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# Pathogen Exposure Patterns among Sympatric Populations of Bighorn Sheep, Mule Deer and Cattle

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ABSTRACT: We sampled sympatric bighorn sheep (*Ovis canadensis*, $n = 31$), mule deer (*Odocoileus hemionus*, $n = 38$), and domestic cattle ($n = 26$) in the San Bernadino Mountains of southern California (USA) for the presence of *Psoroptes* spp. mites and for serologic evidence of exposure to bluetongue virus (BTV) and *Babesia* spp. From 1991 through 1994, *Psoroptes* spp. infestations were found on 12 (44%) of 27 bighorn sheep. No mites were found on mule deer or cattle. The BTV serum antibody prevalence in a cohort of 26 cattle ranged from 17 to 89%. There was no evidence of exposure to BTV in the bighorn sheep or mule deer. The cumulative serum antibody prevalence of *Babesia* spp. during the study was 35% in 26 bighorn sheep and 85% in 20 mule deer, while antibodies were not detected in a cohort of cattle when they were sampled in May ($n = 23$) and December ($n = 22$) of 1992. Based on these results, we concluded that infestation with *Psoroptes* spp. and exposure to BTV was limited to bighorn sheep and cattle, respectively. In contrast, *Babesia* spp. infections appeared to be common in both mule deer and bighorn sheep while there was no evidence of exposure in cattle.

*Key words:* Bighorn sheep, *Ovis canadensis*, mule deer, *Odocoileus hemionus*, cattle, pathogen, disease transmission, serologic testing, bluetongue virus, *Psoroptes* spp., *Babesia* spp.

There is compelling evidence that bighorn sheep (*Ovis canadensis*) frequently undergo epizootics of *Pasteurella* spp. pneumonia following contact with domestic sheep (Goodson, 1982; Foreyt and Jessup, 1982; Jessup, 1985a; Foreyt, 1992). In a recent study by Foreyt and Lagerquist (1996), cattle and bighorn sheep coexisting in pasture conditions seemed to share *Pasteurella haemolytica* biotype A, serotype 2. We are not aware of any report documenting transmission of pathogens between cattle and bighorn sheep under rangeland conditions. We addressed this lack of data by studying pathogens that are of known or potential importance in cattle, bighorn sheep, and mule deer (*Odocoileus hemionus*) in an area where these three ungulate species co-exist. Our objective was to determine and compare the prevalences of bluetongue virus (BTV), *Psoroptes* spp., and *Babesia* spp. among these host species, and to identify patterns of exposure that would indicate whether pathogens were being transmitted among host populations. We combined previously published data concerning exposure to *Babesia* spp. in these bighorn sheep and mule deer (Kjemtrup et al., 1995) with new data concerning BTV and *Psoroptes* spp. exposure in the bighorn sheep, mule deer and cattle, and *Babesia* spp. exposure in the cattle.

The study area was located in the San Bernadino Mountains of southern California, USA (34°1′ to 34°8′N, 116°37′ to 116°48′W), and encompassed approximately 23,600 ha of private land and adjoining public land managed by the Bureau of Land Management and the United States Forest Service. The elevation of this area ranged from 1,000 to over 3,500 m. The native vegetation was composed of mixed conifer, mixed chaparral, desert scrub, oak riparian, and wet meadow habitats.

Thirty-one bighorn sheep and 38 mule deer were captured and sampled between 1991 and 1994 (Table 1) from populations estimated to range in size from 100 to 150, and 100 to 200, respectively (Torres et al., 1994). The animals were captured primarily by net gun and drive net. Several hunter-killed deer were also sampled. All captures were based on convenience, and no individuals were resampled.

A cohort of 26 adult cattle was sampled from a closed herd of approximately 100 animals that grazed in the study area from approximately April to December of every year. These cattle were selected by convenience in December 1991, and 21 to 24 animals in the cohort were sampled six times from December 1991 through April 1994. At each sampling, the cattle were treated with injectable ivermectin (Ivomec®, MSD AgVet, Rahway, New Jersey, USA) and were vaccinated against bovine viral diarrhea, bovine herpesvirus 1, parainfluenza-3 virus, bovine respiratory syncytial virus, and *Leptospira interrogans* serovars *hardjo, pomona, canicola, icterohemorrhagiae,* and *gryppotyphosa* (Discovery®-4L5, Franklin Laboratories, Fort Dodge, Iowa, USA).

