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Susceptibility of Two Rocky Mountain Bighorn Sheep to Experimental Infection with *Anaplasma ovis*

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ABSTRACT: In North America, the role of wild ruminants in the epidemiology of anaplasmosis has not been clearly defined. Such information is particularly meager in regard to bighorn sheep. We report the susceptibility of two Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) to experimental infection with a well characterized field isolate of *Anaplasma ovis* obtained from domestic sheep in Idaho. Both bighorn sheep developed infection resulting in severe clinical disease, with relatively high parasitemias, icterus and anemia. One animal required tetracycline therapy and responded well to treatment, while the other recovered uneventfully without treatment. Both bighorn sheep were spleen-intact, a condition that in *A. ovis*-exposed domestic sheep typically is associated with mild infection. The results indicate that bighorn sheep may be adversely affected if exposed to the organism in nature.

Key words: Anaplasmosis, *Anaplasma ovis*, bighorn sheep, *Ovis canadensis canadensis*, susceptibility, experimental infection.

Anaplasmosis is an infectious, noncontagious disease of ruminants caused by the hematropic rickettsiae, *Anaplasma* spp. Transmission occurs by passage of infected blood to susceptible animals via ticks, biting flies, blood transfers, and iatrogenic accidents (Blood et al., 1979). Also, transplacental transfer has been observed experimentally (Zaugg, 1987b). Infection of susceptible domestic ruminants is characterized by anemia, icterus, depression, anorexia and possibly death (Weinman and Ristic, 1968).

The two *Anaplasma* spp. of importance to livestock in the United States are *A. marginale* of cattle and *A. ovis* of sheep and goats. *Anaplasma marginale* infection in susceptible cattle produces clinical disease (Blood et al., 1979), while *A. ovis* infection in domestic sheep is usually subclinical, with signs of infection exhibited in concert with intercurrent diseases (Kimberling, 1988). However, decreased live weight of market animals due to suboptimal gains is common and accompanies subclinical anaplasmosis in sheep (Kimberling, 1988). Acute anaplasmosis can be experimentally produced in splenectomized sheep (Magonigle et al., 1981). Interestingly, *A. ovis* infection in spleen-intact or splenectomized goats usually results in more severe disease than in sheep, reflecting more the clinical course of *A. marginale* in cattle (Splitter et al., 1956).

Experimental *A. ovis* infections have been described in splenectomized cattle (Kuttler, 1981), white-tailed deer (*Odocoileus virginianus*) (Kreier and Ristic, 1963), mule deer (*Odocoileus hemionus*) (Zaugg, 1988), and pronghorn antelope (*Antilocapra americana*) (Zaugg, 1987a), but apparently have not been attempted in bighorn sheep (*Ovis canadensis*). Howe et al. (1964) attempted experimental *A. marginale* infection of two bighorn sheep, but technical weaknesses produced equivocal results.

Since bighorn sheep and domestic livestock share habitat in certain regions of the United States, it is important to determine the potential role of bighorn sheep as reservoirs of anaplasms. A prerequisite to such investigations is knowledge of bighorn sheep susceptibility to *Anaplasma* sp. infection. The purpose of this experiment was to determine the susceptibility of bighorn sheep to experimental infection with *A. ovis*.

Two spleen-intact Rocky Mountain bighorn sheep were used in this experiment. A 7-mo-old male (Wh717) and 13-mo-old female (Or1) were obtained as wild ani-

mals. Each animal was maintained with other bighorn sheep in an outdoor facility (USDA-ARS, Animal Disease Research Unit, Pullman, Washington 99164, USA) for approximately 3 mo before moving each to an individual indoor isolation facility. Each animal was provided water, mineral salt and high quality alfalfa hay and alfalfa pellets. Each animal was inoculated on separate occasions however, both were housed indoors for 3 wk before the inoculations were done. Each animal was caught and restrained by hand and a hood placed over the head to settle the animal before sampling. Preinoculation restraint and sampling was done once each of the 3 wks before inoculation. Preinoculation blood samples were collected for serology, preparation and examination of Giemsa-stained blood smears, and determination of packed cell volume (PCV).

