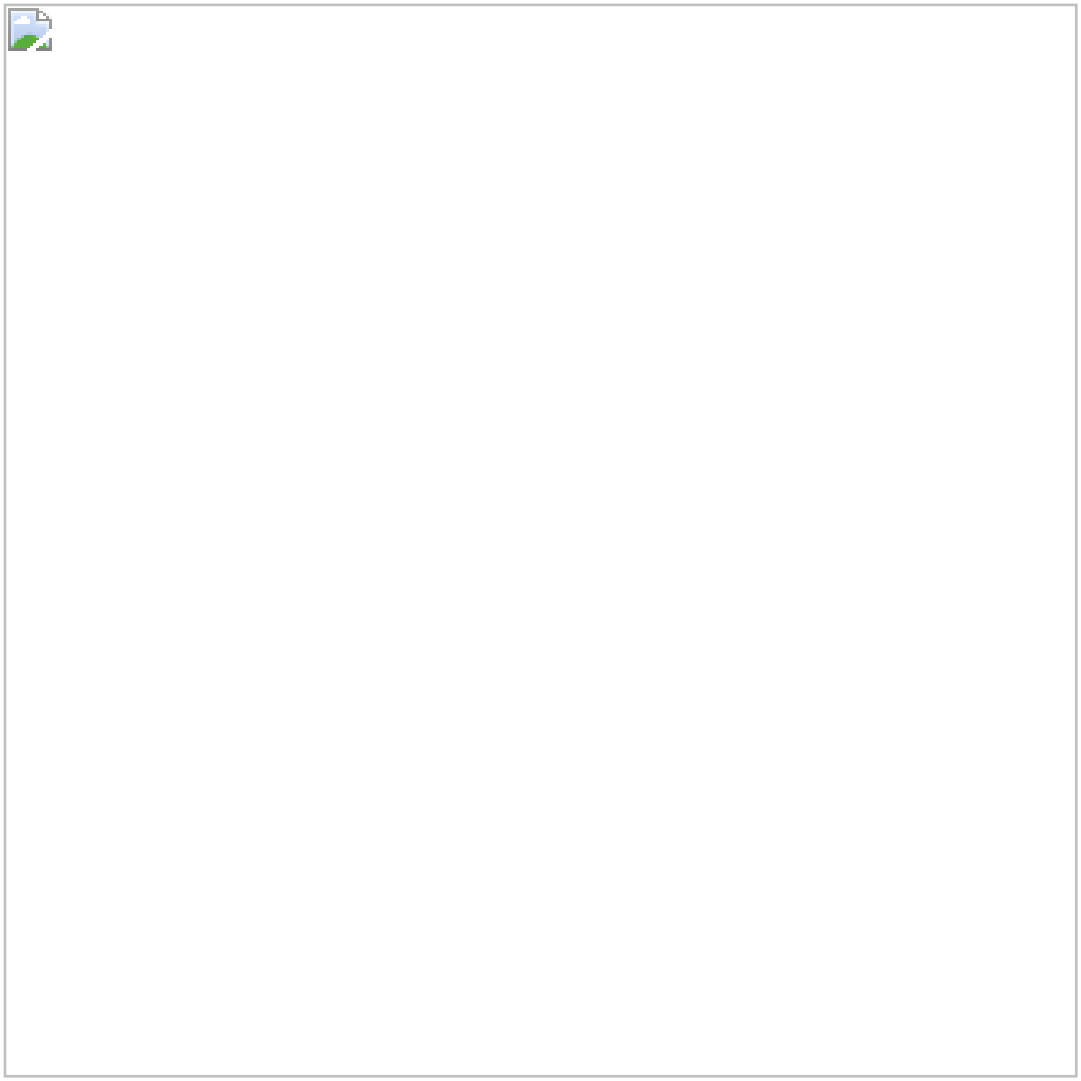

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SEROLOGIC SURVEY FOR *TRICHINELLA* SPP. IN GRIZZLY BEARS FROM ALASKA

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ABSTRACT: Blood was collected from 878 grizzly bears (*Ursus arctos*) in seven geographic areas of Alaska from 1973 to 1987. An enzyme-linked immunosorbent assay procedure was used to test sera for evidence of exposure to *Trichinella* spp. Serum antibody prevalence ranged from 5% (10 positive of 196 tested) in the Southern Region of the state to 83% (355 of 430 tested) in the Northern Region. These major discrepancies may be a result of differing food habits of bears in the major geographic areas. Prevalence was higher in older age cohorts. Neither year-of-collection nor sex had a significant effect on prevalence.

