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SEROPREVALENCE OF *PSOROPTES* SP. MITES IN FREE-RANGING ELK (*CERVUS ELAPHUS*) AS DETERMINED BY KINETIC ELISA

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ABSTRACT: Western blots and a kinetic enzyme-linked immunosorbent assay (ELISA) were used to characterize and quantify the prevalence of antibodies to *Psoroptes* sp. mites in elk (*Cervus elaphus*) from nine herds in North America. Sera from infested (n = 18) and non-infested (n = 22) elk were used to optimize test methodology and to define cut-off values for negative, suspect, and positive samples. Among 357 samples, 35 (9.8%) of the animals were seropositive, 259 (73%) were negative, and 63 (18%) were suspect. Six of nine herds (67%) contained positive animals and two additional herds (22%) had suspect animals. Sex was not associated with prevalence of antibodies, but adults greater than 2 yr old were approximately five times more likely (95% confidence interval = 2.6–15.4) to be seropositive than calves. Based on these results, we propose that exposure to *Psoroptes* sp. mites may be widespread in free-ranging elk of North America.

Key words: Elk, *Cervus elaphus*, mites, *Psoroptes* sp., scabies, immunodiagnosis, antibodies, ELISA, serologic survey.

INTRODUCTION

Clinical scabies due to *Psoroptes* sp. mites (Acari: Psoroptidae) has been an important and potentially devastating disease of both domestic and wild ruminants in North America (Sweatman, 1958). Although the incidence of scabies has been reduced in domestic livestock through the widespread use of acaricides (Meleney et al., 1982), clinical disease has continued to be seen in certain wild populations, including mule deer (*Odocoileus hemionus*) (Boyce et al., 1990), bighorn sheep (*Ovis canadensis*) (Lange et al., 1980), and elk (*Cervus elaphus*) (Worley, 1979). In elk, psoroptic scabies has been reported primarily from northern Idaho and northwestern Wyoming (USA), with mature males the most severely affected (Samuel et al., 1991). The prevalence and distribution of infestations in these and other wild ungulates has been difficult to estimate, largely due to the problems associated with observation and sampling of free-ranging species and the lack of available diagnostic tests. Recently, the presence of antibodies in bighorn sheep exposed to *Psoroptes* sp. mites was determined through the use of Western blotting and kinetic enzyme-linked immunosorbent assay (ELISA) (Boyce et al., 1991a; Mazet et al., 1992). In the present study, we used similar Western blot and ELISA techniques to determine whether elk produce antibodies to *Psoroptes* sp. mites, and to determine the distribution and frequency of antibodies to *Psoroptes* sp. mites in free-ranging elk throughout North America.

MATERIALS AND METHODS

Frozen sera (n = 397) and animal or herd information were obtained from 12 locations in the United States: Concord, California (37°59′N, 122°02′W), Grizzly Island, California (38°09′N, 121°58′W), Foothills Wildlife Research Facility, Colorado (40°35′N, 105°05′W), Dunn, North Dakota (47°21′N, 102°37′W), McKensie, North Dakota (47°46′N, 103°27′W), Pembina, North Dakota (48°46′N, 97°33′W), West Great Salt Lake, Utah (41°00′N, 113°00′W), Colockum, Washington (47°16′N, 120°11′W), West Cascades, Washington (47°34′N, 124°06′W), Finnegans, Wyoming (44°34′N, 106°32′W), Greys River, Wyoming (43°10′N, 111°00′W) and the National Elk Refuge (NER), Wyoming (43°30′N, 110°40′W). All herds sampled, with the exception of the Foothills Wildlife

