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Prevalence of Agglutinating Antibodies to *Toxoplasma gondii* and *Sarcocystis neurona* in Beavers (*Castor canadensis*) From Massachusetts

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ABSTRACT: The present study examined the seroprevalence of *Toxoplasma gondii* and *Sarcocystis neurona* in a population of beavers (*Castor canadensis*) from Massachusetts. Sixty-two blood samples were collected during the field seasons over 3 consecutive years from different animals. Blood was collected onto filter paper and shipped to the Department of Biomedical Sciences, Virginia Tech, Blacksburg, Virginia, for parasite testing. The samples were tested at dilutions of 1:25, 1:50, and 1:100 against each parasite antigen by modified agglutination tests to determine whether antibodies to either parasite were present in the blood. Six of 62 samples (10%) were positive for *T. gondii*, with 2 samples having titers of 1:25 and 4 having titers of 1:50. Four of 62 samples (6%) were positive for *S. neurona*, with 2 samples having titers of 1:25 and 2 having titers of 1:50.

Toxoplasma gondii is an important protozoal pathogen of humans and other warm-blooded vertebrates. Humans become infected by ingesting meat containing tissue cysts or by ingesting oocysts in the environment. Domestic cats and other felines are the only known, definitive hosts for *T. gondii*. Cats can excrete millions of environmentally resistant oocysts in their feces. Sporulated *T. gondii* oocysts have been reported to be able to survive in the environment for 1.5 yr by Frenkel et al. (1975) and for 4.5 yr at 4 C by Dubey (1998). The cat population in the United States was ~57 million in 1991 (Patronek, 1998). The prevalence of antibodies to *T. gondii* is ~58% in free-roaming cats and 37% in pet cats (Dubey, 1994). Outbreaks of *T. gondii* resulting from contaminated water have been described (Dubey, 2004). The prevalence of *T. gondii* antibodies in beavers may reflect the environmental exposure to *T. gondii* in aquatic ecosystems.

Sarcocystis neurona is an obligate, heteroxenous parasite that has the opossum as its definitive host. Intermediate hosts are infected by ingestion of sporocysts passed in opossum feces. *Sarcocystis neurona* is important because it is the causative agent of equine protozoal myeloencephalitis, a severe neurological disease that can occur when a horse accidentally ingests *S. neurona* sporocysts. Horses may become infected by drinking sporocyst-contaminated water.

Little is known about the prevalence of apicomplexan parasites in beavers. The present study was conducted to determine the seroprevalence of *T. gondii* and *S. neurona* in beavers from Massachusetts.

Beavers were live-trapped as part of a larger project on their demographics. Four study areas have been established across Massachusetts to represent a combination of different levels of development and different attitudes of residents regarding management of beavers (Deblinger et al., 1999). The northeastern area of Massachusetts represents heavy suburban development, and a majority of people there are opposed to recreational fur-trapping (based on how residents voted on a statewide trapping referendum). The central area represents light suburban development, and a slight majority of people there are in favor of recreational trapping. The southwestern area is rural, with a slight majority of people opposed to trapping, and the northwestern area is rural, with the majority of residents in favor of recreational trapping. Colonies were surveyed and beavers captured and marked at sites in the eastern, central, and western areas of the state. At least some of these localities are sites included in the state's long-term, beaver colony–monitoring program, which has been ongoing for the last 7–8 yr.

Bobcats (*Felis rufus*) as well as feral and pet domestic cats were present in all study areas (K. Koenen and S. DeStefano, pers. obs.). One of 9 documented mortalities of beavers included predation by a bobcat of a kit during a year of low water levels in the western study area. Bobcats are capable of dealing with human influences, but they tend to avoid agricultural areas with extensive cleared lands that eliminate other vegetative cover types. Bobcats can be classified as being common in central and western Massachusetts, present in the northeast, and rare to absent in southeastern areas of the state (Massachusetts Division of Fisheries and Wildlife, unpubl.).