The *A. ovis* stabilate represented an isolate obtained by feeding adult *Dermacentor andersoni* ticks collected from *Anaplasma* sp. seropositive domestic sheep in the field at the United States Sheep Experiment Station (Dubois, Idaho 83423, USA) on a susceptible domestic sheep (Stiller et al., 1989). When the susceptible domestic sheep developed an ascending parasitemia, blood was collected for stabilate preparation as described by Love (1972), and stored in liquid nitrogen until used. Both bighorn sheep were inoculated intravenously with the *A. ovis* blood stabilate containing 2×10^9 infected erythrocytes per inoculum. After inoculation, blood samples were taken twice weekly for serology, determination of PCV and the evaluation of Giemsa-stained blood smears to determine the percent parasitized erythrocytes (PPE). Following blood smear detection of *Anaplasma* sp. bodies, blood samples were collected daily and processed as previously described. The animals were observed daily for depression, anorexia and icterus.

An indirect immunofluorescence technique (IIF) was used for the detection of *Anaplasma* sp. antibodies in bighorn serum samples. The IIF procedure was done as previously described (Goff et al., 1985) with some modifications. Briefly, the *A. ovis* antigen was prepared by washing infected domestic sheep red blood cells (RBC's) four times at 400 × G in phosphate buffered saline (0.15 M NaCl, 10 mM Na_2HPO_4, 3 mM KH_2PO_4, pH 7.2) (PBS). The pellet was resuspended in PBS containing 1% bovine serum albumin (Fraction V) to a PCV of 20%. This preparation was applied to glass microscope slides to produce a thin smear of infected RBC's. The slides were air dried, covered, and stored at −70 C. Before incubation with test serum, the slides were warmed to room temperature in a desiccator jar and fixed in cold acetone. Recombinant protein-G conjugated with fluorescein isothiocyanate (Zymed, San Francisco, California 94080, USA), diluted 1:80 in PBS was used to detect specific IgG. It was determined by gel diffusion and immunoelectrophoresis that recombinant protein-G would bind IgG of bighorn sheep (T. Tibbitts, unpubl. obs.). Positive and negative IIF control sera were obtained from *A. ovis*-infected and naive domestic sheep, respectively. A dilution of the positive control serum that consistently produced a weak reaction was used as a reference. A 1/100 dilution of the same serum and a negative control serum, along with the weak positive reference dilution were applied to each antigen slide. All test sera were initially screened at a 1/100 dilution and titers of all positive sera determined following 2-fold serial dilutions. A positive was defined as any reaction ≥the weak positive control.

Preinoculation serum samples from both bighorn sheep were IIF negative. *Anaplasma* sp. bodies were not seen in preinoculation blood smears and preinoculation PCV's (Or1 = 50, Wh717 = 53) were considered to be within normal limits for bighorn sheep (Franzmann, 1970; Woolf and Kradel, 1970).

Both bighorn sheep became infected with *A. ovis*. The prepatent period was 20

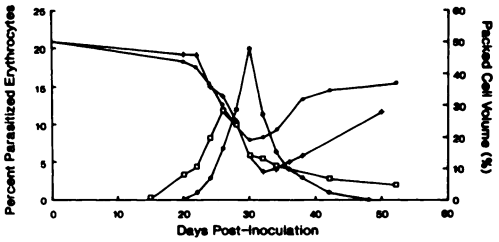

FIGURE 1. Packed cell volume (PCV) and percent parasitized erythrocytes (PPE) from two bighorn sheep resulting from experimental infection with *Anaplasma ovis*. ●——●, PCV for Or1; |——|, PCV for Wh717; □——□, PPE for Or1; •——•, PPE for Wh717.

and 21 days in Or1 and Wh717, respectively (Fig. 1). These prepatent periods are typical for livestock infections, and prepatent periods of 20–40 days have been noted in experimental wild ruminant infections (Howe et al., 1964; Kreier and Ristic, 1963; Zaugg, 1987a; Zaugg, 1988). The highest PPE for Or1 was 12 which occurred on post-inoculation day (PID) 26, and for Wh717 was 20 which occurred on PID 30. Both animals developed severe anemia (9% PCV, 83% reduction for Wh717, and 19% PCV, 62% reduction for Or1), icterus, and lethargy. Wh717 was treated with oxytetracycline (LA-200, Pfizer, New York, New York 10017, USA) at 25 mg/kg body weight, i.v. when the PCV dropped to 9%. The infection of Or1 resolved without antibiotic treatment.

