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Source: Journal of Wildlife Diseases, 26(4) : 518-521

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.4.518

Journal of Wildlife Diseases, 26(4), 1990, pp. 518–521

SEROLOGIC SURVEY FOR *ACTINOBACILLUS CAPSULATUS* IN FREE-RANGING SNOWSHOE HARES (*LEPUS AMERICANUS*) FROM ALASKA AND ALBERTA

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ABSTRACT: A plate agglutination method was developed to test sera from free-ranging snowshoe hares (*Lepus americanus*) captured in Alaska (USA) or Alberta (Canada) for antibody against *Actinobacillus capsulatus*. Antiserum against *A. capsulatus* was prepared in a domestic rabbit. A concentrated suspension of formalin-killed *A. capsulatus* was prepared for use as an antigen. Serum antibody prevalence for hares was 98 of 239 (41%) in Alaska and 51 of 111 (46%) in Alberta. Prevalence in Alaska peaked in 1981 corresponding to a peak in hare population density. Seasonal prevalence peaked in May in Alaska. Prevalence at one capture site in Alaska was significantly higher than at four other sites. There was no difference in sex-specific prevalence for either Alaska or Alberta.

Key words: *Actinobacillus capsulatus*, serology, snowshoe hare, *Lepus americanus*, survey.

INTRODUCTION

Various *Actinobacillus* spp. are pathogens of domestic animal species (Phillips, 1984). Members of this genus are capable of causing suppurative or granulomatous lesions in cattle, sheep, horses and swine (Phillips, 1984). *Actinobacillus* spp. have been implicated in diseases of wildlife species including cases of pneumonia in both bighorn sheep (*Ovis canadensis*) and pronghorn (*Antilocapra americana*), splenic abscesses in pronghorn, and jaw abscesses in elk (*Cervus elaphus nelsoni*) from Wyoming, USA (Thorne, 1982).

Actinobaccillus capsulatus was originally isolated from caged Angora and mixed breed rabbits in Sri Lanka (Arseculeratne, 1961, 1962). When *A. capsulatus* was injected intravenously into domestic rabbits, infection spread throughout the body and death occurred within 3 wk (Arseculeratne, 1962). A closely related variant of *A. capsulatus* was isolated from lung, liver and spleen of free-ranging snowshoe hares (*Lepus americanus*) in Alaska (USA) (Zarnke and Schlater, 1988). Little is known about natural host range, natural means of exposure or other aspects of the epizootiology of *A. capsulatus* infection in free-ranging wildlife species. The purposes of the present study were to (1) develop a serologic test to aid in the diagnosis of exposure to *A. capsulatus* and, (2) utilize the test to conduct a serologic survey in Alaska and Alberta (Canada).

MATERIALS AND METHODS

Antiserum was prepared by injecting 2 ml of a suspension of *A. capsulatus* in phosphate buffered saline (PBS) containing 2×10^9 colony forming units/ml intramuscularly into the thigh of a domestic rabbit (*Oryctolagus cuniculus*) on two occasions 3 days apart. The bacterial culture was originally isolated from a snowshoe hare (Zarnke and Schlater, 1988) and had been passaged several times on sheep blood agar. Serum samples were collected on 4, 7, 14, and 20 days following the initial injection.

Actinobacillus capsulatus was grown on slant tubes of sheep blood agar from 24 to 36 hr. Bacterial growth was flushed from the surface of the agar slant with approximately 2 ml of 1% formalin in PBS. Bacterial suspensions from numerous tubes were pooled and centrifuged at $5{,}000 \times g$ in a refrigerated centrifuge (Sorval model RC-2B, Dupont Medical Products, Wilmington, Delaware 19880, USA) for 10 min. The supernatant was discarded and the pellet was resuspended in approximately ¼ of the volume of formalin PBS as was used for the original suspension. The resultant suspension was again centrifuged at $5{,}000 \times g$ for 10 min. The supernatant was discarded and the pellet was resuspended in a quantity of formalin PBS sufficient to create a suspension with an optical

density reading of approximately 580 units as determined by a spectrophotometer (model 800-3, Klett Manufacturing Co., Inc., New York, New York 10001, USA). The volume of diluent required to attain this reading was approximately 20 times the volume of the pellet. The antigen suspension was therefore considered to be a 1:20 dilution. Based upon comparison to standard McFarland nephelometer turbidity solutions, this antigen suspension contained approximately 9×10^9 bacteria per ml. Antigen suspensions were tested for sterility before use.

