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SEROLOGIC SURVEY FOR *TOXOPLASMA GONDII* IN SELECTED WILDLIFE SPECIES FROM ALASKA

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ABSTRACT: Blood was collected from selected wildlife species in specific areas of Alaska (USA) during 1976–96. A modified agglutination test was used to test sera for evidence of exposure to *Toxoplasma gondii*. Serum antibody prevalence was 43% (62 positive of 143 tested) for black bears (*Ursus americanus*), 9% (11/125) for wolves (*Canis lupus*), 7% (22/319) for Dall sheep (*Ovis dalli*), 6% (14/241) for caribou (*Rangifer tarandus*), 1% (3/240) for moose (*Alces alces*), and 1% (2/241) for bison (*Bison bison*). A predictive model was developed to determine the effect of sex, age, location, and year of collection on antibody prevalence for each species. Prevalence was higher in older black bears, caribou, and wolves. For black bears, prevalence was highest in the southeast region of the state. For caribou, prevalence was lowest on the Alaska Peninsula.

Key words: *Alces alces*, bison, *Bison bison*, black bear, *Canis lupus*, caribou, Dall sheep, moose, *Ovis dalli*, *Rangifer tarandus*, serologic survey, *Toxoplasma gondii*, *Ursus americanus*, wolf.

INTRODUCTION

Toxoplasma gondii is an apicomplexion with worldwide distribution. Domestic and free-ranging felids are the only recognized definitive hosts for *T. gondii*. The parasite multiplies in the gastrointestinal tract of cats. 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 means of transmission (Dubey, 1994). Carnivores and omnivores may be exposed to *T. gondii* via either route. Clinical manifestations of toxoplasmosis in humans include lymphadenopathy, myalgia, neuralgia, mental retardation and loss of vision. Toxoplasmosis is a major cause of abortion in domestic sheep and goats, and mortality in Australian marsupials (Dubey and Beattie, 1988).

Several serologic tests 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 procedure for detection of latent *T. gondii* infections in black bears (*Ursus americanus*) (Dubey et al., 1995a).

Serum antibody prevalence of *T. gondii* was 23% (25 positive of 110 tested) for moose (*Alces alces*) collected from 1974–82 from southern portions of Alaska (USA) (Kocan et al., 1986). This survey employed the indirect hemagglutination test (IHAT). Antibody prevalence was 28% in 1,572 Alaska Natives tested during the early 1970s (Peterson et al., 1974). This survey used the indirect fluorescent antibody test and the IHA. Antibody prevalence was 18% (87 positive of 480 tested) for brown/grizzly bears (*Ursus arctos*) and 15% (six positive of 40 tested) for black bears captured in Alaska from 1988 to 1991 (Chomel et al.,1995). This survey used the latex agglutination test (LAT). Antibody prevalence in brown/grizzly bears ranged from 9% (18/196) in the southern portion of Alaska to 37% (162/433) in the northern portion from 1973 to 1987, as determined by the MAT (Zarnke et al., 1997).

The objectives of the current project were to determine (1) the serum antibody prevalence of *T. gondii* in selected terrestrial big game species from Alaska and (2)

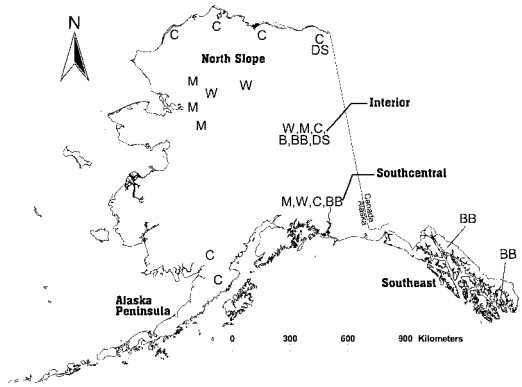

FIGURE 1. Capture areas for wildlife species tested for serologic evidence of exposure to *Toxoplasma gondii* in Alaska. Species tested include bison (B), black bear (BB), caribou (C), Dall sheep (DS), moose (M), and wolf (W).

the relationship between antibody prevalence and sex, age, location, and year of collection.

MATERIALS AND METHODS

Blood samples were collected by personnel of the Alaska Department of Fish and Game (Fairbanks, Anchorage, and Juneau, Alaska, USA) and the U.S. Fish and Wildlife Service (Fairbanks and Anchorage, Alaska, USA) during population ecology studies conducted from 1976–96 (Fig. 1). Sera were stored temporarily at −12 C and subsequently at −40 to −50 C for up to 21 yr until the time of testing. Ages of bears (in yr) were determined by examining cementum annuli of premolar teeth (Craighead et al., 1970). Ages of Dall sheep (*Ovis dalli*) were determined by counting horn growth rings.

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."

