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SEROPREVALENCE OF TWO *BABESIA* SPP. ISOLATES IN SELECTED BIGHORN SHEEP (*OVIS CANADENSIS*) AND MULE DEER (*ODOCOILEUS HEMIONUS*) POPULATIONS IN CALIFORNIA

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ABSTRACT: Sera from 111 bighorn sheep (*Ovis canadensis*) and 95 mule deer (*Odocoileus hemionus*) were tested using an indirect immunofluorescence assay for antibodies to two isolates of *Babesia* spp. recently obtained from these hosts in California (USA). The study populations were from six locations: three areas of real or potential sympatry of bighorn sheep and deer, one area with deer only, and two areas with bighorn sheep only. Antibody titers from seroreactive individuals were similar with both babesial isolate antigens ($P < 0.05$), and seroprevalence was highest in the areas of host sympatry. A moderate to high seroprevalence ($\geq 30\%$) in some of the study populations was evidence that babesial parasites may be common in bighorn sheep and mule deer in some areas of California.

Key words: *Babesia* spp., bighorn sheep, *Ovis canadensis*, mule deer, *Odocoileus hemionus*, serological survey, seroprevalence.

INTRODUCTION

Parasites of the genus *Babesia* are tick-transmitted intraerythrocytic protozoans that infect and sometimes cause hemolytic anemia in a variety of animals (Telford et al., 1993). Among wildlife in the United States, *Babesia* spp. have been documented in caribou (*Rangifer tarandus caribou*) in a Minnesota zoo (Holman et al., 1994a), and in elk (*Cervus elaphus*) in Texas (Holman et al., 1994b). *Babesia odocoilei* has been studied in white-tailed deer (*Odocoileus virginianus*) in Texas (Emerson and Wright, 1968) and Oklahoma (Waldrup et al., 1989). Goff et al. (1993) isolated and described a *Babesia* sp. organism from bighorn sheep (*Ovis canadensis*) in the San Bernardino Mountains, California (USA), and Thomford et al. (1993) isolated and characterized other babesial isolates from both bighorn sheep and mule deer (*Odocoileus hemionus*) from the same location. These *Babesia* spp. differed from *B. odocoilei* morphologically and differed somewhat in parameters of infection in deer and bighorn sheep (Goff et al., 1993). In addition, mule deer erythrocytes supported in vitro growth of these *Babesia* spp., but not *B. odocoilei* (Thomford et al., 1993). Indirect fluorescent antibody (IFA) tests commonly have been used to assess antibody responses to babesial parasites (Yamane et al., 1993). We used an IFA test to compare the prevalence of antibodies among deer and bighorn sheep in California to isolates of *Babesia* spp. (Thomford et al., 1993) obtained from these hosts.

MATERIALS AND METHODS

Sera were collected by California Department of Fish and Game personnel from indigenous mule deer and bighorn sheep populations in California (Fig. 1) from October 1988 until August 1992 as part of a health surveillance program. At the time of collection, age (based on dentition or annular horn ring), sex, and general health status of the animals were estimated and recorded. Sera were stored at −20 C until tested. Samples from sympatric populations included deer ($n = 45$) and bighorn sheep ($n = 43$) from the San Bernardino Mountains, San Bernardino County (116°43′W, 34°4′N), and bighorn sheep ($n = 14$) from Carrizo Gorge in Anza Borrego Desert State Park, San Diego County (116°11′W, 32°47′N). No samples were