Agglutination reactions were performed on glass slides with 30 individual 2 cm inside diameter raised ceramic rings (American Scientific Products, McGaw Park, Illinois 60085, USA). A standard volume (0.04 ml) of antigen suspension was placed on the slide and mixed with the serum to be tested. A combination of equal volumes (0.04 ml) of antigen and serum was selected as our screening dilution. This dilution was assigned a value of 1:40. After 4 min of gentle rocking, the mixture was evaluated for degree of agglutination and assigned a value from negative (0) to four (4+). Sera which exhibited agglutination at ≥1:40 dilution were considered to provide evidence of previous exposure to *A. capsulatus*, and were classified as "positive." All others were classified as "negative."

Sera from apparently healthy, free-ranging hares from interior Alaska were collected from 1979 to 1986 by Alaska Department of Fish and Game personnel (Fairbanks, Alaska 99701, USA) or wildlife professionals from other agencies. Trapping effort was not uniform between years or collection sites. Hare sera from near Rochester, Alberta, Canada (113°30′W; 54°15′N) were collected during 1976 by staff and graduate students from the University of Wisconsin–Madison (Madison, Wisconsin 53706, USA).

Nineteen hare sera (representing a broad range of agglutination titers) were tested by means of enzyme-linked immunosorbent assay (ELISA) and western blot methods (Manning et al., 1986). The purpose of these tests were to (1) confirm agglutination results and (2) evaluate the degree of cross-reactivity relative to *Pasteurella multocida*.

Actinobacillus capsulatus antigen was tested for agglutination against *Francisella tularensis* antiserum (Difco Laboratories, Detroit, Michigan 48232, USA) and *A. capsulatus* antiserum was tested for agglutination against *F. tularensis* antigen (Difco Laboratories). All sera from free-ranging hares were also tested against *F. tularensis* antigen (Difco Laboratories). A titer of ≥1:20 was considered positive.

Differences in antibody prevalence related to sex, capture location, and month and year of collection were tested for significance by means of the chi-square test (Johnson, 1980).

RESULTS

Antibody titer for the known-infected domestic rabbit reached 1:8,000 4 days postinoculation (PI) and was still at this level 20 days PI. Titers in snowshoe hares ranged from 1:40 to 1:3,200. There was an inverse relationship between titer and number of specimens which exhibited that titer; high frequency of low titers and low frequency of high titers. Overall serum antibody prevalence for *A. capsulatus* in hares from Alaska was 98 of 239 (41%). Corresponding values for hares from Alberta were 51 of 111 (46%). These values were not significantly different ($P > 0.25$).

Prevalences based upon year of capture (hereafter referred to as "annulized" prevalences) exhibited significant variation between years ($P < 0.005$). Serum antibody prevalence for *A. capsulatus* in snowshoe hares captured in interior Alaska during 1979 was 38% (22 positive of 58 sera tested); 1980, 24% (24 of 100); 1981, 81% (9 of 11); 1982, 67% (22 of 33); 1983, 63% (12 of 19); 1984, 44% (7 of 16); for a total of 41% (96 of 237). Hare population density routinely cycles over an 8 to 11 yr period (Keith and Windberg, 1978). Based upon an Alaska Department of Fish and Game survey (Fairbanks, Alaska 99701, USA), hare population density in interior Alaska reached a peak in 1980–1981 (R. Beasley, unpubl.). Antibody prevalence and population density appeared somewhat synchronous during the 1979 to 1984 period (Fig. 1). Antibody prevalence in hares from both Alberta and Alaska showed a seasonal pattern (Fig. 2) with significant differences between months ($P < 0.005$).

Sample sizes for hares from five areas of Alaska were adequate to allow comparison based upon capture location. Serum antibody prevalence for *A. capsulatus* in snowshoe hares which were captured at Fort Wainwright (64°50′N; 147°40′W) was 85% (11 positive of 13 tested). Corresponding values for Creamer's Field Refuge

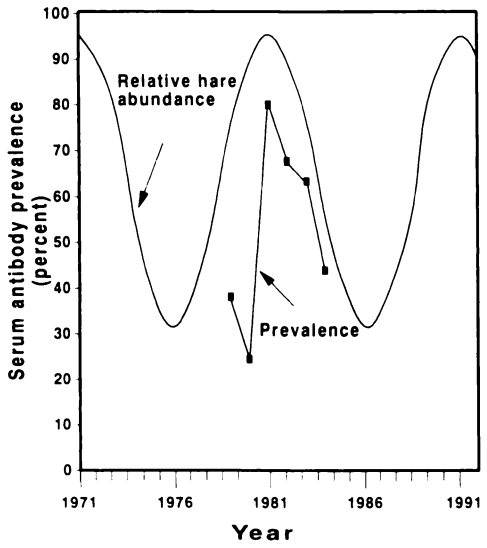

FIGURE 1. Comparison of serum antibody prevalence for *Actinobacillus capsulatus* in snowshoe hares (*Lepus americanus*) captured in interior Alaska with relative hare abundance.

