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SERODIAGNOSTIC ANTIBODY RESPONSES TO *PSOROPTES* SP. INFESTATIONS IN BIGHORN SHEEP

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ABSTRACT: The antibody responses of bighorn sheep (*Ovis canadensis*) infested with *Psoroptes* sp. mites were investigated by enzyme linked immunosorbent assay on western blots of *P. cuniculi* antigens. Serum from 20 *Psoroptes* sp.-infested bighorn sheep (*O. canadensis mexicana, O. canadensis nelsoni, O. canadensis canadensis*) from New Mexico, Nevada, California, and Idaho reacted strongly with mite antigens ranging from 12 to 34 kd. Serum from 35 *Psoroptes* sp.-free bighorn sheep of unknown tick infestation status and from three *Psoroptes* sp.-free bighorn sheep infested with *Dermacentor hunteri* ticks did not react with these antigens. *Psoroptes* sp.-specific antibody responses were present throughout a 16 mo period in one infested bighorn sheep, but were not detectable 8 mo following successful treatment. These results demonstrate that specific serodiagnosis of *Psoroptes* sp. infestation is feasible in bighorn sheep and suggest that antibody responses are indicative of current or recent infestation.

Key words: *Psoroptes* sp., mites, *Dermacentor hunteri*, ticks, scabies, bighorn sheep, *Ovis canadensis*, immunodiagnosis, antibodies.

INTRODUCTION

Ectoparasitic mites in the genus *Psoroptes* infest a variety of domestic and wildlife species resulting in conditions ranging from inapparent infestation to severe clinical disease (Sweatman, 1958; Meleney, 1985). Although it is not clear which species of *Psoroptes* infest wild ungulates, *Psoroptes* sp. infestations can be particularly devastating in bighorn sheep (*Ovis canadensis*) (Lange et al., 1980), and recent reports suggest that the prevalence of psoroptic scabies in bighorn sheep may be increasing (Clark et al., 1988). Currently, *Psoroptes* sp. infestations are diagnosed in bighorn sheep by recovering mites from skin scrapings of lesions on the body or from the ears. However, this technique was found to be inadequate for diagnosis in cattle and sheep when lesions were inapparent or when small numbers of mites were present (Meleney and Christy, 1978; National Research Council, 1979). The development of immunoassays for *Psoroptes ovis* infestations in domestic livestock provided an alternative method for detecting small numbers of mites (Fisher, 1983; Fisher et al., 1986; Wassall et al., 1987), and the utility of these tests suggested that similar assays would also be useful for the diagnosis of psoroptic scabies in bighorn sheep. The purpose of the present study was to improve our ability to detect *Psoroptes* sp. infestations in bighorn sheep by identifying serodiagnostic antibody responses on western blots of *Psoroptes cuniculi* antigens. *Psoroptes cuniculi* mites were used because of their demonstrated antigenic similarities with other species of *Psoroptes* (Rafferty and Gray, 1987), and because their ready availability made it possible to prepare a large supply of stock antigen for future studies.

MATERIALS AND METHODS

Study animals

Free-ranging and captive bighorn sheep were examined for skin and/or aural lesions caused by *Psoroptes* sp. mites and infestations were confirmed by identifying mites recovered from ear swabs and/or skin scrapings. Negative control serum was collected from mite-free bighorn