Key words: Grizzly bear, serology, survey, *Trichinella* spp., trichinellosis, *Ursus arctos*.

INTRODUCTION

Trichinella spp. are nematode parasites which are capable of infecting virtually all warm-blooded animals (Dick, 1983). Prevalences vary between different host species. *Trichinella* spp. can only be transmitted by ingestion of infected muscle tissue from another host (Bailey and Schantz, 1990). Therefore, strict carnivorous species generally have higher prevalences than omnivorous species (Franchimont et al., 1993; Oivanen and Oksanen, 1993).

Human trichinellosis is frequently associated with ingestion of infected pork. Since 1975 the number of human cases acquired from pork in the United States has decreased. Concurrently, the number of cases acquired from other species has increased (Bailey and Schantz, 1990). Historically, the most common source of infection for humans in Alaska (USA) has been bear meat (Williams, 1946; Maynard and Pauls, 1962; Clark et al., 1972). Alaska has the highest per capita incidence of human trichinellosis in the United States (Schantz et al., 1977; Stehr-Green et al., 1986).

Diagnostic testing for trichinellosis can be performed by several methods. One method involves direct microscopic observation of larvae in musculature. This method is known as the compression method. A second method involves observation of larvae from digested muscle tissue. This method is known as the digestion method. A third general category involves testing serum for presence of *Trichinella* spp. antibodies. One specific method in this group is known as the enzyme-linked immunosorbent assay (ELISA) (Gamble and Murrell, 1992).

The ELISA is an effective tool for detecting *Trichinella* spp. infection in a variety of host species including humans (*Homo sapiens*) (Ivanoska et al., 1989), domestic pigs (*Sus scrofa*) (Murrell et al., 1986), horses (*Equus caballus*) (Fetzner et al., 1991; Gamble et al., 1996), black bears (*Ursus americanus*) (Dubey et al., 1994) and polar bears (*Thalarctos maritimus*) (H. R. Gamble, unpubl. data). The ELISA was 100% accurate in identifying infection in six of 319 black bear sera from Pennsylvania (USA) (Dubey et al., 1994).

The objective of the current study was to determine the relationship of the following four host parameters to serum antibody prevalence of *Trichinella* spp. in grizzly bears from Alaska to: 1) sex, 2) age, 3) year-of-collection, and 4) geographic location.

MATERIALS AND METHODS

Personnel of the Alaska Department of Fish and Game and the U.S. Fish and Wildlife Ser-