For all species, a generalized linear model, with a logit link (McCullagh and Nelder, 1989) and a binomial distribution, was used to determine if there was significant dependence of serologic test result on the variables of (1) age, (2) sex, (3) year of collection, and (4) location. Serologic test result is a binary response variable. For some species age could be determined to the nearest year. In these cases, age was treated as a continuous variable. Otherwise, age was grouped in classes (e.g., pup, yearling, and adult) and analyzed as a categorical variable. Sex and geographic location were treated as categorical variables. Year of collection was treated as a continuous variable. 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 software package was used to fit the model with maximum likelihood parameter estimates (SAS Institute, Inc., Cary, North Carolina, USA).

The generalized linear model allows evaluation of all effects simultaneously. It also incorporates continuous data and categorical data (and their interactions) into a single model. Alternatives such as contingency tables allow evaluation of only a single variable at one time. In addition, they require modification of continuous variables into categorical variables. Thus, the generalized linear model was chosen for the type of data involved in the current study.

RESULTS

A summary of serologic test results is presented in Table 1. One half of the 62 positive black bear sera had titers ≥500. This titer is well beyond the threshold and eliminates concern regarding false positive results. The fitted model for black bear included the two covariates of age and location. The best model is $\mu = -1.9120 + 0.2198x + \tau_i$ where x is age, and τ_i is −1.6231 if the location is Southcentral, 0 if location is Interior, and 2.8747 if location is Southeast; and where the predicted value is $p(\mu) = \exp(\mu)/[1 + \exp(\mu)]$. For example, if an animal were 10-yr-old and from the Interior, then $\mu = -0.018$, so the probability of a positive test result is predicted to be $p(\mu) = 0.4955$. Both age and location in the model has significance values of $P < 0.0001$.

For bison (*Bison bison*), only two of 241 animals had positive results. Both were females. No age data was available. All ani-

TABLE 1. Serum antibody prevalence of *Toxoplasma gondii* in six wildlife species from Alaska.

Species	Prevalence[a]	Sample size at selected titers		
		25	50	≥500
Black bear (*Ursus americanus*)	62/143 (43)[a]	6	25	31
Wolf (*Canis lupus*)	11/125 (9)	6	4	1
Dall sheep (*Ovis dalli*)	22/319 (7)	10	9	3
Caribou (*Rangifer tarandus*)	14/241 (6)	3	6	5
Moose (*Alces alces*)	3/240 (1)	3	0	0
Bison (*Bison bison*)	2/241 (1)	1	1	0

[a] Number positive/number tested (%).

mals came from the same location. None of the covariates significantly improved the model, so the fitted model is simply $\mu = -4.7833$ and $p(\mu) = 0.0083$ (this is equal to 2/241).

The fitted model for caribou (*Rangifer tarandus*) included two covariates, age and location. The best model is $\mu = -29.1294 + \alpha_i + \tau_i$ where α_i is 0 if age is calf or yearling and 27.3864 if age is adult, and τ_i is −26.798 if from the Alaska Peninsula and 0 if from any other region in Alaska.

This model establishes essentially two groups; if a caribou is a yearling or younger, or from the Alaska Peninsula, then $p(\mu)$ is very near zero (because there were zero positive results in these age classes and this location), whereas if a caribou is an adult from the Interior or northern Alaska, then $\mu = -1.743$ and $p(\mu) = 0.1489$. In the model, the significance of age was $P < 0.0001$ and location was $P = 0.0103$.

None of the covariates significantly improved the model for Dall sheep. There were 22 positive results out of 319 animals tested. As for bison, then, the model is very simple, $\mu = -2.6027$ and $p(\mu) = 0.0690$ (this is equal to 22/319).

Three of the 240 animals were positive. Two of these positive animals were adults. One was a calf. None of the covariates significantly improved the model for moose. Similar to the situation for bison and sheep, the model for moose is very simple, $\mu = -4.3694$ and $p(\mu) = 0.0125$ (this is equal to 3/240).

Four of the positive wolf (*Canis lupus*) sera had titers of 50. One sample had a titer ≥500. The fitted model for wolf included the single covariate age. The best model is $\mu = -27.3653 + \alpha_i$, where α_i is 0 if the age is pup or yearling and 25.6201 if age is adult. As for caribou, this probability of a pup or yearling showing a positive result is near 0, whereas for an adult $\mu = -1.7452$ and $p(\mu) = 0.1487$. The significance of age in the model was $P = 0.0005$.

DISCUSSION

The MAT titer indicative of actual *T. gondii* infection varies among host species. For example, a titer <100 may be nonspecific in domestic cattle. Conversely, *T. gondii* has been isolated from domestic swine and black bears with titers as low as 25 (Dubey et al., 1995a,b; Dubey, 1997). This threshold titer of 25 was selected based on extensive validation of MAT in domestic pigs (Dubey et al., 1995a; Dubey, 1997). This interspecies variability and the lack of large-scale experimental infections of wildlife species often makes it difficult to interpret serologic test results.