Both bighorn sheep seroconverted to *A. ovis* by PID 20. Peak serum titers of 1/20,000 were reached on approximately PID 30, which coincided with peak parasitemia in both animals (Fig. 1).

The limited studies that have been done with *Anaplasma* sp. in wild ruminants (mainly *A. marginale*) indicate that these hosts may be refractory to serious clinical disease (Kuttler, 1984). However, to our knowledge, this is the first study involving bighorn sheep and *A. ovis*. One or a combination of 3 factors may have influenced the severity of the infections in these bighorn sheep. First, the inoculation dose of 2×10^9 *A. ovis* infected erythrocytes used in this study was high. However, we wanted to maximize the chances of infection because the susceptibility of bighorn sheep to *Anaplasma* sp. infection was unknown, and because we were using spleen-intact animals. It is well established that splenectomized animals are more susceptible to Anaplasma infection than spleen-intact animals (Kuttler, 1981; Roby et al., 1961; Splitter et al., 1956). In addition, it is difficult, if not impossible, to determine the number of infectious parasites in blood stabilates. For example, in a study using susceptible cattle, *A. marginale* infective titers were reported in terms of numbers of infected erythrocytes from blood stabilates. However, there was an inverse relationship between titer and length of time that the stabilates were stored in liquid nitrogen (Kliewer et al., 1973).

Second, the wild bighorn sheep were, by necessity, subjected to unnatural and extremely stressful conditions of indoor confinement and handling. It has been shown that this kind of stress in bighorn sheep leads to an increase in adrenal activity, with elevated levels of serum cortisol (Harlow et al., 1987). It is well established that prolonged exposure to, or increased levels of, glucocorticosteroids can lead to immunosuppression (Balow et al., 1975; Cohen, 1971). In studies involving bighorn sheep, immunosuppression has been demonstrated as a result of stress-induced increase in cortisol levels (Hudson, 1973; Spraker and Hibler, 1982). In this regard, it would be useful to undertake a comparative pathogenicity study involving domestic sheep and bighorn sheep that have been born in, and well adjusted to, captivity.

Third, the isolate of *A. ovis* may have been relatively virulent. It is important that well characterized, low passage level field isolates be used in susceptibility and pathogenicity studies. This typically has not been true of previous studies involving wild ungulates and *Anaplasma* spp. This oversight must be corrected if the role of wildlife species in Anaplasma epizootiology is to be accurately assessed. The blood stabilate used in this study was a true field isolate

not subjected to selective pressure due to passage, and which was not maintained for a prolonged period of liquid nitrogen (<1 yr). Although no critical studies concerning the pathogenicity of *A. ovis* in domestic sheep have been reported, the general consensus is that *A. ovis* in spleen-intact sheep results in a mild infection (Splitter et al., 1956). This isolate appears to be less virulent in spleen-intact domestic sheep where, under similar conditions, a milder infection has resulted (<1 PPE and <30% PCV reduction). However, only a few domestic sheep have been observed after infection with this particular *A. ovis* isolate, and thus no general comparative conclusions can be reached.

The present study demonstrates the susceptibility of bighorn sheep to experimental infection with *A. ovis*. Based on the results, continued investigations seem warranted. Natural exposure of bighorn sheep to *A. ovis*, including transmission by ticks, seems to merit investigation, as does the potential of bighorn sheep to act as a reservoir of the parasite. Most importantly, natural exposure of free-roaming bighorn sheep to *A. ovis* either alone, or in combination with other disease agents or stress factors, could pose a health hazard to these animals.

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