sheep maintained at the Red Rock Refuge, New Mexico (35°27′N, 108°45′W; *O. canadensis mexicana*; $n = 9$), the Bighorn Research Institute, California (33°56′N, 116°36′W; *O. canadensis cremnobates*; $n = 9$), and a captive herd at Washington State University, Washington (46°43′N, 117°09′W; *O. canadensis canadensis*; $n = 17$). Serum and mites were collected during 1988–1989 from free-ranging bighorn sheep in the San Andres Mountains, New Mexico (33°04′N, 106°38′W; *O. canadensis mexicana*; $n = 9$); Morgan Creek, Idaho (44°36′N, 114°09′W; *O. canadensis canadensis*; $n = 1$); Black Mountains, Nevada (36°12′N, 114°28′W; *O. canadensis nelsoni*; $n = 2$); Last Chance Mountains, California (37°08′N, 117°36′W; *O. canadensis nelsoni*; $n = 1$); Dead Mountains, California (34°59′N, 114°44′W; *O. canadensis nelsoni*; $n = 1$); San Bernardino Mountains, California (34°12′N, 116°58′W; *O. canadensis nelsoni*; $n = 5$); and Panamint Butte, California (36°26′N, 117°21′W; *O. canadensis nelsoni*; $n = 1$). All of the San Andres animals had severe skin and aural lesions; two animals from Nevada and two from California had clinically inapparent aural infestations detected by finding <10 mites on ear swabs; all of the remaining infested animals had obvious aural lesions containing large numbers of mites.

Tick infestations were occasionally noted on both mite-infested and noninfested bighorn sheep, but an accurate determination of the presence or absence of ticks on each animal was not always made. To determine whether or not antibodies from tick-infested bighorn sheep cross-reacted with *P. cuniculi* mite antigens, serum was collected from bighorn sheep (36°32′N, 117°30′W; *O. canadensis nelsoni*, $n = 3$; Hunter Mountains, California) infested with *Dermacentor hunteri* ticks, but determined to be *Psoroptes* sp.-free after careful clinical and parasitological examination.

Serum was also collected from one bighorn sheep (*O. canadensis nelsoni*) captured in the Panamint Mountains, California (36°26′N, 117°21′W), in November, 1987, March, 1988, March, 1989, and November, 1989. Aural lesions and mites were present during the first three captures, while no mites or lesions were present in November, 1989, after successful treatment with ivermectin at 2,000 μg/kg body weight (Merck, Sharpe and Dohme Inc., Rahway, New Jersey 07065, USA) in March, 1989.

Mite antigens

Aural scabs containing mites (*P. cuniculi*) were collected from naturally infested domestic rabbits (New Zealand White; Antibodies Inc., Davis, California 95616, USA) and placed in glass jars. Live mites (larvae, nymphs and adults) were aspirated by vacuum suction as they crawled away from the scabs and frozen at −20 C in sterile water. Mites were thawed, washed in sterile water, and homogenized in phosphate-buffered saline (PBS) using a Ten-Broeck tissue grinder (Corning Glass Works, Corning, New York 14831, USA). The homogenate in PBS was kept for 24 h at 4 C and then centrifuged at 14,000 G for 30 min. Soluble proteins present in the supernatant were quantified using a protein assay (Bio Rad, Richmond, California 98404, USA), and frozen at −40 C in aliquots adjusted to a concentration of 1 mg/ml in PBS.

SDS-PAGE, western blotting and enzyme immunoassay

Sodium dodecyl sulfate polyacrylamide gel electrophoresis, silver staining and western blotting were performed exactly as described by Boyce et al. (1988) with minor modifications. Briefly, *P. cuniculi* mite proteins (200 μg/15 cm gel) were reduced and denatured by preparing and heating samples in sample buffer containing mercaptoethanol (Sigma Chemical Co., St. Louis, Missouri 63178, USA) as recommended by the electrophoresis equipment manufacturer (Bio Rad). Proteins were separated on 12% polyacrylamide gels with 4% stacking gels (Bio Rad), and then electrophoretically transferred overnight onto nitrocellulose (NC; Bio Rad) under previously described conditions (Boyce et al., 1988). Apparent molecular weights of mite proteins were determined by comparison with protein standards stained with amido black (Bio Rad). Enzyme-linked immunosorbent assays were then performed on NC strips, each containing approximately 5 μg bound protein, using serum from *Psoroptes* sp.-infested and non-infested bighorn sheep. Unbound sites on NC strips were blocked with 3% gelatin (Sigma Chemical Co.) in Tris buffered saline (TBS; 20 mM Tris, 500 mM NaCl, pH = 7.5; Sigma Chemical Co.) for 30 min and the strips probed for 3 hr with bighorn sera at a 1:100 dilution in 1% gelatin in Tween Tris buffered saline (TTBS; 0.05% Tween-20 in TBS; Bio Rad). Strips were incubated for 1 hr with a 1:500 dilution (1% gelatin in TTBS) of rabbit anti-sheep IgG conjugated with horseradish peroxidase (Kirkegaard Perry Laboratories, Gaithersburg, Maryland 20879, USA), and color development was performed using 4-chloro-1 naphthol (Bio Rad). To ensure that the rabbit anti-sheep conjugate did not react with mite antigens in the absence of bighorn sheep antibodies, control strips were prepared and treated exactly as described except that bighorn sheep serum was omitted during the 3 hr incubation in TTBS.