The source(s) of *T. gondii* exposure for wildlife species in Alaska are unknown. Felids infected with *T. gondii* shed infective oocysts in feces. Wildlife could be exposed via ingestion of food or water contaminated by felid feces. Domestic cats are uncommon in rural Alaska villages. In most areas of mainland Alaska, feral domestic cats do not survive outside of es-

tablished communities. Therefore, domestic cats are not believed to represent a major source of exposure for wildlife.

Lynx (*Lynx canadensis*) are the only free-ranging felids in Alaska. Thus, this species is the logical replacement for the domestic cat in the transmission cycle of *T. gondii* in Alaska. Lynx are found throughout most of the mainland. They do not occur on Kodiak Island or the islands of the southeastern region. In addition, population density of lynx is low on the southeastern mainland, on the Alaska Peninsula and north of the Brooks Range (68°N) (Bee and Hall, 1956). A recent serologic survey revealed an antibody prevalence of 15% (39/255) in lynx from Interior Alaska (R. L. Zarnke and J. P. Dubey, unpubl. data). We are unaware of any previous studies.

Antibody prevalence was highest in wolf and black bear (Table 1). Carnivore/omnivore species such as these can be exposed to *T. gondii* via ingestion of infected meat. Thus, the higher prevalence in these two species is reflective of their higher position in the food chain. Potential sources of *T. gondii* for wolves and black bears include all herbivore species included in this survey, as well as other carnivores. No tissues were examined histologically to confirm *T. gondii* exposure.

Antibody prevalence in black bears had a distinct geographic pattern. Prevalence was highest in bear populations from the southeastern region of Alaska (26 positive of 33 tested; 78%). Prevalence was intermediate in the Interior (21/76; 28%) and lowest in the southcentral region (5/34; 15%). No explanation for this pattern is readily apparent.

The high antibody prevalence in black bears from the southeastern region is in direct contrast to the low prevalence in brown/grizzly bears from this region (Zarnke et al., 1997). Most of the black bears in the current survey were captured on the mainland, whereas most of the brown/grizzly bears in the previous study were from large islands adjacent to the mainland. Lynx and feral cats are found in small numbers on the mainland. Free-ranging felids are essentially nonexistent on the islands. Apparently, opportunities for exposure to *T. gondii* are significantly higher on the mainland than on the islands. The only other geographic pattern was a prevalence of 0% for caribou from the Alaska Peninsula.

Antibody prevalence was directly related to age for black bears, wolves, and caribou. This is a common pattern for many host species and disease agents. Apparently, opportunity for exposure to *T. gondii* is routinely available. As an animal ages, its cumulative likelihood for exposure increases.

Antibody prevalence of *T. gondii* in bison was <1% (2/241). Prevalence in bison from Montana was similarly low (2/93; 2%) (Dubey, 1985). Both bison and cattle have been experimentally infected with *T. gondii* (Dubey, 1983). However, *T. gondii* has not been isolated from naturally exposed bison. In addition, the parasite has only rarely been isolated from naturally exposed cattle. For this reason, cattle are considered to be a relatively resistant host. Bison may be biologically similar to cattle with respect to *T. gondii* infection. Both objective results and subjective interpretation indicate that bison are also a relatively resistant host.

Reindeer (*Rangifer tarandus*) are highly susceptible to *T. gondii* infection (Oksanen et al., 1996). They develop both clinical illness and high levels of antibody (Oksanen et al., 1996). We are not aware of any previous studies of *T. gondii* exposure in caribou from Alaska. However, caribou and reindeer are conspecific. Therefore, we assume that the susceptibility of caribou is similar to that of reindeer. An outbreak of toxoplasmosis in pregnant women from northern Quebec, Canada was linked to consumption of raw caribou meat (McDonald et al., 1990). Consumption of raw caribou meat is common in some rural areas of Alaska. A public information campaign should be implemented to inform

the Alaskan public of the human health risk of this practice.

Antibody prevalence of *T. gondii* in moose was 1% (3/240). Two of these animals were captured in the southcentral portion of the state; one during 1979 and the other during 1980. The third animal was captured in the northwestern portion of the state during 1994. This prevalence was much lower than the 23% (25/110) reported for moose from southern portions of Alaska (Kocan et al., 1986). In addition, the parasite was isolated from one of seven moose from Montana (Dubey, 1981). Survival of oocysts declines at low temperatures (Dubey and Beattie, 1988). Perhaps the decreasing prevalence in moose at higher latitudes is related to colder winter temperatures.

Humans routinely eat meat from black bears and all of the herbivorous species included in this survey. Results of the current study indicate that this meat could serve as a source of *T. gondii* exposure for humans and other mammals. In order to reduce the potential for transmission of *T. gondii* to humans, meat of wildlife species should be thoroughly cooked prior to consumption.

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