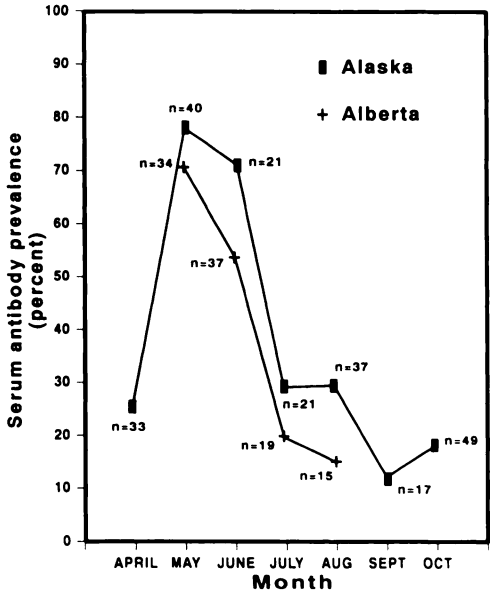

FIGURE 2. Serum antibody prevalence for *Actinobacillus capsulatus* in snowshoe hares (*Lepus americanus*) captured in Alaska or Alberta.

(64°50'N; 147°45'W) were 50% (9 of 18); Eielson Air Force Base (64°40'N; 147°00'W) 50% (20 of 39); Washington Creek (65°10'N; 147°55'W) 39% (33 of 84); and Delta Junction (63°55'N; 145°45'W) 26% (5 of 19). Total prevalence for these five major trapping sites was 45% (78 of 173). All sites were within 175 km of Fairbanks (Alaska, USA). Collection areas were at least 40 km from each other except Fort Wainwright and Creamer's Field Refuge which are approximately 5 km apart. Prevalences were similar at all locations, with the exception of Fort Wainwright, where prevalence was significantly higher than at the other locations ($P < 0.005$).

There was no significant difference in sex-specific prevalence for Alaska where 22 of 60 (37%) and 25 of 64 (39%) male and female, respectively, were positive ($P > 0.90$) or Alberta where 26 of 53 (49%) males and 24 of 57 (42%) females were positive ($P > 0.25$).

ELISA test results revealed no significant cross-reactivity of hare sera with *P. multocida*. ELISA mean optical density (at 492 nm) for five sera with no evidence of agglutinating antibody were 0.22 ± 0.06 (standard error) for *A. capsulatus* and 0.10 ± 0.02 for *P. multocida*. Corresponding values for 14 sera with agglutination titers ranging from 1:160 to 1:3,200 were 1.14 ± 0.14 for *A. capsulatus* and 0.28 ± 0.09 for *P. multocida*. Western blot test results (utilizing whole cell lysates and proteinase K lysates in conjunction with hare sera) revealed antibody activity to several *A. capsulatus* antigens and few *P. multocida* antigens.

No agglutination occurred when *A. capsulatus* reagents were tested against *F. tularensis* reagents, nor was there any evidence of exposure to *F. tularensis* in any of the free-ranging hare sera.

DISCUSSION

Members of the genus *Actinobacillus* are serologically cross-reactive (MacInnes and Rosendal, 1987). It is possible that the antibody which we detected in sera from free-ranging hares was actually produced in response to exposure to an *Actinobacillus* spp. other than *A. capsulatus*. However, isolation of *A. capsulatus* from three hares (Zarnke and Schlater, 1988) and the

results of ELISA and western blot tests support our contention that the serologic reactions reported here indicate exposure to *A. capsulatus*.

A comparison of prevalences between Alaska (41%) and Alberta (46%) suggests that opportunity for exposure to *A. capsulatus* may be widespread in North America. The apparent decline in antibody prevalence as summer progresses (Fig. 2) may reflect the addition to the population of large numbers of new-born hares which have not yet been exposed to *A. capsulatus*.

Disease has long been considered one of many possible factors in the regular decline in hare density (Keith and Windberg, 1978). The apparent correlation of annualized antibody prevalences and hare population density suggests the possibility that exposure to *A. capsulatus* may be density-dependent. Effects of *A. capsulatus* infection on hare populations are difficult to evaluate. This bacterium is associated with mortality in hares (Zarnke and Schlater, 1988). However, the high antibody prevalences reported here (Fig. 2) indicate that large numbers of hares either (1) suffer no overt disease as a result of exposure, or (2) recover from infection.

ACKNOWLEDGMENTS

The authors wish to thank Robert Mowrey for collecting and submitting sera for use in this study, Linda Schlater and Soren Rosendal for providing advice and reagents, and Jesse Venable for computer and statistical assistance. This study was supported in part by Federal Aid in Wildlife Restoration Projects Number W-22-0 and W-23-1 and National Institutes of Health grant RRO 1234.

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Received for publication 5 April 1988.