Research Facility, were free-ranging. Eighteen of the samples from animals at the NER were proven to be infested with *Psoroptes* sp. by direct examination of the skin by personnel of the Wyoming Game and Fish Department. These samples were used as known mite-positive samples. Samples (n = 22) from elk at Concord and Grizzly Island, California and the Foothills Wildlife Research Facility, Colorado, were provided by the respective state wildlife agencies, and were used as known mite-negative samples. Based on a deep swabbing of ears of all sampled animals, these three herds were negative for mites; also the herds had no history of *Psoroptes* sp. mites infestation. *Dermacentor* sp. ticks were found on elk from California, as well as those from the NER. All available sex, age, and subspecies information was collected for tested animals. Age was categorized for this study as being either adult (greater than 2 yr old), yearling (1 to 2 yr old), or calf (less than 1 yr old). By subspecies, the California herds were composed of Tule elk (*C. elaphus nannodes*), the Washington herds composed of Roosevelt elk (*C. elaphus roosevelti*), and the remaining herds were comprised of Rocky Mountain elk (*C. elaphus nelsoni*).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed with sera from known positive and negative elk as described by Boyce et al. (1988) with slight modification. Briefly, homogenized *Psoroptes cuniculi* mite proteins were denatured, electrophoretically separated on 12% polyacrylamide gels and 4% stacking gels (Bio Rad, Richmond, California), and transferred to nitrocellulose strips. Unbound sites were blocked using 3% gelatin, and strips were probed using elk sera at 1:100 dilution in 1% gelatin/Tween-TBS (Bio Rad) and recombinant protein G horseradish peroxidase (Zymed Laboratories, San Francisco, California) at 1:100 dilution in nonfat powdered milk (NPM) as a conjugate.

The microtiter-based kinetic ELISA was developed for use with elk sera by modifying an assay previously described by Boyce et al. (1991b) for bighorn sheep. Briefly, 96-well polystyrene microtiter plates (Corning Glass Works, Corning, New York) were sensitized with homogenized *P. cuniculi* mite proteins diluted in carbonate buffer. The NPM (0.5%) in phosphate-buffered saline was used to reduce non-specific protein binding. Sera from elk of known mite-infestation status were then assayed in two-fold serial dilutions in NPM (1:12.5 to 1:800) to determine optimal test parameters. Samples from representative groups were then pooled to form positive, suspect, and negative control samples. Recombinant protein G horseradish peroxidase conjugate in NPM was tested at dilutions ranging from 1:125 to 1:8000, and 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS) (Sigma Chemical Co., St. Louis, Missouri) was used as the substrate. The absorbance at 405 nm (A_{405nm}) and maximal kinetic rates (V_{max}) were determined with an automated plate-reader and associated software (UVMax, Molecular Devices Corporation, Menlo Park, California).

Optimization of the kinetic ELISA was done by determining those serum and conjugate concentrations that gave maximum ratios between known positive and negative values (P/N ratios). Assay sensitivity and specificity were maximized by the standard methods of Martin et al. (1987), in which samples from known mite-infested and mite-free elk were assayed and classified as either positive or negative by adding four or two standard deviations, respectively, to the average V_{max} of the known-negative samples. Values between these cut-off levels were classified as suspect.

Test samples (n = 357) were assayed in quadruplicate, and corrected V_{max} values were calculated by multiplying the mean V_{max} for each sample by a correction factor to ensure plate-specific biases would not influence final results. This factor was determined by dividing the average positive control value (APCV) over all plates into the APCV for the positive control on the same plate as the sample in question. Intra-plate variation was monitored by ensuring the coefficient of variation (the ratio of the standard deviation to the mean) for each set of quadruplicate samples was <10%.

Crude odds ratios (OR) and 95% confidence intervals (CI) were used to measure the strength of association between herd location, age, gender, and seropositivity. The elk herd at the NER was selected as the reference population due to prior knowledge regarding *Psoroptes* sp. mite infestation. Logistic regression was used to calculate adjusted ORs for the relationship between location (as a fixed effect) and both seropositivity and suspect seropositivity accounting for age and sex. All analyses were done using proprietary statistical software (BMDP, Los Angeles, California).

RESULTS

Sera from known-mite positive animals reacted strongly on Western blot tests with antigens ranging from 12 to 164 kilodaltons (Fig. 1), while sera from known negative animals had no such banding activity. Sera from those herds known to be infested with *Dermacentor* sp. did not cross-react with *P. cuniculi* mite antigens. Optimal