RESULTS

Many *P. cuniculi* proteins, ranging from 10 to >200 kd, were visualized by silver staining of SDS-PAGE gels, and these proteins were successfully transferred onto nitrocellulose as indicated by staining with amido black. Antibody responses to these mite proteins were examined using serum from a population of *Psoroptes* sp.-infested bighorn sheep (*O. canadensis mexicana*) from the San Andres National Wildlife Refuge in New Mexico (Fig. 1). Serum from each infested animal reacted with at least seven different antigens ranging from 12 to 164 kd, and in some cases as many as 20 different antigens were recognized. Serum from *Psoroptes* sp.-free bighorn sheep (*O. canadensis mexicana*) from the Red Rock Refuge, New Mexico, also reacted weakly with *P. cuniculi* antigens >34 kd, with the most prominent reactions occurring with antigens >116 kd (Fig. 1). After accounting for these cross-reactions, *Psoroptes* sp.-specific antibody responses were found to be directed against at least three antigens in the 12 to 34 kd range for each mite-infested San Andres bighorn sheep. No reactions were seen on the conjugate control strips.

With minor exceptions, antigen-antibody reactions were very similar among the three subspecies and seven populations of infested bighorn sheep sampled from New Mexico, Nevada, California and Idaho (Fig. 2). Each infested bighorn sheep produced antibodies which reacted with three or more antigens in the 12 to 34 kd range, while serum from the three groups of mite-free bighorns reacted only with antigens >34 kd. Serum from *Psoroptes* sp.-free, tick-infested bighorn sheep also recognized those antigens >34 kd (Fig. 2, lane H), but did not cross-react with *P. cuniculi* antigens in the 12 to 34 kd range. No qualitative or quantitative differences (intensity or number of bands) were detected in antibody responses from mite-infested animals which could be correlated with the numbers of mites or the extent of lesions.

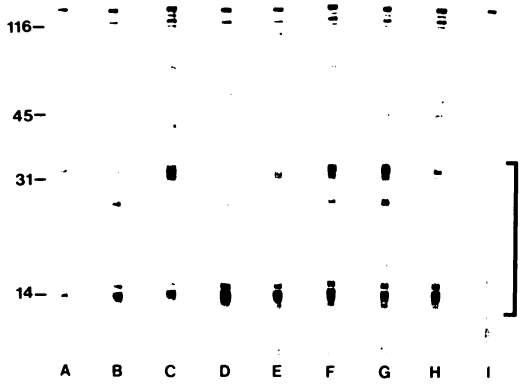

FIGURE 1. Antibody recognition of *Psoroptes cuniculi* antigens by *Psoroptes* sp.-infested bighorn sheep (*O. canadensis mexicana*) from the San Andres Mountains, New Mexico (A to H), and by a non-infested bighorn sheep (*O. canadensis mexicana*) from the Red Rock Refuge, New Mexico (I). Molecular weights in kd are indicated on the left, and *Psoroptes* sp.-specific antibody responses (12 to 34 kd) are indicated by a bracket on the right.