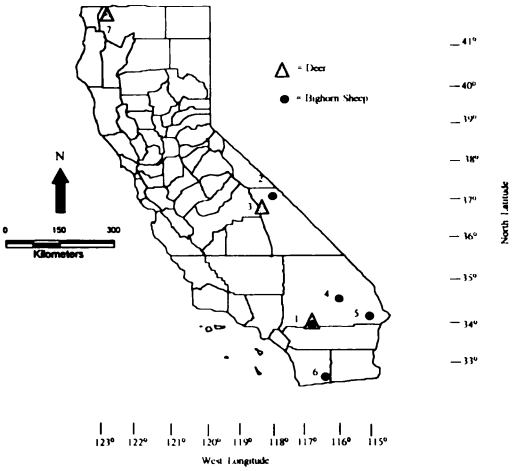

FIGURE 1. Location and classification of bighorn sheep (*Ovis canadensis*) and deer (*Odocoileus hemionus*) study populations in California. Locations are: 1. San Bernardino Mountains, San Bernardino County; 2. White Mountains, Inyo County; 3. Inyo Mountains, Inyo County, 4. Old Dad Mountains, San Bernardino County; 5. Turtle Mountains, San Bernardino County; 6. Anza Borrego Desert State Park, San Diego County; 7. Happy Camp, Siskiyou County.

available from sympatric deer in Carrizo Gorge. Samples also were tested from a population of deer (n = 18) from the Inyo Mountains, Inyo County (118°20'W to 118°30'W, 37°00'N to 37°8'N) and an adjacent bighorn sheep population (n = 15) from the White Mountains, Inyo County (118°11'W, 37°23'N). Sera from isolated populations included deer (n = 32) from Happy Camp, Siskiyou County (123°23'W, 41°48'N), bighorn sheep (n = 25) in the Old Dad Mountains, San Bernardino County (115°48'W, 34°46'N), and bighorn sheep (n = 14) from the Turtle Mountains, in San Bernardino County (114°50'W, 34°18'N). Also tested were sera from two white-tailed deer (*Odocoileus virginianus*) known to be infected with *B. odocoilei*, provided by Dr. Ken Waldrup of Texas A&M University.

Antigen slides for the IFA were prepared using two distinct *Babesia* spp. isolates obtained by in vitro cultivation of blood collected for a general health survey of bighorn sheep and deer captured in San Bernardino County, California (Thomford et al., 1993). A small babesial parasite, BH1, was isolated from a pooled collection of blood from five bighorn sheep captured in December 1990. A larger *Babesia* sp., MD1, was isolated from pooled blood collected from 10 mule deer sampled in April 1991. These isolates were maintained using donor erythrocytes from a captive mule deer. When cultures reached a rapidly growing phase and had a parasitemia of at least 3% they were considered suitable for immunofluorescent antibody slide preparation. Slides were prepared and tests were performed as described by Yamane et al. (1993) with two modifications. The secondary antibody used was a fluorescein isothiocyanate (FITC) labeled rabbit anti-sheep IgG (H + L) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Maryland, USA), and sera that were positive at 1:80 were assayed at two-fold dilutions to 1:10,240 to determine the endpoint titer.

Initially, slides were read by two people; however, after agreement of endpoints was unequivocally established, the slides were read by one person. Samples with a titer of ≥1:640 to BH1 and MD1 were considered seropositive. Positive and negative controls were included on each slide. Negative-control sera were collected from captive bighorn sheep or deer (n = 8) that had no visible parasitemias on multiple Giemsa-stained blood smears and whose blood remained negative for *Babesia* spp. after 1 mo of in vitro cultivation. Positive control serum for deer included serum from a deer from the original pool with a titer of 1:5120, and serum from a captive mule deer inoculated with a *Babesia* spp. isolate from the San Bernardino Mountains bighorn sheep herd (Goff et al., 1993) that had an IFA titer to MD1 of 1:640. The positive control serum for BH1 was collected from a bighorn sheep in the San Bernardino Mountains that was culture positive for *Babesia* spp. and had a titer to BH1 of 1:5120. Samples originally were tested in duplicate but because the titers were in almost perfect agreement between duplicates, half of the samples were screened as single samples. Statistical analysis was performed using Epi-Info (Dean et al., 1994) and STATISTIX (Analytical Software, St. Paul, Minnesota) software packages. Odds ratios and 95% confidence intervals (CI) were used to assess the strength of the relationships between sex of the animals, season of capture, and likelihood of having antibodies. The Wilcoxon signed rank test (Daniel, 1991) was used to assess whether the magnitude of mean differences between BH1 and MD1 titers was significantly ($P < 0.05$) different from zero. As a measure of potential cross-reactivity, the Spearmann rank correlation coefficient (Daniel, 1991) was used to assess correspondence of MD1 and BH1 titers. For calculation of the correlation coefficient, titers were ranked from one to eight, corresponding to serial two-fold dilutions ranging from 1:80 to 1:10,240.

