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Source: Journal of Wildlife Diseases, 37(1) : 36-38

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-37.1.36

Journal of Wildlife Diseases, 37(1), 2001, pp. 36–38

SEROLOGIC SURVEY FOR *TOXOPLASMA GONDII* IN LYNX FROM INTERIOR ALASKA

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ABSTRACT: Two hundred fifty-five lynx (*Felis lynx*) carcasses were collected from trappers in Interior Alaska (USA). Serosanguinous fluids were collected from the chest cavity of each carcass. These fluids were tested for evidence of exposure to *Toxoplasma gondii* by means of a modified agglutination test using formalin fixed tachyzoites and mercaptoethanol. Thirty-nine of the samples had titers greater than or equal to the threshold (≥25). Antibody prevalence differed between areas, and was directly related to age of the host.

Key words: *Felis lynx*, lynx, serology, survey, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasma gondii is a protozoan parasite with worldwide distribution (Dubey and Beattie, 1988). Clinical signs of toxoplasmosis in humans and other animals include lymphadenopathy, myalgia and neuralgia (Dubey and Beattie, 1988). Infection can cause pregnant domestic sheep and goats to abort (Dubey, 1994).

Domestic and free-ranging felids are the only recognized definitive hosts for *T. gondii*. The parasite multiples in the gastrointestinal tract of cats. *T. gondii* oocysts are excreted in feces. Other mammals can become infected by ingesting food or water contaminated with oocysts. The parasite multiplies in the gastrointestinal tract of these secondary hosts. The resulting developmental stages circulate via the blood and lymphatic systems. Tissue cysts form in various organs. Ingestion of these tissue cysts provides another form of transmission (Dubey, 1994). Lynx (*Felis lynx*) may be exposed to *T. gondii* via either route.

Several test procedures have been employed to detect *T. gondii* antibody in mammal sera (Peterson et al., 1974; Kocan et al., 1986; Chomel et al., 1995). The modified agglutination test (MAT) is the most sensitive and specific procedure for detection of latent *T. gondii* infections in domestic swine (Dubey et al., 1995). For these reasons, the MAT was selected for use in the current study.

Serum antibody prevalence of *T. gondii* was 23% (25 positive of 110 tested) for moose (*Alces alces*) from southern parts of Alaska from 1974 to 1982 (Kocan et al., 1986) using the indirect hemagglutination test (IHA). Antibody prevalence was 18% (87 positive of 480 tested) for grizzly bears (*Ursus arctos*) and 15% (six positive of 40 tested) for black bears (*Ursus americanus*) captured in Alaska from 1988 to 1991 (Chomel et al., 1995) using the latex agglutination test (LAT). Antibody prevalence in grizzly bears captured from 1973 to 1987 ranged from 9% (18/196) in southern Alaska to 37% (162/433) in northern areas (Zarnke et al., 1997). Antibody prevalence was 43% for black bears (62/143), 9% for wolves (*Canis lupus*) (11/125), 7% for Dall sheep (*Ovis dalli*) (22/319), 6% for caribou (*Rangifer tarandus*) (14/241), 1% for moose (*Alces alces*) (3/240), and 1% for bison (*Bison bison*) (2/241) from Alaska (Zarnke et al., 2000). In humans, *T. gondii* infection may be common in demographic groups associated with the use and consumption of wildlife. Among 1,572 Alaska Natives tested in the early 1970's using the indirect fluorescent and IHA tests, antibody prevalence was 28%.

Previous studies of *T. gondii* in Alaska wildlife focused on secondary host species. The current project focused on the presumed definitive host species. The objectives of the current project were to deter-

mine (1) the serum antibody prevalence of *T. gondii* in lynx from Interior Alaska and (2) the relationship between antibody prevalence and sex, age, and location.

MATERIALS AND METHODS

Lynx carcasses were purchased from trappers in Interior Alaska. These carcasses were considered a random sample of the population in each area. The large study area was subdivided into four smaller areas based on geologic characteristics. Area A (20,658 mi^2 with a central point at lat 64°N, long 142°30′W) contains large areas of poorly drained lowlands. Area B (5,637 mi^2 with a central point at lat 64°N, long 144°45′W) consisted of upland foothills and mountains. Area C (6,796 mi^2 with a central point at lat 64°10′N, long 147°45′W) was comprised entirely of poorly drained lowland flats. Area D (9,114 mi^2 with a central point at lat 65°N, long 147°W) was predominately upland forest covering low hills.

Sex of lynx was determined by examination of internal organs. Age was determined by counting cementum annuli of a canine tooth (Crowe, 1972). Serosanguinous fluids were collected from 255 carcasses. Fluids were frozen at −50 C for approximately 30 days until the time of testing. Carcasses had been frozen for 30–120 days prior to examination.