Antibody responses were virtually identical each time serum was collected from a *Psoroptes* sp.-infested ewe over a 16 mo period (Fig. 3). Eight months after administration of an apparently successful treatment, the antigen-antibody recognition pattern was indistinguishable from that of non-infested bighorn sheep (Figs. 2 and 3).

DISCUSSION

Severe infestations with *Psoroptes* sp. mites in wild and domestic ungulates are easily detected by the presence of mites and lesions on the body and/or ears. However, diagnosis may be quite difficult when mites are present in small numbers and cause little or no detectable pathologic effects (National Research Council, 1979). Clinically inapparent infestations are particularly common in domestic livestock during those times of the year when host/environmental conditions cause the mites to enter a period of latency (Meleney, 1985). A similar phenomenon may occur in bighorn sheep and thus cause investigators to underestimate the actual prevalence of infestation.

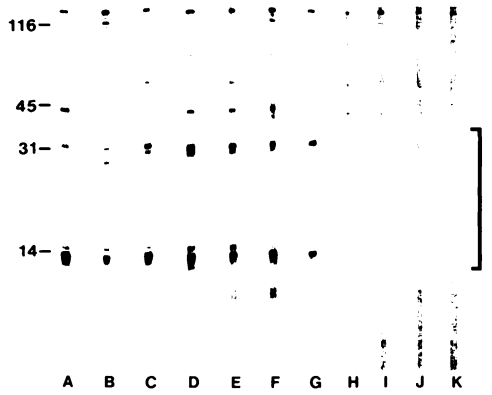

FIGURE 2. Antibody recognition of *Psoroptes cuniculi* antigens by bighorn sheep from different populations. *Psoroptes* sp.-infested bighorn sheep labeled A to G are *O. canadensis canadensis* from Morgan Creek, Idaho; *O. canadensis mexicana* from San Andres, New Mexico; *O. canadensis nelsoni* from Black Mountains, Nevada; *O. canadensis nelsoni* from Last Chance Mountains, California; *O. canadensis nelsoni* from Dead Mountains, California; *O. canadensis nelsoni* from San Bernardino Mountains, California; and *O. canadensis nelsoni* from Panamint Mountains, California, respectively. A mite-free, *Dermacentor hunteri*-infested bighorn sheep labeled H is *O. canadensis nelsoni* from Hunter Mountains, California. Mite-free captive bighorn sheep labeled I to K are *O. canadensis mexicana* from Red Rock Refuge, New Mexico; *O. canadensis cremnobates* from Bighorn Research Institute, California; and *O. canadensis canadensis* from Washington State University, Washington, respectively. Molecular weights in kd are indicated on the left, and *Psoroptes* sp.-specific antibody responses (12 to 34 kd) are indicated by a bracket on the right.

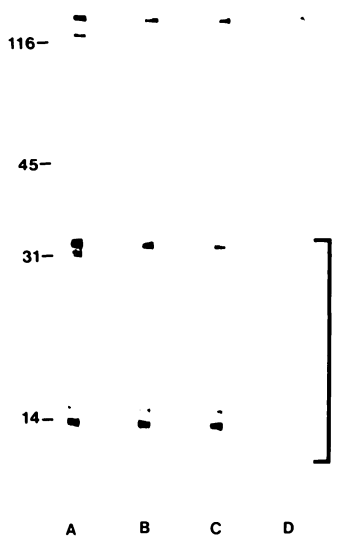

FIGURE 3. Antibody responses to *Psoroptes cuniculi* antigens by a *Psoroptes* sp.-infested bighorn sheep (*O. canadensis nelsoni*, Panamint Mountains, California) before and after treatment. Pretreatment samples are labeled A to C and were collected in November, 1987, March, 1988, and March, 1989, respectively. The animal was successfully treated in March, 1989, and the posttreatment sample, labelled D, was collected in November, 1989. Molecular weights in kd are indicated on the left, and *Psoroptes* sp.-specific antibody responses (12 to 34 kd) are indicated by a bracket on the right.

