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Prevalence of *Toxoplasma gondii* Infections in Pennsylvania Black Bears, *Ursus americanus*

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ABSTRACT: Serum samples from 665 hunter-killed black bears killed in 1989 to 1992 throughout Pennsylvania (USA) were tested for *Toxoplasma gondii* antibodies by the agglutination test in dilutions of 1:25, 1:50, and 1:500. *Toxoplasma gondii* antibodies were found in 535 of 665 (80%) bears. Considering the highest dilutions at which antibodies were detected, prevalences were 10% at 1:25, 37% at 1:50 and 33% at 1:500. No significant difference in antibody prevalence was found between males (79%) and females (80%), but a significant difference was found between juvenile (65%) and adult (83%) bears.

Key words: *Toxoplasma gondii*, toxoplasmosis, prevalence, antibodies, *Ursus americanus*, black bear.

Toxoplasma gondii is an intracellular parasitic protozoan that infects a wide variety of vertebrates including mammals and birds and is the causative agent of toxoplasmosis, a serious disease of humans and livestock. Infections in immunocompetent individuals are usually asymptomatic, although fatal *Toxoplasma* encephalitis may develop in humans with the acquired immune deficiency syndrome (AIDS) or in patients receiving immunosuppressive therapy for organ transplantation or treatment for tumors. Congenital infection can cause serious disease to the fetus resulting in hydrocephaly, mental retardation, and birth defects (Dubey and Beattie, 1988; Frenkel, 1990).

The role of wildlife in the transmission of the disease is not fully understood, though wildlife can serve as intermediate, reservoir hosts for the organism (Dubey and Beattie, 1988; Dreesen, 1990). Encysted *Toxoplasma* can survive for years in the skeletal muscle of these animals and cause an acute *T. gondii* infection when the infected meat is consumed by humans or other wildlife (Dubey and Beattie, 1988).

Little is known regarding prevalence of the parasite in the black bear. Northern Pennsylvania (USA) has a large black bear (*Ursus americanus*) population, and game management programs maintain the population at approximately 7,500 animals. Hunters, in a 3-day season, kill an average of 1,500 bears each year (Alt, 1990). Since these bears are used as a human food source, our objective was to determine the prevalence of *T. gondii* in Pennsylvania black bears.

The Pennsylvania Game Commission (PGC) requires that all hunter-killed black bears be examined and tagged at check stations located throughout the state. Most bears have been field-dressed by the hunters but blood collects in the body cavity during the trip to the check station. During the 1989 to 1992 bear hunting seasons, blood samples were removed from the body cavity of bears at check stations. Blood was kept on ice in the field; at the laboratory it was centrifuged and serum was removed and frozen at −20 C. Information recorded included the sex of the animal, age of the animal (juvenile or adult, as determined by length of canine teeth) (Willey, 1974) and the county and township where each animal was killed. Sera were examined for *T. gondii* antibodies using an agglutination test (Desmonts and Remington, 1980) as modified by Dubey and Desmonts (1987). Whole formalin-preserved *T. gondii* tachyzoites used as an antigen in the agglutination test were prepared at the Institute de Puericulture, Paris, France, as described by Desmonts and Remington (1980). All testing was done at the Zoonotic Diseases Laboratory, Beltsville, Maryland (USA).

Serum from each bear was diluted 1:25,

1:50, and 1:500, respectively, in pH 7.2 phosphate-buffered saline (PBS). The selection of 1:25 dilution of serum as the threshold titer for the detection of *T. gondii*-specific antibodies was based on experience with agglutination testing with human and animal sera (Dubey and Beattie, 1988). Positive and negative controls were included in each test and all sera were tested by one individual.

The chi-square test (Sokal and Rohlf, 1981) was used to determine statistical associations between prevalence of *T. gondii* antibodies in male versus female bears and between juvenile and adult bears.

Toxoplasma gondii antibodies were found in 535 of 665 (80%) of bear sera collected during 1989 to 1992. For 10% of the bear sera tested, the highest titer was 1:25, for 37% it was 1:50, and for 33% of the bear sera, it was 1:500. All 27 counties sampled had seropositive bears (ranging from 25 to 100% of the bears tested). The bear sera were analyzed in two lots corresponding to samples collected during 1990 to 1991, and 1992. In both lots, the antibody prevalence was 80% and adults had a higher prevalence than juveniles (1990 to 1991: $n = 350$, adults = 80%, juveniles = 67%; 1992: $n = 315$, adults = 83%, juveniles = 65%; $P < 0.05$). From these data it appears that the prevalence remained constant from 1990 to 1992, and that the serological methods employed were consistent.

In two previous surveys for *T. gondii* antibodies in sera of bears in the United States, indirect hemagglutination antibodies were found in 27% of 147 bears from California (USA) (Ruppanner et al., 1982) and 8% of 303 bears from Idaho (USA) (Binninger et al., 1980); the sera of these studies were tested at a 1:64 dilution. Although the data from these two surveys are not strictly comparable to our findings because of different serological tests used and different dilutions tested, our 80% prevalence is definitely one of the highest prevalences of *T. gondii* antibodies among animals and humans in the United States, especially in the eastern United States. We are not aware of any large scale *Toxoplasma* antibody study in humans and animals from Pennsylvania. Feldman and Miller (1956) found dye test *T. gondii* antibodies in 35% of 144 humans from Pittsburgh, Pennsylvania. Using the same dilutions and the same serological test as for the black bears, agglutination antibodies were found in 28% of 474 pigs (Dubey et al., 1991) from the eastern United States, including Pennsylvania and in 24 of 28 raccoons (*Procyon lotor*) (Dubey et al., 1992) from Pennsylvania.

Toxoplasma gondii usually is transmitted postnatally by ingesting water or food contaminated with oocysts passed in feces of infected cats or tissue cysts in animal tissues. Black bears and raccoons are omnivores and feed on vegetation, carrion, rodents, and garbage. *Toxoplasma gondii* infection in them may serve as a potential source for human infection and these wild animals also act as indicators for environmental contamination with this organism. Although persistence of *T. gondii* tissue cysts in tissues of bears is not known, there is evidence that in other animal species, *T. gondii* encysts in several organs including viscera, skeletal muscle, and the central nervous system (Dubey et al., 1980, 1992; Dubey and Beattie, 1988; Dreesen, 1990; Lindsay et al., 1991). Because of the high antibody prevalence of *T. gondii* in bears, hunters should be educated about toxoplasmosis. Burial of bear viscera will reduce predation by carnivores, especially felids. All meat should be cooked so that the internal temperature of meat has reached 66 C for 3 min (Dubey et al., 1990). The cooking temperature recommended to kill *T. gondii* also kills *Trichinella spiralis*.

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