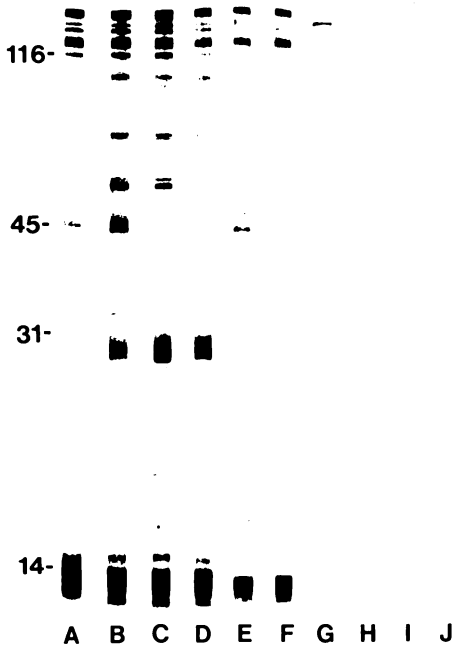

FIGURE 1. Western blot analyses showing antibody recognition of *Psoroptes cuniculi* antigens by *Psoroptes* sp.-infested elk (A to F, National Elk Refuge, Wyoming), non-infested elk (G to I, Concord, California), and conjugate control (J, no sera added). Molecular weights in kilodaltons are on the left.

P/N ratios and minimal background reactivity were obtained in the kinetic ELISA using pooled control sera (positive and negative) and conjugate at dilutions of 1:50 and 1:3000, respectively. Kinetic rates (V_{max}) were most linear 5 to 10 min. after the addition of substrate, giving values for pooled positive and negative control sera of 85.79 (SD = 7.01) and 4.05 (SD = 0.31), respectively. Appropriate negative and positive cutoff levels were calculated and are given in Table 1.

Of 357 test samples assayed, 35 (9.8%) were positive, 259 (73%) were negative, and 63 (18%) were classified as suspect (Table 2). All areas in which animals were tested, except West Cascades, Washington, had at least one animal that was either positive or suspect, and six of the nine areas had seropositive elk. Adults had a greater chance of being seropositive (OR = 5.18, 95% CI = 1.69 to 15.90) or

TABLE 1. Accuracy of the kinetic enzyme-linked immunosorbent assay (ELISA) for correctly classifying *Psoroptes* sp.-infested and non-infested elk of North America.

Classification value[a]	V_{max}[a]	Sensitivity (95% CI)[b]	Specificity (95% CI)[b]
$\bar{x}$ + 2 SD	28.8	94% (71%–99.7%)	91% (69%–98%)
$\bar{x}$ + 3 SD	37.0	78% (52%–93%)	100% (87%–100%)
$\bar{x}$ + 4 SD	45.2	61% (36%–82%)	100% (87%–100%)

[a] $\bar{x}$ + Y SD = Mean kinetic rate (V_{max}) of known negative controls plus Y standard deviations.
[b] CI = Confidence interval.

be classified in a combined group of seropositive and suspect seropositive (OR = 4.89, 95% CI = 2.65 to 9.00), compared to calves; however, yearlings were not at increased risk (OR = 1.05, 95% CI = 0.23 to 4.81 and OR = 0.54, 95% CI = 0.18 to 1.61, respectively). No significant association was found between sex and either antibody presence (OR = 0.23, 95% CI = 0.08 to 0.65) or the combined category of antibody presence and suspected antibody presence (OR = 0.77, 95% CI = 0.45 to 1.31). After adjustment for age and sex, animals from McKensie, North Dakota, had a significantly increased risk of being seropositive or suspect seropositive (adjusted OR = 5.21, 95% CI = 1.15 to 23.6) compared to all other herds. When suspect animals were not included in the analysis, however, this herd was not found to be at increased risk. Two other areas, West Great Salt Lake, Utah, and West Cascades, Washington, had crude odds ratios that we interpreted to mean increased risk in both antibody presence alone (OR = 5.65, 95% CI = 2.64 to 12.1 and OR = 9.72, 95% CI = 3.88 to 24.4, respectively) and antibody presence and suspected antibody presence combined (OR = 4.51, 95% CI = 2.50 to 8.14 and OR = 6.10, 95% CI = 2.51 to 14.8, respectively). However, these estimates could not be adjusted because of missing age and sex data.

TABLE 2. Seroprevalence of *Psoroptes* sp. mite infestation in elk and odds ratio (OR) by geographic location.

