct agglutination test. We also examined whole blood samples from automobile-killed Virginia opossums to determine whether naturally infected Virginia opossums had antibodies to *S. neurona*.

Serum samples were collected from experimentally infected Virginia opossums (*D. virginiana*). Three of the opossums had been fed muscle tissue from nine-banded armadillo (*D. novemcinctus*) naturally infected with *Sarcocystis* (Cheadle, Tanhauser et al., 2001), and 5 were fed muscle from striped skunks (*M. mephitis*) experimentally infected with *S. neurona* (Cheadle, Yowell, et al., 2001).

Twenty-six Virginia opossums killed by automobiles were obtained in Alachua County, Florida. The blood from the opossums had coagulated; therefore, a filter-paper method was developed and used. An 11.0-cm^2 piece of round filter paper was soaked in blood left in the thoracic cavity, absorbing approximately 0.10 ml of blood. The filter paper was labeled and frozen at −20 C until it was submitted to the Department of Biomedical Sciences and Pathobiology, Virginia Tech, for detection of antibodies via the *S. neurona* direct agglutination test (SAT) (Lindsay and Dubey, 2001). Serum samples from experimentally inoculated opossums were examined as previously described for experimentally infected rodents (Lindsay and Dubey, 2001). Blood samples from automobile-killed opossums were examined using the following modifications of the SAT: briefly, a square (~16 mm) that was soaked in blood was cut from the larger piece of filter paper. The square was added to 200 ml

of phosphate-buffered saline (PBS) in a 1.5-ml microfuge tube. The tube was then vortexed for approximately 30 sec. The solution stood at 20 C for 15 min, was vortexed again, and the remaining filter paper was removed from the solution. A 1:1 dilution of this solution was used for the SAT. All sera from experimentally infected opossums were examined at a 1:50 dilution, and the examiner was blind as to the source. Sera from mice experimentally infected with *S. neurona* were used as a positive control (1:50 dilution). Negative controls consisted of normal mouse sera (1:50 dilution) and sera from mice experimentally infected with *Neospora caninum* (1:50 dilution) and *Toxoplasma gondii* (1:50 dilution).

Filter-paper samples from 2 armadillos (DN65 and DN69) containing *S. neurona*-like sarcocysts and whose muscle tissue induced *S. neurona* sporocyst excretion in opossums (Cheadle, Tanhauser, et al., 2001) were examined in the SAT to determine whether the SAT modification worked in naturally infected animals. Samples from these armadillos were examined at dilutions of 1:10, and serial dilutions were endpoint titrated.

None of the serum samples collected from the experimentally fed opossums was found to contain antibodies to *S. neurona* at a 1:50 dilution using the SAT (Table I). All 3 (100%) armadillos fed opossums had sporocysts in their intestines (1 identified as *S. neurona*) and 5 of 5 (100%) skunks fed opossums had sporocysts in their intestines (5 identified as *S. neurona*). None of the serum samples collected from automobile-killed opossums was found to contain antibodies to *S. neurona* at a 1:1 dilution. Seven of 26 (30%) automobile-killed opossums had sporocysts in their intestines. Naturally infected armadillo DN65 had a titer of 1:1,000, and naturally infected armadillo DN69 had a titer of 1:10,000 in the SAT.

Unlike an opossum, which developed antibodies to *S. neurona* after parental inoculation with *S. neurona* merozoites (Cheadle, Yowell et al., 2001), none of the opossums in this study developed antibodies to *S. neurona* in their serum, even after *S. neurona* had completed its life cycle in the intestinal tracts. Although it is likely that opossums will seroconvert to other species of *Sarcocystis*, such as *Sarcocystis greineri*, where asexual stages are present in extra-intestinal tissues of the opossum intermediate host (Cheadle, 2001), diagnostic tests are not currently available to detect antibodies to that species. Muscle sections were not observed to determine whether sarcocysts of *S. greineri* were present; therefore, it is not known whether antibodies to *S. greineri* were present, or whether they will cross-react with the SAT. Further testing to determine whether the opossums developed antibodies to other species of *Sarcocystis* that use it as a definitive host were not performed.

The lack of antibody production to *S. neurona* by opossums is probably due to a lack of host immune response to the sexual stages of the parasite in intestinal tissues. It also indicates a lack of migration of asexual stages of *S. neurona* in the opossum. Another reason for the lack of antibody detection may be that the antigen in this test is not recognized by antibodies produced against the sexual stages of the parasite contained in the intestine. The lack of antibody production will not allow the screening of sera using the SAT to determine whether an opossum is infected with *S. neurona*; however, alterations to the test may allow for the detection of antibodies against sexual stages.

Our finding that the filter-paper modification of the SAT is valid has implications in epidemiological studies. This simple method can be used to collect samples in the field for transport back to the laboratory. The filter-paper samples require less space and will not break as will glass test tubes or collection vials. A recent study on beavers using this filter-paper method indicated that 4 (6%) of 62 beavers from Massachusetts had antibodies to *S. neurona* (Jordan et al., 2005).

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