The 23,600 ha that comprised our study area constituted the entire range of the bighorn sheep and mule deer. The cattle were seasonally rotated through portions of the area, and their home range for a given month was considerably smaller. While no field studies were conducted to observe the degree of home range overlap among these three species, the bighorn, deer and cattle were observed to be sympatric within these smaller monthly grazing sections. Observations by biologists with the California Department of Fish and Game confirmed that mule deer, bighorn sheep, and cattle all utilized common areas within this study site, particularly near riparian corridors.

Aliquots of whole blood from each sampled animal were inoculated into embryonating chicken eggs and BHK cell culture (baby hamster kidney) for isolation of BTV at the National Veterinary Services Laboratory (NVSL, Ames, Iowa). Isolates of BTV were first differentiated from epizootic hemorrhagic disease virus (EHDV) by fluorescent antibody staining and then serotyped through virus neutralization (Afshar, 1994) at NVSL. Serum samples were collected from each animal for detection of antibodies to BTV and *Babesia* spp. Deep ear swabs were collected to recover *Psoroptes* spp. Serum samples were tested in our laboratory for antibodies to BTV using the competitive enzyme-linked immunosorbent assay (c-ELISA) (BluePlate Special®, Diagxotics Inc., Wilton, Connecticut, USA). In this assay, antibodies in the sample compete with a biotinylated monoclonal antibody (MAb) for binding to inactivated BTV antigen. Positive samples resulted in greater than 30% inhibition of the binding of the MAb to the inactivated BTV antigen. We utilized an indirect immunofluorescent antibody test (IIF) and appropriate controls as described by Kjemtrup et al. (1995) to test sera for the presence of antibodies to *Babesia* spp. The antigens used in this assay were derived from *Babesia* spp. previously isolated from bighorn sheep and mule deer in the San Bernadino Mountains (Thomford et al., 1993). Initially, the samples were diluted 1:80, and samples with a final titer of at least 1:640 were considered positive. Finally, ear swabs collected from each animal's ears were examined under a dissecting microscope for the presence of *Psoroptes* spp.

Prevalences of exposure to BTV, *Babesia* spp., and *Psoroptes* spp. were calculated for each sampling period (Table 1). Since different bighorn sheep and mule deer were sampled at each time period, we calculated an overall prevalence for each pathogen for the entire study period. Antibodies to BTV were present only in cattle, *Psoroptes* spp. mites were found only on bighorn sheep, and antibodies to *Babesia* spp. were present in both bighorn sheep and mule deer.

We believe that this is the first study in which the potential disease transmission between bighorn sheep, mule deer, and cattle is assessed under rangeland conditions. The fact that the cattle were sampled repeatedly while the individual bighorn and mule deer were sampled only once was a major limitation of this study. However, we sampled between 20 and 30% of the bighorn sheep and mule deer populations during the study. In addition,

TABLE 1. Prevalence of exposure to selected infectious agents in bighorn sheep (*Ovis canadensis*), mule deer (*Odocoileus hemionus*), and cattle, San Bernadino Mountains, California, 1991 through 1994.

| | Sample date | Number sampled | Infectious disease agents | | |
|---|---|---|---|---|---|
| | | | BTV[a] | Psor[b] | Bab[c,d] |
| Bighorn sheep | Mar. 1991 | 5 | 0 (0/5)[e] | 60 (3/5) | 100 (5/5) |
| | June 1991 | 3 | 0 (0/3) | ND[f] | 33 (1/3) |
| | Dec. 1991 | 3 | 0 (0/3) | 33 (1/3) | 33 (1/3) |
| | Dec. 1992 | 6 | 0 (0/6) | 33 (2/6) | 17 (1/6) |
| | Nov. 1993 | 2 | 0 (0/2) | 0 (0/2) | 0 (0/2) |
| | Dec. 1993 | 11 | 0 (0/11) | 60 (6/10) | 14 (1/7) |
| | Jan. 1994 | 1 | 0 (0/1) | 0 (0/1) | ND |
| | TOTAL | 31 | 0 (0/31) | 44 (12/27) | 35 (9/26) |
| Mule deer | Mar. 1991 | 3 | 0 (0/3) | 0 (0/3) | 67 (2/3) |
| | Apr. 1991 | 10 | 0 (0/10) | ND | 80 (8/10) |
| | Jan. 1992 | 3 | 0 (0/3) | ND | 100 (3/3) |
| | June 1992 | 4 | 0 (0/4) | 0 (0/4) | 100 (4/4) |
| | Nov. 1992 | 10 | 0 (0/10) | ND | ND |
| | Jan. 1994 | 8 | 0 (0/8) | 0 (0/8) | ND |
| | TOTAL | 38 | 0 (0/38) | 0 (0/15) | 85 (17/20) |
| Cattle | Nov. 1991 | 21 | 24 (5/21) | 0 (0/21) | ND |
| | May 1992 | 23 | 17 (4/23) | 0 (0/23) | 0 (0/23) |
| | Dec. 1992 | 22 | 41 (9/22) | 0 (0/22) | 0 (0/22) |
| | May 1993 | 19 | 47 (9/19) | 0 (0/19) | ND |
| | Nov. 1993 | 18 | 89 (16/18)[g] | 0 (0/18) | ND |
| | Apr. 1994 | 20 | 75 (15/20) | 0 (0/20) | ND |