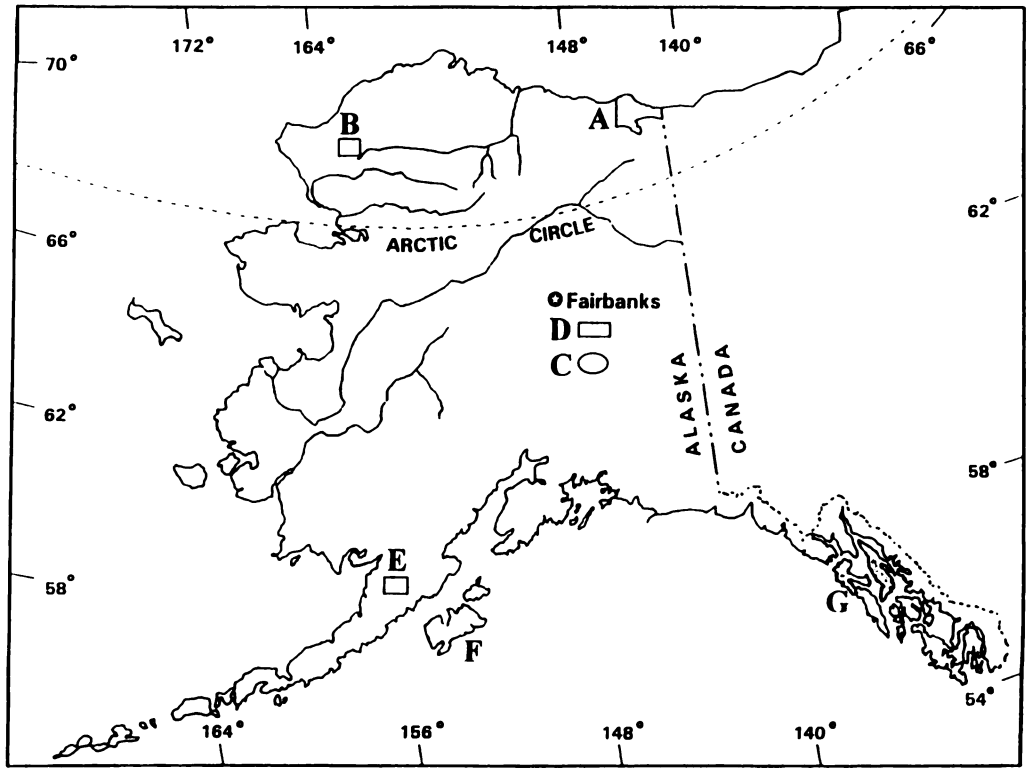

FIGURE 1. Location of collection sites for grizzly bears (*Ursus arctos*) included in *Trichinella* spp. serologic survey. A, Northeast Arctic (69° to 70°N; 141° to 146°W); B, Northwest Arctic (69° to 70°N; 158° to 162°W); C, Southcentral Interior (62°30′ to 63°30′N; 146° to 148°W); D, Central Interior (62°45′ to 64°15′N; 146°30′ to 148°30′W); E, Alaska Peninsula (57° to 58°N; 156° to 157°W); F, Kodiak Island (57° to 58°N; 152° to 155°W); G, Southeastern Islands (57° to 58°N; 134° to 135°W).

vice collected 878 grizzly bear sera from seven sites during studies of bear population ecology (Fig. 1). Sera were stored temporarily at −12 C and then at −40 to −50 C for up to 22 yr until the time of testing. Ages of bears (in yearly increments) were determined by examining cementum annuli of premolar teeth (Craighead et al., 1970).

Sera were tested according to the method of Dubey et al. (1994). *Trichinella spiralis* larval excretory-secretory antigen (5 μg/ml) was diluted in carbonate buffer (pH 9.6). Each well in polystyrene microtitration plates (Dynatech Laboratories, McLean, Virginia, USA) was coated with 100 μl of this antigen suspension. Plates were incubated for 60 min at 37 C. Wells were washed three times with 50 mM Tris buffer (Gibco BRL Life Technologies, Gaithersburg, Maryland, USA) (pH 8.0) which contained 150 mM sodium chloride, 5% nonfat dry milk and 1% Triton X-100 (Fisher Biotech, Fair Lawn, New Jersey, USA). Sera were diluted 1:10 in this Tris buffer. Then 100 μl of the diluted serum was added to antigen-coated wells for 30 min at 20 to 22 C. Wells were again washed as described. Peroxidase conjugated goat-anti-black bear serum (IgG fraction; Jackson Immuno Research Laboratories, West Grove, Pennsylvania) was diluted 1:500 in Tris buffer. Then 100 μl of the conjugate was added to each well and incubated for another 30 min. A final wash in Tris buffer and a distilled water rinse followed. Then 2,2′-azino-di-3-ethylbenzthiazoline sulfate (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) substrate was added. After 15 min an automated microplate reader was used to determine optical density at 405 nm.

A value of five times the mean optical density for negative controls was used to separate grizzly sera into two categories. Sera with optical densities greater than or equal to this value were considered evidence of previous exposure. Such samples will be referred to as positive. Sera with values below this level were considered to be from uninfected bears. These samples will be referred to as negative.

Black bear sera from a previous study (Dub-

TABLE 1. Serum antibody prevalence for *Trichinella* spp. in grizzly bears (*Ursus arctos*) from seven areas of Alaska, 1973 to 1987.

