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Source: Journal of Wildlife Diseases, 34(4) : 811-815

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-34.4.811

Journal of Wildlife Diseases, 34(4), 1998, pp. 811–815

Seroprevalence of Antibodies Against *Toxoplasma gondii* in Free-ranging Mammals in Iowa

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ABSTRACT: Serum samples from raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), Virginia opossums (*Didelphis virginiana*), and free-ranging house cats trapped in Iowa between 1984 and 1988 were tested for antibodies against *Toxoplasma gondii* using the modified direct agglutination test (MAT). Antibody titers ≥1:32 were considered indicative of infection. Prevalence rates by species were estimated for raccoons at 134/885 (15%), skunks at 38/81 (47%), opossums at 12/53 (23%), and cats at 16/20 (80%).

Key words: Epidemiology, field study, prevalence, serologic survey, *Toxoplasma gondii*, wild mammals.

Toxoplasma gondii is a protozoan parasite belonging to phylum Apicomplexa, class Sporozoasida, and family Eimeriidae (Levine, 1982). The geographic distribution of *T. gondii* is so broad, susceptible species so numerous, and infection so common, that one specialist was moved to declare that "... there is a sea of toxoplasma infection around us" (Jacobs, 1957). *Toxoplasma gondii* has a complex life cycle with both sexual and asexual replication phases (Jacobs and Frenkel, 1981). The asexual replication cycle takes place in the tissues of susceptible species and appears to be capable of parasitizing essentially all warm-blooded animals, including mammals, marsupials, and birds. Parasites encysted in the tissues remain viable throughout the lifetime of the non-felid host (Quinn and McCraw, 1972). Toxoplasmosis is considered to be an increasingly important disease of humans. Infections in healthy children and adults are usually mild and generally pass unnoticed. However, fetal infections will occur in 10 to 15% of the pregnant women exposed to the organism for the first time and toxoplasmosis also is recognized as a problem in immunocompromised individuals (Gleason and Hamlin, 1974; Frenkel et al., 1975; Gerberding, 1988).

The objectives of this study were to (1) estimate the serologic prevalence of toxoplasmosis in raccoons (*Procyon lotor*), striped skunks (*Mephitis mephitis*), Virginia opossums (*Didelphis virginiana*), and free-ranging house cats in Iowa and (2) examine the effects of age, sex, and season on seroconversion. Animals were trapped in two areas of the state of Iowa. In Guthrie County (41°36′ to 41°39′N, 94°19′ to 94°28′W), raccoons were trapped between 1984–88 as part of a long term study of raccoon population dynamics (Hasbrouck et al., 1992). Trapping occurred in two 10 wk periods each year starting in March and August. Blood samples were collected and the animal's sex, weight, age, and date of capture were recorded. Ages were determined by tooth extraction, sectioning and cementum annuli analysis (Klevezal and Kleinenberg, 1967). In Cerro Gordo county (43°07′N, 93°27′W), raccoons, skunks, opossums, and free-ranging cats were trapped over a 5 mo period (March–July) each year for 5 yr (1984–88). Animals were classified by sex and date of capture. All animals were adults. All sera were heat inactivated (56 C for 30 min), treated with a 25% kaolin preparation for 30 min to absorb non-specific inhibitors, and stored at −20 C until tested.

Sera were tested in the modified direct

agglutination test (MAT) (Desmonts and Remington, 1980). Studies have shown that the MAT is the most sensitive test for the serodiagnosis of toxoplasmosis (Dubey and Beattie, 1988; Patton et al., 1991). In addition, the MAT does not require living *T. gondii*, expensive equipment, or species specific conjugates. The formalin-fixed tachyzoites used as antigen were supplied by bioMerieux Laboratory Reagents (Lyon, France). The test was performed as previously described with mercaptoethanol (Sigma Chemical Company, Saint Louis, Missouri, USA) added to the serum samples to remove IgM (Dubey and Desmonts, 1987; Patton et al., 1990). Trypan blue dye (J. T. Baker, Inc., Phillipsburg, New Jersey, USA) was added to the diluent solution before it was mixed with the antigen to enhance the visibility of the agglutination reaction.

Sera were diluted in phosphate-buffered saline (PBS), pH 7.2, and screened for antibodies at dilutions of 1:16 and 1:512. Sera positive for anti-*T. gondii* antibodies at the 1:512 dilution were then diluted 2-fold and titered to endpoint. Sera positive for anti-*T. gondii* antibodies at the 1:16 dilution, but negative at 1:512 also were titered to endpoint. Control sera (positive and negative) were included with each batch of serum tested to assure accuracy and reproducibility of results. As a further control, anti-*Sarcocystis* serum prepared in rabbits (Granstrom et al., 1990) also was diluted 2-fold in PBS, from 1:4 to 1:8192, and checked for antibodies that would cross react with the formalin-fixed *T. gondii* tachyzoites used as antigen in the MAT. Two samples were checked. Anti-*Sarcocystis* serum from one of the rabbits was positive at a titer of 8; the other rabbit serum was negative. Because of this and results from previous studies, antibody titers of <1:32 were considered nonspecific reactions (Dubey, 1988; Dubey and Beattie, 1988; Patton et al., 1991).

Data were categorized to study the relationship between factors such as species, season, and sex on presence of *T. gondii* antibodies in the population. In Guthrie county, relationships to age were evaluated. Statistical analyses were performed by the chi-square test (SAS 6.09, SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA).