[a] BTV = bluetonge virus antibodies determined by competitive enzyme-linked immunosorbent assay.
[b] Psor = *Psoroptes* spp. infestation determined by presence in ear swabs.
[c] Bab = *Babesia* spp. antibodies determined by immunofluorescent antibody test.
[d] Data for *Babesia* spp. in bighorn sheep and mule deer previously published in Kjemtrup et al. (1995).
[e] Prevalence in percent with number positive/number tested in parentheses.
[f] ND = not done.
[g] BTV serotype 13 was isolated from one individual in this group.

most of these animals were adults. Consequently, we feel that the cumulative information collected during this study can be used reliably to make some preliminary conclusions.

Because incident sampling was used in the cattle (that is, a cohort of cattle was sampled repeatedly), we were able to monitor serologic conversion to BTV during the course of the study. The time interval in which seroconversion to BTV occurred was known for nine of the cows. The proportions of cows that seroconverted in the April to December period and the December to April period were compared with a two-tailed z-test of proportions assuming that equal proportions of seroconversions were expected in the two time intervals (Zar, 1984). During the course of this study, significantly ($P < 0.05$) more cattle ($n = 8$) seroconverted during the April to December period than during the December to April period ($n = 1$). None of the BTV-seropositive cows converted to a seronegative status during the course of the study. Bluetongue virus (serotype 13) was isolated from the whole blood from one cow in the November 1993 sampling.

Since BTV is an important and relatively common pathogen among all three ungulate species in California (Hoff and Trainer, 1978; Clark et al., 1993; Barratt-Boyes and MacLachlan, 1995), it was surprising that antibodies to BTV were detected in cattle, but not mule deer or bighorn sheep. Based on the seroconversion data, we concluded that at least eight of the cattle were exposed to BTV in the period from April to December. This is the same period

when the cattle shared rangeland with bighorn sheep and mule deer. Bluetongue virus typically can be isolated from infected hosts only during the first few weeks following infection (Leudke et al., 1969, Barratt-Boyes and MacLachlan, 1995). Therefore, isolation of BTV from one cow in November 1993 provides additional evidence for the conclusion that BTV transmission occurred while cattle were in the study area. Clark et al. (1993) reported serologic detection and isolation of BTV from one bighorn sheep in the same population we studied. We reviewed the original data on which the Clark et al. (1993) report was based and discovered that there never was a confirmed isolate of BTV obtained from bighorn sheep in this population. In fact, this animal was seronegative, and the animal's inclusion as a positive animal was based solely on the supposed virus isolation. Therefore, we concluded that there has not been any documented exposure to BTV among bighorn sheep in our study area. Bluetongue virus transmission occurred only among the cattle in the study area, even though they shared rangeland with bighorn sheep and mule deer.

