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Brucellosis in Captive Rocky Mountain Bighorn Sheep (*Ovis canadensis*) Caused by *Brucella abortus* Biovar 4

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ABSTRACT: Nine (four female, five male) captive adult Rocky Mountain bighorn sheep (*Ovis canadensis*) contracted brucellosis caused by *Brucella abortus* biovar 4 as a result of natural exposure to an aborted elk (*Cervus elaphus*) fetus. Clinical signs of infection were orchitis and epididymitis in males and lymphadenitis and placentitis with abortion in females. Gross pathologic findings included enlargement of the testes or epididymides, or both, and yellow caseous abscesses and pyogranulomas of the same. *Brucella abortus* biovar 4 was cultured in all bighorn sheep from a variety of tissues, including testes/epididymides, mammary gland, and lymph nodes. All bighorn sheep tested were positive on a variety of standard *Brucella* serologic tests. This is the first report of brucellosis caused by *B. abortus* in Rocky Mountain bighorn sheep. It also provides evidence that bighorn sheep develop many of the manifestations ascribed to this disease and that infection can occur from natural exposure to an aborted fetus from another species. Wildlife managers responsible for bighorn sheep populations sympatric with *Brucella*-infected elk or bison (*Bison bison*) should be cognizant of the possibility of this disease in bighorn sheep.

Key words: Brucella abortus, Cervus elaphus, elk, *Ovis canadensis,* Rocky Mountain bighorn sheep.

Brucellosis is a contagious bacterial disease that causes abortion in animals and undulant fever in humans. Several species of the genus *Brucella* can infect wildlife, including *Brucella abortus, Brucella suis, Brucella canis, Brucella melitensis, Brucella neotomae,* and *Brucella ovis* (Hunter and Kreeger, 1998). There are few reports, however, of North American wild sheep having exposure to or disease caused by these *Brucella* species. No serologic reactors for *B. abortus* were found in 43 Rocky Mountain bighorn sheep (*Ovis canadensis*) from Arizona (USA; Davis, 1990) or in nine bighorn sheep from Alberta (Canada; Zarnke and Yuill, 1981). Three (0.4%) of 783 desert bighorn sheep (*O. canadensis nelsoni*) from California (USA) were considered suspect reactors to *Brucella* serologic tests (Drew et al., 1992), and three (4%) of 73 Dall's sheep (*Ovis dalli dalli*) in Alaska (USA) were found to be serologically positive to a *Brucella* (Foreyt et al., 1983). No reports could be found of culture-positive *Brucella* infections or of evidence of clinical disease in any North American wild sheep.

Nonetheless, brucellosis exists in other wildlife occupying bighorn sheep habitat. Rocky Mountain elk (*Cervus elaphus*) and bison (*Bison bison*) have been infected with *B. abortus* for more than a half century in the greater Yellowstone area (GYA), an area encompassing Yellowstone and Grand Teton National Parks and surrounding areas of Wyoming, Montana, and Idaho (USA; Cheville et al., 1998). Caribou (*Rangifer tarandus*) in Alaska are infected with *B. suis* (Dieterich, 1998). If wild bighorn sheep can contract brucellosis from these other species, it must be a rare event or, alternatively, bighorn sheep might be like moose (*Alces alces*; Hunter and Kreeger, 1998) in that they are extremely susceptible to brucellosis and few survive to provide serologic evidence of exposure. Herein, we report on brucellosis in captive Rocky Mountain bighorn sheep resulting from exposure to naturally infected captive elk.

This report describes events occurring at the Sybille Wildlife Research and Conservation Education Unit (Sybille) of the Wyoming Game and Fish Department (WGFD, Wheatland, Wyoming, 41°45.778′N, 105°22.605′W). The WGFD conducts research on a variety of wildlife diseases, including brucellosis in elk,

chronic wasting disease in cervids, and *Pasteurella* pneumonia in bighorn sheep. The bighorn sheep in this report were progeny of wild bighorn sheep caught in the 1970s and 1980s from northwestern Wyoming and transported to Sybille. At Sybille, the bighorn sheep lived in a natural 97-ha enclosure containing meadows, creek, and steep rocky bluffs and hillsides. In winter, bighorn sheep were fed alfalfa hay supplemented with a high-energy pelleted supplement. This feed was provided adjacent to corrals containing research elk.