Serodiagnostic assays which detect antibodies to infectious diseases can be extremely valuable in diagnosing infections in which the etiologic agent is difficult to isolate and identify. In this study, serum antibodies from 20 *Psoroptes* sp.-infested bighorn sheep in New Mexico, Nevada, Idaho and California consistently recognized *P. cuniculi* antigens in the 12 to 34 kd range, while serum from 35 noninfested bighorn sheep reacted weakly, if at all, with a small number of antigens ranging from 34 to 164 kd. Using the primarily qualitative technique of western blotting, a quantitative relationship could not be established between the severity of infestation and the number of antigen-antibody bands. However, antibody responses to the 12 to 34 kd antigens proved to be a sensitive indicator of *Psoroptes* sp.-infestation in each affected animal, including the four asymptomatic animals from which fewer than ten mites were recovered.

With one exception, the animals were sampled only once, and it was not possible to determine whether an animal had just acquired an infestation or whether it was in the process of eliminating its infestation. One infested bighorn captured on four occasions recognized similar 12 to 34 kd antigens over a 16 mo period, and these responses were not detectable 8 mo following successful treatment. These results agree with those of Fisher et al. (1986), and suggest that bighorn sheep are similar to cattle in that antibody responses to *Psoroptes* sp. mites are detectable only during current or recent infestations.

Tick infestations were noted on many bighorn sheep and antibodies against tick antigens could conceivably cross-react with mite antigens. However, serum from three mite-free bighorn sheep infested with *D. hunteri* ticks did not react with *P. cuniculi* antigens ranging from 12 to 34 kd, and antibody recognition of antigens >34 kd was identical to mite-free bighorn sheep of uncertain tick status. The specificity of antibody responses to 12 to 34 kd mite antigens is further supported by the findings of DenHollander and Allen (1986) who reported that antibodies from tick-infested (*Dermacentor variabilis*) mice did not cross-react with *P. cuniculi* antigens.

The mites recovered from bighorn sheep in this study could not be positively identified to species using the criteria established by Sweatman (1958). Adult males recovered from both the ears and bodies of bighorn sheep had outer opisthosomal setae lengths within the range established for *P. ovis*. However, Sweatman (1958) did not consider *P. ovis* to be an ear mite of bighorn sheep. Accordingly, many recent reports have tentatively identified ear and body mites from bighorn sheep as *P. ovis*, while noting the need for taxonomic revision (Lange et al., 1980; Wright et al., 1981, 1984; Kinzer et al., 1983; Foreyt et al., 1985).

The choice of *P. cuniculi* mites as the source of antigen for this study was based upon availability and demonstrated antigenic and morphologic similarities between *P. cuniculi* and *P. ovis* mites. Precipitating antibodies were first reported in serum from domestic sheep, cattle, and bighorn sheep infested with *P. ovis* using the agar gel diffusion test (Fisher, 1972; Fisher and Wilson, 1977; DeVos et al., 1980). These studies showed that two or three antigens of unknown molecular weight were recognized within crude extracts of both *P. ovis* and *P. cuniculi* mites. Based on cross-reactions observed in immunofluorescent studies, Rafferty and Gray (1987) also suggested that *P. ovis* and *P. cuniculi* mites shared the same antigens. These observations and the results of the present study indicate that *P. cuniculi* antigens are suitable for detecting *Psoroptes* sp.-specific antibody responses in bighorn sheep.

MANAGEMENT IMPLICATIONS

The western blot assay for antibodies against *Psoroptes* sp. mites provides wildlife managers with a tool to determine which bighorn sheep populations and which individuals are infested, regardless of whether or not the animals are showing clinical signs. We are currently using a modification of this test to screen 1,600 serum samples from >60 populations of bighorn sheep in North America. These results, coupled with additional serologic screening of appropriate populations, should be useful in reducing the risk of relocating *Psoroptes* sp. mites to new sites and into new populations of bighorn sheep and perhaps domestic livestock. It also appears that this test may be used to indicate when mite infestations have been eliminated, thus providing a means for evaluating the success/failure of treatment and control measures.

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