There was serologic evidence of exposure to *T. gondii* in every species. In Guthrie county 814 blood samples were collected from 775 raccoons. Thirty-four animals were sampled more than once, five of which were trapped in the same season of the same year. The second sample was excluded so that only one sample per season was considered. The number of samples collected each year were 147 (1984), 164 (1985), 148 (1986), 193 (1987), and 157 (1988). Five-hundred-seventy-two (71%) of the samples were collected during the summer/fall trapping period. Distribution of samples by age group were similar; 406 (50%) were from adults and 403 (50%) from juveniles <1-yr-old. The majority of the juveniles 398 (99%) were collected during the second trapping period. The sex distribution of animals trapped over the 5 yr period was 432 (53%) male and 377 (47%) female. The distribution of age groups within sexes was similar. The seropositive frequency rate was 14%, i.e., 111 of 809 raccoon samples demonstrated evidence of exposure to *T. gondii*. Of the 31 animals which were captured and sampled more than once in subsequent seasons or years, 13 were sampled as juveniles and then later as adults. All 13 animals were sampled as juveniles in the summer/fall trapping periods of the years prior to 1988. Eleven of these juveniles were seronegative, two of which became seropositive as an adult. The other two were seropositive as juveniles, with one seronegative and one seropositive when sampled as adults. Seropositive frequency rates varied among the years, seasons, sexes and age groups. Rates by year were 19% in 1984, 12% in 1985, 15% in 1986, 15% in 1987 and 8% in 1988. On a seasonal basis, significantly more ($P = 0.001$) of the samples from the spring trapping period

were seropositive (26%) than from the fall trapping period (9%) when all ages where considered. However, among adults, the frequency rate of seropositive samples was similar ($P = 0.083$) for spring and fall, 26% and 19% respectively. A comparison of age groups showed that the seropositive rate was significantly higher ($P = 0.001$), among adults (23%) as compared to juveniles (4%). Seropositive adults ranged in age from one to seven years. Differences in prevalence rates were observed between chronological age categories for adult animals. Seropositive rates by chronological age were 16% (1-yr-old), 24% (2-yr-old), 15% (3-yr-old), 47% (4-yr-old), 30% (5-yr-old), 100% (6-yr-old), and 100% (7-yr-old). The seropositive frequency rate for adult animals of unknown age was 34%. Seropositive frequency rates were similar ($P = 0.503$) among males (13%) and females (15%) when all ages were considered. Among juveniles, the number of seropositive females (6%) was similar ($P = 0.204$) to males (3%). Adult males (22%) and females (25%) were similar ($P = 0.474$) in seropositive frequency rates. When comparing rates among season and sex groups for adults, adult males trapped during the spring/early summer trapping showed nearly equal ($P = 0.879$) rates (27%) as females (26%). In adults trapped in the summer/fall period, females had a higher seropositive rate (24%) than males (15%) but the difference was not significant ($P = 0.138$).

In Cerro Gordo county 76 blood samples were collected from raccoons. Sample size on a yearly basis ranged from a low of three to a high of 26. The seropositive frequency rates varied from 14% to 100% by year. The overall seropositive frequency rate was 30%. Some raccoons were sampled multiple times in the same year. Only the first sample taken per year was utilized in the analysis. A total of 81 samples from 80 skunks were assayed, with one skunk sampled in two consecutive years. The sample size ranged from two to 38 samples per year. Seropositive frequency rates ranged from 18% to 63% with an overall rate of 47%. Fifty-three opossums were sampled 57 times. Three opossums were sampled multiple times in the same year. Only the first sample collected was used in the analysis. One to 27 opossums were sampled per year. The seropositive frequency rates varied from 0% to 44% with an overall rate of 23%. The number of cats sampled over the years ranged from one to nine per year with a total of 20 samples taken. The seropositive frequency rates ranged from 57% to 100% with an overall frequency rate of 80%. The seropositive frequency rates within each species were analyzed with animals grouped by season or sex. No differences were noted, except for opossums, in which males had a higher ($P = 0.009$) seropositive rate (47%) than females (13%).

These results indicated that many species of wild mammals trapped from different areas in Iowa had been exposed to *T. gondii*. The prevalence rates among free-ranging mammals in Iowa is similar to previously reported rates (Brillhart et al., 1994; Zimmermann, 1975; Tizard et al., 1978; Burridge et al., 1979; Dubey et al., 1992; Smith et al., 1992; Dubey et al., 1995). Infection without serologic response has been reported (Walton and Walls, 1964). Thus, it is likely these prevalence rates underestimated the actual level of exposure of free-ranging Iowa mammals to the organism. The major factors associated with seroprevalence were sex, age, and species. Seropositive rates were most variable for skunks, opossums and cats, however sample size during any particular trapping period was quite small for these species. Seropositive frequency rates for the Guthrie county raccoons varied among age groups, sex, season, and year. Distribution of positive animals varied between adults and juveniles. Having significantly more adults seropositive than juveniles is consistent with length of time in the population being correlated with risk of exposure. The significant decrease in prevalence rates from the spring to the fall

trapping coincided with the large number of negative juveniles entering the population during the second trapping period. This study also suggested that a public health threat exists for persons hunting and consuming meat from wildlife species. This reinforces the importance of thoroughly cooking wildlife meat before consumption.

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Received for publication 19 December 1997.