Location	Estimated herd size	Number sampled	Number positive	Number suspect	Positive (%)	Positive crude OR (95% CI)[a]	Positive and suspect (%)	Positive and suspect crude OR (95% CI)[a]
Dunn, North Dakota	100	38	1	7	2.6	0.25 (0.03–1.86)	21	0.68 (0.30–1.54)
McKensie, North Dakota	100	8	1	4	13	1.43 (0.17–12.1)	63	4.57 (1.07–19.6)[b]
Pembina, North Dakota	70	6	0	1	0.0	N/A[c]	17	0.52 (0.06–4.56)
West Great Salt Lake, Utah	650	58	15	17	26	5.65 (2.64–12.1)[e]	65	4.51 (2.50–8.01)[d]
Colockum, Washington	6,500	6	0	0	0.0	N/A	0.0	N/A
West Cascades, Washington	22,500	24	10	6	42	9.72 (3.88–24.4)[e]	67	6.10 (2.51–14.8)[d]
Finnegans, Wyoming	3,300	16	0	1	0.0	N/A	6.3	0.17 (0.02–1.29)
Greys River, Wyoming	3,800	29	3	1	10	0.33 (0.04–2.55)	14	0.40 (0.13–1.18)
NER, Wyoming[e]	16,000	172	5	26	2.9	Reference	18	Reference

[a] CI = Confidence interval.
[b] Statistically significant at $P = 0.05$ when adjusted for age and gender (adjusted OR = 5.21, 95% CI = 1.15–23.6).
[c] N/A = Not available.
[d] Age and sex information unavailable.
[e] NER = National Elk Refuge.

DISCUSSION

Based on the Western blotting analysis, *Psoroptes* sp.-infested elk produced antibodies to *P. cuniculi* mite antigens (Fig. 1). Elk infested with *Dermacentor* sp. ticks did not produce cross-reactive antibodies, and these results coupled with earlier studies are evidence that *P. cuniculi* antigens are appropriate for the immunodiagnosis of mite infestations in elk and several other species (Boyce and Brown, 1991; Boyce et al., 1991a).

From the kinetic ELISA results, eight of nine elk herds in North America contained either seropositive or serosuspect animals (Table 2). In assessing the geographic risk of exposure to mites, only the McKensie, North Dakota herd had a statistically significant increased risk (OR = 5.21) compared with the NER after adjustment for age and sex. However, only eight animals were sampled from a herd of 100 at this location and this herd was not found to be at increased risk when analyzed for antibody presence alone. In fact, when suspect animals were excluded, only six of nine herds had seropositive animals, and two of these herds had a single positive individual. The West Great Salt Lake and West Cascades herds had an increased risk of exposure when analyzed for antibody presence but adjustments for potential confounding factors were not possible due to missing animal information. Surprisingly, elk from Wyoming herds, where clinical scabies has been most evident, were not at increased risk when compared with other areas, even after adjustment for age and gender. However, these results may have been affected by sampling bias, since samples were primarily collected from healthy females and calves selected for translocations.

Scabies in elk has been identified more frequently in adult males than adult females and calves (Samuel et al., 1991), and Murie (1951) suggested that clinical disease in elk occurs secondarily to predis-

posing factors, such as post-rut debilitation. We found no evidence of a sex-related risk of exposure, but adults had about a five-times higher risk of exposure than calves. These results probably reflect an age-dependent risk of exposure.

In this study, we found that exposure to *Psoroptes* sp. mites is more widespread in elk herds than previously reported. Because of the legal and economic implications of mite infestation in livestock (Sweatman, 1958), as well as impacts on susceptible wild ungulate populations such as bighorn sheep (Lange et al., 1980), determination of the true prevalence and distribution of these parasites in free-ranging animals is important. Although the source of exposure to *Psoroptes* sp. mites in these elk was unknown, seropositive elk may have been exposed to mites either via contact with other infested animals or subclinical infestation may have persisted in herds after translocation from a source herd already infested with *Psoroptes* sp. mites. For example, the three North Dakota elk herds examined in this study originally were translocated from Wyoming where elk were known to be exposed to *Psoroptes* sp. mites. On the other hand, due to relatively large confidence intervals for assay sensitivity and specificity, the single seropositive results from Dunn and McKensie may have been misclassified. Although *Psoroptes* sp. mites have been seen in wild ungulates in Washington and other northwestern states, the relatively high prevalence estimated in this study was surprising. Additional studies, particularly in the Cascade and western Rocky Mountain herds, should be undertaken to attempt to further classify the extent to which *Psoroptes* sp. mites are present in these free-ranging elk populations. The possible management implications of translocating *Psoroptes* sp. infected elk should be considered, and acaricidal treatments should be administered prior to elk relocation.

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