RESULTS

The IFA endpoint antibody titers ranged from <1:80 to >1:10,240 for both isolate

TABLE 1. Seroprevalence of two isolates of *Babesia* (BH1 and MD1) in selected mule deer (*Odocoileus hemionus*) and bighorn sheep (*Ovis canadensis*) populations in California, from 1988 to 1992, using 1:640 as the positive cutoff titer.

Location	Species	Number sampled	Seroprevalence (%)	
			BH1	MD1
San Bernardino[a]	Deer	45	40	42
	Bighorn	43	60	56
Anza Borrego[b]	Bighorn	14	7	29
Inyo Mountains[c]	Deer	18	33	39
White Mountains[c]	Bighorn	15	7	7
Siskiyou[d]	Deer	32	6	13
Desert (Turtle Mtns. and Old Dad Mtns.)[e]	Bighorn	39	3	5

[a] The difference in seroprevalence of the two isolates was not significant ($P \geq 0.67$).
[b] Sera from sympatric deer were unavailable for testing.
[c] Bighorn sheep and deer were potentially sympatric.
[d] Only mule deer present.
[e] Only bighorn sheep present.

antigens. No background reactions with negative control deer and bighorn sheep sera occurred at dilutions >1:80. There was no evidence of cross-reactivity between antisera from *B. odocoilei* infected white-tailed deer and the California *Babesia* spp. isolate antigens. The seroprevalence of deer and bighorn sheep using MD1 and BH1 isolate antigens ranged from 3% to 60% (Table 1). The sympatric and potentially sympatric bighorn sheep and deer populations had the highest seroprevalence, while the isolated populations had a lower seroprevalence. There was no significant difference ($P = 0.11$) in seroprevalence for both MD1 and BH1 between the bighorn sheep and deer in San Bernardino mountains regardless of the isolate antigen used. Over all deer populations, neither season of capture ($P = 0.33$ for BH1 and $P = 0.30$ for MD1) nor sex of the animal ($P = 0.73$ for BH1 and $P = 0.58$ for MD1) increased the likelihood of having antibodies. Bighorn sheep were 3.6 (95% confidence interval = 1.1 to 13.8) times more likely to be positive to BH1 and 6.0 (95% confidence interval = 7.7 to 26.4) times more likely to be positive to MD1 in the fall and winter than in the spring and summer. Bighorn sheep males were 3.6 (95% confidence interval = 1.3 to 10.5) times more likely to be positive to BH1 than the females. There was not a significant difference ($P = 0.45$) in seroreactivity between males and females to MD1. Based on the Wilcoxon signed rank test, the ranks of titer values were not significantly different ($P > 0.05$), with the exception that deer from Siskiyou had significantly different ($P = 0.01$) titer values between MD1 and BH1 antigens. The Spearmann rank correlations for antigenic comparison between BH1 and MD1 for each population except Siskiyou had r_s values ranging from 0.22 to 0.76; this was a significant correlation between the two titer values ($P < 0.05$). Data were reanalyzed to determine whether findings were robust to changes in cutoff values. When using cutoff values of 1:320 or 1:1,280 as positive, the results did not change significantly.