Region	Area	Sample collection period	Prevalence
Northern	Northwest Arctic[a]	1977–80, 1983–87	194/224[b] (87%)
	Northeast Arctic	1973–74, 1982–87	161/206 (78%)
	Subtotal		355/430 (83%)
Interior	Southcentral Interior	1978–87	40/153 (26%)
	Central Interior	1981–87	22/99 (22%)
	Subtotal		62/252 (25%)
Southern	Alaska Peninsula	1986	4/18 (22%)
	Southeast Islands	1984–87	4/27 (15%)
	Kodiak Island	1984–87	2/151 (1%)
	Subtotal		10/196 (5%)

[a] Study areas shown in Fig. 1.
[b] Number positive/number tested.

ey et al., 1994) were used as positive and negative controls. Positive sera were from bears confirmed to be infected as determined by the digestion method. Negative sera were from bears with no *Trichinella* spp. larvae as determined by the digestion method.

Serologic test results were analyzed using a generalized linear model with a logit link and binomial distribution (McCullagh and Nelder, 1989). Four host factors were entered into the model: 1) location of capture, 2) year-of-collection, 3) age, and 4) sex.

These factors were ranked in importance by determining their effect on improving the fit of the model to the data. This process provided an initial ranking of importance. Factors were then entered into the model in sequence to determine if additional factors improved the fit of the model. Significance ($\alpha = 0.05$) was determined by comparing the increase in the log-likelihood to a chi-square distribution with the appropriate degrees of freedom (McCullagh and Nelder, 1989). Quadratic terms were added for the continuous variables of age and year. All second order interactions were also evaluated. For purposes of comparison, individual study areas were combined into three major regions: 1) Northern Region composed of Northeast Arctic and Northwest Arctic, 2) Interior Region composed of Southcentral Interior and Central Interior, and 3) Southern Region composed of Alaska Peninsula, Southeast Islands and Kodiak Island.

RESULTS

Bears ranged in age from 0 to 26 yr. Serum antibody prevalences for *Trichinella* spp. in bears increased dramatically from 5% in the Southern Region to 83% in the Northern Region (Table 1). Based on the statistical model, the order of importance for host parameters was: 1) location, 2) age, 3) year-of-collection, and 4) sex. The final model included only location ($P < 0.0001$) and age ($P < 0.0001$). Neither year-of-collection ($P = 0.0713$) nor sex ($P = 0.6037$) was significantly related to prevalence. No second order interactions were significant. The quadratic term for age was not significant. The following formula was used to predict the probability that a bear had been exposed to *Trichinella* spp.: Probability = $e^f/1 + e^f$; where $f = -4.1667 + (0$ if Southern$) + (2.3283$ if Interior$) + (4.9401$ if Northern$) +$ age $\times 0.0861$. In all three regions, the probability of a bear having been exposed to *T. spiralis* increased with age (Fig. 2). Probability of exposure increased dramatically from the Southern Region to the Northern Region.

DISCUSSION

No attempt was made to identify the species of *Trichinella* infecting bears in this study. However, the geographic distribution of this genus has been well-documented (Pozio et al., 1989). Based on

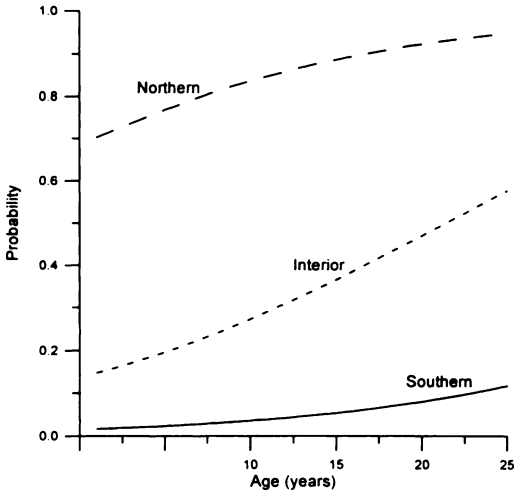

FIGURE 2. Relationship between grizzly bear (*Ursus arctos*) age and predicted probability of a bear serum sample from one of three regions of Alaska exceeding threshold optical density (> five times mean OD for negative control) for *Trichinella* spp. ELISA.

available information, *T. nativa* is the only species present in arctic regions. The ELISA used in the present study is effective in detecting all species of *Trichinella* (Gamble and Murrell, 1986; Gamble, 1993). Therefore, the prevalence estimates reported here are independent of species type present.

From 1987 through 1989, muscle samples were collected from 23 grizzly bears which were killed by hunters in the Central Interior. These samples were tested by means of enzymatic digestion. Six (23%) contained *Trichinella* spp. larvae (D. Worley, pers. comm.). Serologic tests on different bears from this same area revealed an antibody prevalence of 22% (Table 1). Thus, the ELISA procedure provided an accurate estimate of prevalence compared to the digestion method.