Sera were tested by means of the MAT (Dubey and Desmonts, 1987). Mercaptoethanol was incorporated with whole formalinized tachyzoites in the test procedure. Sera which agglutinated the antigen suspension at a serum dilution ≥1:25 were considered indicative of previous natural exposure to *T. gondii*. Sera with a titer ≥25 will be referred to as positive. All others will be referred to as negative. No attempt was made to confirm serologic test results by means of either histologic examination or attempted isolation of *T. gondii* from lynx tissues because tissues had been frozen.

A generalized linear model (McCullagh and Nelder, 1989), with a logit link and a binomial distribution, was used to determine if there was significant dependence of serologic test result on the following variables: (1) age, (2) sex, and (3) location. Serologic test result is a binary response variable. Age was treated as a continuous variable. Sex and geographical location were treated as categorical variables. All main and interaction effects of these variables were examined. During the modeling process, all higher order terms were removed from the model if they did not substantially ($P > 0.05$) increase the fit of the model based on the deviance function compared to a chi-squared distribution (McCullagh and Nelder, 1989). The GENMOD procedure of version 6.12 SAS statistical package was used to fit the model with maximum likelihood parameter estimates (SAS Institute, Cary, North Carolina, USA).

RESULTS

Thirty-nine sera (15%) had titers of ≥25. Ten samples (4%) had titers of 25, 10 (4%) had titers of 50, nine (4%) had titers of 100, seven (3%) had titers of 200, and three (1%) had titers of 800. Two hundred sixteen (85%) had titers of <25.

The probability that an individual serum would be positive increased with the age of the lynx and varied between locations. Antibody prevalence was 13/67 (19%) in Area A, 3/27 (11%) in Area B, 13/61 (21%) in Area C, and 5/80 (6%) in Area D. Age of host was the parameter with the greatest predictive value for a positive serologic test result. The predictive value of geographic area was also significant. The fitted model included these two covariates. The best model is: $\mu = \tau_i + 0.5536 \times$ age; where τ_i is −2.1119 if the animal was from Tok, −2.1437 if the animal was from the Tanana Flats, −3.6875 if the animal was from Fairbanks, and −2.7582 if the animal was from Delta Junction. Because the model is on the logit scale, the predicted value is: $p(\mu) = \exp(\mu)/1 + \exp(\mu)$.

For example, if an animal were 4-yr-old and from the Tanana Flats, then $\mu = 0.0707$, so the probability of a positive serologic test is predicted to be $p(\mu) = 0.5177$. The significance of age in the model was $P < 0.0001$, and the significance of location in the model was $P = 0.0262$. Sex was not significant ($P = 0.3091$), nor was the interaction between location and age ($P = 0.6143$). None of the other higher order interactions were significant. The modeled probability of positive serologic test, as a function of age for each location is presented in Figure 1.

DISCUSSION

Antibody prevalence exhibited an age-specific pattern (Fig. 1). Prevalence increased from nearly 0% in the kitten co-

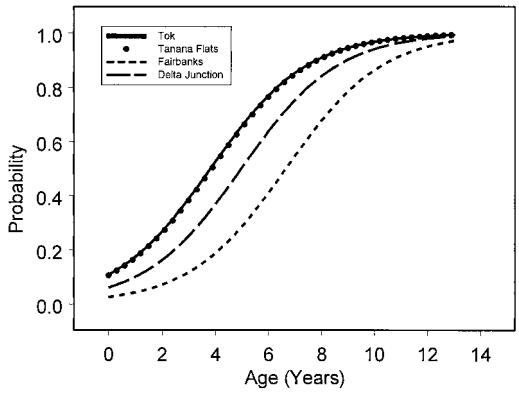

FIGURE 1. Probability of a positive serologic test for *Toxoplasma gondii* in lynx as a function of age and location.

hort to nearly 100% in animals >10 yr. This pattern is typical for many host species and disease agents. It apparently reflects the cumulative opportunity for exposure during an animal's life.

Antibody prevalences also varied between locations (Fig. 1). Those locations with higher prevalence (Areas A and C) have a greater proportion of wetlands (Gallant et al., 1995). Perhaps the habitat and microclimate conditions in these areas enhance survival of shed *T. gondii* oocysts and therefore promote increased transmission.

Domestic cats are uncommon in rural Alaska villages. In most areas of the Interior, feral domestic cats do not survive outside of established communities. Therefore, domestic cats are not believed to represent a major source of *T. gondii* for lynx. Presumably, the infection is enzootic within the lynx population.

Trappers occasionally have direct contact with lynx feces while handling and skinning carcasses. This feces could serve as a route of transmission from lynx to humans if oocysts have an opportunity to sporulate. Therefore, trappers should be advised to wear disposable gloves during the skinning process. Trappers and family members routinely eat lynx meat. This also could serve as source of infection for humans. Lynx meat should be thoroughly cooked prior to eating.

ACKNOWLEDGMENTS

This study was supported in part by Federal Aid in Wildlife Restoration Study 18.71, Grant W-27-2. The authors thank C. Gardner, S. DuBois, and the trappers of Interior Alaska for collecting and submitting carcasses.

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Received for publication 8 January 2000.