There are several possible explanations for the lack of seropositive bighorn sheep and mule deer in this study, aside from the possibility that our sample sizes were too small to detect a low seroprevalence. One possible explanation is that all exposed bighorn and mule deer died of bluetongue disease following infection. This situation is extremely unlikely for two reasons. First, we were not able to locate a case in which an observed hemorrhagic disease epizootic in a wildlife population resulted in a 100% mortality rate (Jessup, 1985b; Barratt-Boyes and MacLachlan, 1995). Some seropositive individuals survive, and antibodies persist in these animals for an extended period of time. Second, all the bighorn and deer in this study were radiocollared and tracked, so it was known that none of the sampled animals died during the study. Thus, even if all of the BTV-infected bighorn sheep and mule deer in this area did die post infection, then under the logic of 100% mortality, every animal we sampled could be considered to have remained unexposed for the duration of the study. Given the active infection of the cattle throughout the study, the high seroprevalence within the cattle herd by the conclusion of the study, the fact that antibodies to bluetongue are extremely long-lived (greater than a year), the fact that we sampled between 20 and 30% of the wildlife populations, and the fact that most of the animals sampled were adults, the probability would be low that the true prevalences in the bighorn and deer populations differed from zero. Other possible explanations for the difference in bluetongue virus exposure among the cattle and the wildlife species include a host specificity of the vectors of bluetongue virus (*Culicoides* spp.), or a spatial or temporal separation of the host species such that the bighorn and deer were not bitten by infected vectors.

Psoroptic scabies is an important disease of bighorn sheep and cattle in the western United States. *Psoroptes* spp. have been found on mule deer adjacent to a bighorn sheep population in New Mexico, and the infested bighorn sheep population was decimated by the mite (National Research Council, 1979; Boyce and Brown, 1991). Mite infestations were previously found to be common among bighorn sheep populations in California, including the San Bernadino population that was the subject of this study (Mazet et al., 1992). *Psoroptes ovis* infestations on cattle have resulted in generalized loss of fitness and productivity (Cole et al., 1984), and consequently, these *Psoroptes* spp. infestations on cattle are reportable to the California Department of Food and Agriculture. (CDFA). We anticipated that mites might be found on mule deer, cattle, and bighorn sheep. However, no mites were found on deep ear swabs from mule deer or cattle, and no lesions were seen on either of these species that were suggestive of prior infestation. The treatment with ivermectin should not have

interfered with our ability to detect infestations with *Psoroptes* spp. The residual activity of injectable ivermectin is relatively short (Meleney et al., 1982), and consequently, after several weeks, these cattle would again be susceptible to infestation. Any mites that the cattle acquired since their last ivermectin treatment would then be identified at the time of resampling. Therefore, we concluded that cross transmission of mites from bighorn sheep to mule deer and cattle did not occur during this study, despite the fact that 44% of the bighorn sheep were infested with mites. Because of the efforts to eradicate *Psoroptes* spp. infestations in cattle by the United States Department of Agriculture and the CDFA (Hourrigan, 1979), the finding of no *Psoroptes* spp. on the cattle is important.

*Babesia* spp. were first isolated from bighorn sheep and mule deer in the San Bernadino Mountains in 1991 by Goff et al. (1993) and Thomford et al. (1993). Kjemtrup et al. (1995) later showed that the seroprevalence of these parasites ranged from 40% to 60% in these two host populations. We initiated our comparative study to address the question of whether cattle were also being exposed to *Babesia* spp. Based on our data, we concluded cattle were not exposed to *Babesia* spp., and that infections were limited to bighorn sheep and mule deer. Because the IIF used in this study is potentially cross-reactive with other organisms, such as other *Babesia* spp. and potentially other protozoa such as *Theileria* spp., positive results on this assay need to be interpreted with caution. However, isolates of *Babesia* spp. were obtained from the bighorn sheep and mule deer in this study area, and the isolates were well-characterized (Goff et al., 1993; Kjemtup et al., 1995). Because the cattle were negative on the IIF, we were confident that the cattle were not exposed to the isolated *Babesia* spp., nor to any of the organisms to which the IIF could be cross-reactive. We suspect that the tick, *Ixodes pacificus,* may be the vector of *Babesia* spp. in this area since this tick species was found on bighorn sheep and mule deer, but not cattle, during the study. However, we cannot conclude that *I. pacificus* was absent from the cattle since we were unable to perform systematic whole-body examinations of each cow for ticks. The use of ivermectin was unlikely to influence our ability to detect *Ixodes* spp. infestations for the same reasons described for *Psoroptes* spp.

This study provided strong circumstantial evidence that exposure patterns to BTV, *Psoroptes* spp., and *Babesia* spp. differed among sympatric cattle, mule deer, and bighorn sheep in the San Bernardino Mountains. Despite the limitations of our study, it was clear that cattle in our study site did not share similar patterns of exposure to BTV, *Babesia* spp. or *Psoroptes* spp. with the wild ungulate populations. We encourage other investigators to examine pathogen sharing among other sympatric populations of cattle, bighorn sheep, and mule deer to see if our findings are substantiated in different areas.

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