Beavers were captured at multiple colonies within each of the 3 study areas. Box traps (Koenen et al., in press) and Bailey traps were used to capture beavers, and each animal was immobilized with an intramuscular injection of ketamine hydrochloride (10–13 mg/kg) and acepromazine maleate (2.5 mg) (Lancia et al., 1978). Age and sex were determined for each animal, and individuals were marked with metal and plastic ear tags as well as a tail-mounted radio transmitter. Given the difficulty of venipuncture in beavers, an alternative method for collecting samples was employed. Drops of blood were blotted onto filter paper following a cutaneous tail stick using a hypodermic needle. This is a reliable method of collecting blood for use in toxoplasmosis testing, as described in congenitally infected infants (Hsu et al., 1992; Patel and Holliman, 1994; Paul et al., 2001). It also has been used in sheep (Uggla and Nilsson, 1987) and cats (Nogami et al., 1992). After marking, beavers were placed back into box traps in a shady, protected area near the water, and individuals were not released until they had recovered fully from the drugs. Recovery was judged on the basis of the beaver's alertness, mobility, and ability to hold up its head and to move within the box trap (Koenen et al., in press).

The filter-paper blood blots were kept on ice packs and mailed to the Center for Molecular Medicine and Infectious Diseases, Department of Biomedical Sciences and Pathobiology, Virginia–Maryland Regional College of Veterinary Medicine, Virginia Tech, for testing. Sections (1 cm^2) containing the dried blood were cut from the filter-paper blots and submerged in 200 μl of phosphate-buffered saline (PBS). The paper was soaked overnight at 4 C to allow blood proteins to dissolve into the PBS. Then, the eluate was removed and placed into clean tubes for use in agglutination testing.

The modified direct agglutination test (MAT) was used to examine beaver eluates for agglutinating immunoglobulin G antibodies to *T. gondii* (Dubey and Desmonts, 1987) and *S. neurona* (Lindsay and Dubey, 2001). A dilution of 1:25 was used to screen sera. Thirty-one serum samples were tested from beavers in 2002, 17 in 2003, and 14 in 2004. Positive beaver sera were examined further at dilutions of 1:50 and 1:100.

Positive *T. gondii* MAT results were found in 6 of 62 beavers (10%) from 2002. Four samples were positive at 1:50, and 2 samples were positive only at 1:25. Four of 62 beavers (6%) from 2002 were positive for *S. neurona* by MAT. Two were positive at 1:50, and 2 were positive only at 1:25. No samples from 2003 or 2004 were positive for *T. gondii* or *S. neurona*.

Toxoplasma gondii has been isolated from a beaver in Kansas (Smith and Frenkel, 1995). The prevalence of *T. gondii* in beavers reported here is low compared to that in other surveys of aquatic wildlife in the United States. One study in North Carolina found that 45% of river otters sampled were seropositive for *T. gondii* using a latex agglutination test (Tocidlowski et al., 1997).

The infected beavers in the present study may be ingesting *T. gondii* oocysts excreted by wild or domestic cats from contaminated water or foliage. Bobcats are the only wild cats that live in Massachusetts, where they are most common in the central and western regions. Although to our knowledge no prevalence data exist for *T. gondii* in bobcats from Massachusetts, we are aware of studies conducted in other parts of the United States. Kikuchi et al. (2004) tested serum from 52 bobcats from various states for antibodies to *T. gondii*, and 50% of the animals were seropositive.

We are unaware of any previous reports of *S. neurona* infection in beavers. However, a recent study examined other wildlife species from Connecticut for agglutinating antibodies to *S. neurona*. Eleven of 24 skunks (46%) and 12 of 12 raccoons (100%) had positive titers to *S. neurona* (Mitchell et al., 2002). Another study found that 24 of 25 raccoons (96%) from Massachusetts were seropositive for *S. neurona* (Lindsay et al., 2001). Compared to these and other studies, the prevalence of *S. neurona* infection in beavers observed in the present study is low.

Opossums are the definitive host for *S. neurona*, and they are abundant throughout Massachusetts. Prevalence data are not available for *S. neurona* infections in opossums from Massachusetts, but studies have been conducted in Maryland and Michigan. These studies found that 6 of 11 opossums (55%) from Maryland (Dubey, 2000), and 31 of 206 opossums (15%) from Michigan (Elsheikha et al., 2004), were infected with *S. neurona*.

The prevalence of *T. gondii* and *S. neurona* in beavers from Massachusetts is lower than what has been observed in other wildlife species. However, even a small number of infected animals indicates the presence of parasites in the area.

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