DISCUSSION

Based on our findings, exposure to *Babesia* spp. was common in bighorn sheep and deer herds in California. Although seropositive animals were identified in isolated herds, antibody prevalence was higher in the sympatric populations, such as the deer and bighorn sheep in the San Bernardino Mountains and the bighorn sheep

in Anza Borrego Desert State Park. Exposure to *Babesia* spp. may be more frequent in areas where a wide range of species can support either the parasite or its vector. The slightly increased seroprevalence (BH1 antigen) in bighorn sheep males may also be evidence for a mechanism where greater movement of male animals may increase the risk of exposure in nearby, but not completely sympatric, populations. In future studies, more populations of sympatric deer and bighorn sheep should be evaluated to determine the importance of host sympatry as a risk factor for exposure to *Babesia* spp.

The vectors for the *Babesia* spp. isolates used in this study are unknown. However, since *Ixodes scapularis* transmits *Babesia odocoilei* of white-tailed deer in the southeastern United States (Waldrup et al., 1992), the western black-legged tick, *Ixodes pacificus* is a likely vector of *Babesia* spp. in California. *Ixodes pacificus* has been reported from the study areas (San Bernardino County, Inyo County and Anza Borrego) where we found the highest seroprevalence (Furman and Loomis, 1984). Additionally, the slight increase in BH1 antibody prevalence in bighorn sheep sera collected during the fall and winter coincides with adult *I. pacificus* activity (Furman and Loomis, 1984). However, *Dermacentor* spp. ticks, present in all of the study sites (Furman and Loomis, 1984), also may be capable of transmission, since all populations had some antibody positive individuals, regardless of overlap with other host species or the presence or absence of *I. pacificus*.

Serum dilutions used for defining a positive reaction have ranged from 1:80 (Waldrup et al., 1990) to 1:1,240 (Yamane et al., 1993) in previous *Babesia* spp. IFA studies. This variation may be due to differences in parasite isolates, immunological responses of infected animals, or IFA test technique such as the choice of the secondary FITC-conjugated antibody. We chose a 1:640 cutoff since the experimentally infected positive control deer had a 1:640 titer. Moreover, reanalysis of our data using cutoffs of 1:320 or 1:1,240 did not affect our results; hence, 1:640 was a reasonable choice for this preliminary study.

Cross-reactivity between *Babesia* spp. has been well documented (Krause et al., 1994). Despite significant morphological differences seen in in vitro culture (Thomford et al., 1993), the Wilcoxon signed test and the Spearmann rank test provided evidence of a strong correlation in titer values between BH1 and MD1. Similar antibody reactions may be due to taxonomically related parasites having shared or structurally similar immunodominant antigens (Gray et al., 1991). Moreover, these similar titer values also could be a reflection of the IFA test itself. A low specificity of the test for an isolate could artificially increase the number of test positive animals. Since we did not have sufficient numbers of known positive and known negative sera, we could not calculate test sensitivity and specificity. Consequently, we could not determine if an individual animal was infected with one or both parasites. Until these parasites are more completely characterized, the IFA test is useful to obtain estimates of seroprevalence, and should not be relied upon to distinguish between isolates. Since morphological characteristics used to distinguish these parasite isolates may not be reliable for the differentiation of *Babesia* spp. in vitro (Thomford et al., 1993), further antigenic and genetic studies of these isolates are needed to determine their relatedness.

Goff et al. (1993) produced clinical disease in a captive bighorn sheep and a white-tailed deer with their babesial isolate from a bighorn sheep. However, the pathogencity of the BH1 and MD1 isolates has not been evaluated in either captive or wild populations. Since most babesial species are host-adapted (Telford et al., 1993), disease may not occur in deer or bighorn sheep unless the animals are immunocompromised or stressed. However, since some populations of bighorn sheep in California have declined due to disease (Torres et al.,

1994), knowledge of parasite prevalence and risk factors for transmission of infection are important for appropriate herd management.

Recently, three cases of human infection with *Babesia* spp. were reported in California (Persing et al., 1995). At least two of these cases were associated with a history of activity in *I. pacificus* and deer enzootic areas. If human and wildlife isolates are antigenically and molecularly similar, our present findings may have important public health implications.

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