There are few published reports regarding the occurrence of trichinellosis in grizzly bears from Alaska. Prevalence was 50% in a sample of 20 animals (Rausch et al., 1956). Location of collection was not reported. Additional records from the Arctic Health Research Laboratory at the University of Alaska indicated prevalences of: 1) 86% (12 positive of 14 tested) for the Alaska Peninsula, 2) 50% (four positive of eight tested) for the Arctic, 3) one positive of three tested for Kodiak Island, and 4) 29% (seven positive of 24 tested) for the Interior (R. L. Rausch, pers. comm.). Samples for this second survey were collected during the 1960s and 1970s. Specimens in both of these prior Alaska studies were analyzed by means of the compression method. This technique is considered highly reliable if an adequate portion of meat is tested or if the animal is heavily parasitized. No previous studies reported a clear geographic pattern of prevalence. Therefore, comparison of current results with prior data is difficult.

Three independent surveys have been conducted for evidence of trichinellosis in polar bears from coastal areas of Alaska. Prevalences were as follows: (1) 53% (nine positive of 17 tested) during the 1950s (Rausch et al. 1956), (2) 64% (188 of 292) from 1967 to 1970 (Lentfer, 1976), and (3) 61% (56 of 92) from 1985 to 1991 (Weyermann et al., 1993). Polar bears from Greenland had a prevalence of 24% (56 of 231) during the 1950s (Madsen, 1961). A prevalence of 23% (54 of 237) was reported in a subsequent study in Greenland (Henriksen et al., 1993). All of these studies used the enzymatic digestion method.

For many infectious and parasitic agents, prevalence increases in successive age cohorts. Prevalence of trichinellosis is directly related to age of polar bears from Greenland (Henriksen et al., 1993). Results of the current survey are evidence for a similar pattern in grizzly bears (Fig. 2). This data indicates the opportunity for exposure to *Trichinella* spp. is present throughout a bear's lifetime. Probability of previous exposure increases as a bear becomes older. Almost all of the bears included in the Arctic Health Research Laboratory survey were older-age animals. Investigators speculated that this skewed age structure provided a biased estimate of prevalence higher than expected (R. L. Rausch, pers. comm.). Estimates of trichi-

nellosis prevalence provided by that study are indeed higher than values for the Southern and Interior Regions (Table 1). Thus, results of the current study support their theory.

Trichinellosis is transmitted by ingestion of parasitized meat. Thus, discrepancies in location-specific prevalence (Table 1) may be attributable to differences in food habits of bears from different geographic regions. Bears from Kodiak Island and Southeast Alaska have easy access to deer (*Odocoileus hemionus sitkensis*) and salmon (*Onchorhynchus* spp.). Bears in the northern portion of the state have access to larger prey species such as moose (*Alces alces*) and caribou (*Rangifer tarandus*). None of these prey items is considered a source of trichinellosis.

On a long-term basis, less biomass is available to bears in the Northern Region from moose and caribou as compared to biomass available to bears in the Southern Region from deer and salmon. In addition, adult moose and caribou are larger and more difficult to kill than deer and salmon. Therefore, bears in northern areas may be forced to seek alternate sources of meat. Perhaps these alternatives include other predator and scavenger species (including other bears) which may serve as sources of trichinellosis.

Alternatively, high prevalence in the Northern Region may be at least partially due to colder ambient temperatures. Strains of *Trichinella* spp. found in temperate climates are killed by freezing (Pozio et al., 1992). However, the arctic strain of *T. nativa* is resistant to cold temperatures (Dick and Belosevic, 1978; MacLean et al., 1989). Trichinae present in frozen carcasses remain viable for extended periods. Thus, the colder temperatures in the Northern Region may provide increased opportunities for exposure.

Population genetics may provide another potential explanation for the geographic discrepancies in antibody prevalence (Table 1). Analysis of mitochondrial DNA has shown that subpopulations of bears within Alaska are genetically distinct (Talbot and Shields, 1996). Perhaps these subpopulations have developed genetically-based differences in immune capability. The ELISA results would then provide a false picture of exposure rates to *Trichinella* spp.

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