In January 1998, approximately 80 female elk calves were captured in corral traps at the National Elk Refuge (Jackson, Wyoming). These calves were transported to Sybille for brucellosis vaccine research. There, elk were housed in 0.4-ha corrals and fed alfalfa hay supplemented with a pelleted ration. Water and a trace mineral block were provided ad libitum. Elk were blood tested monthly for *Brucella* antibodies with the use of buffered acidified plate antigen, card, standard plate, complement fixation, and rivanol serologic tests (MacMillan, 1990). Any elk having a positive reaction to two or more of these tests was considered a reactor and euthanized.

In March 1998, elk were randomly divided into several groups to test the efficacy of strain RB51 (RB51) brucellosis vaccine (Kreeger et al., 2002b) and to test serologic responses to biobullet-administered strain 19 (S19) vaccine (Herriges et al., 1989). Groups were vaccinated in March 1998 and blood tested monthly through February 2000 for *Brucella* antibodies.

In September 1999, a bull elk was placed with each group for breeding. Subsequently, elk were examined for pregnancy by pregnancy-specific protein B assay (Huang et al., 2000). In March 2000, a cow in an S19 group aborted prior to any challenge or other testing. The aborted fetus was collected and cultured as described previously (Kreeger et al., 2002b). With the use of techniques outlined by Alton et al. (1988), the isolate cultured from the aborted fetus was identified as *B. abortus* biovar 4.

The elk that aborted was housed in a holding corral adjacent to where the bighorn sheep were being fed for the winter. This elk was serologically negative for *Brucella* from time of capture until she was vaccinated with S19 in March 1998. She was serologically positive as a result of the vaccination until February 1999, when she tested negative and remained so through February 2000. Subsequent to the abortion and in preparation for scheduled vaccine trials, the elk in this group were moved to another corral for isolation and observation.

Feeding of the bighorn sheep ceased in spring but started again the following winter. During this interval, they lived in a semiwild state in the 97-ha enclosure, often going unobserved for a wk or more. In December 2001 at the winter feeding station, a mature ram was observed with a swollen testis. The ram was anesthetized with carfentanil and xylazine (Kreeger et al., 2002a) and the testis examined. The ram recovered uneventfully after antagonism of the carfentanil and xylazine but was found dead 3 hr later. The testes were removed and submitted for examination. On gross examination, the right testis was greatly enlarged and firm. It contained multifocal yellow caseous abscesses of the epididymis and diffuse thickening and adhesions of the vaginal tunics of the testis. The center of the testis contained a granuloma (Fig. 1). The left testis was of normal size and texture, but contained a small yellow caseous abscess of the epididymis. Bacteriologic culture of both testes identified *B. abortus* biovar 4. Portions of both testes and epididymides were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Microscopic examination of the testes and epididymides revealed large multifocal to coalescing pyogranulomas effacing normal tubular architecture. The pyogranulomas were composed of abundant degenerate

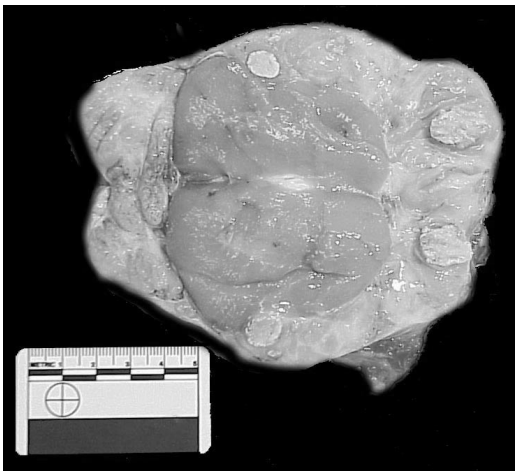

FIGURE 1. Testis from a male Rocky Mountain bighorn sheep infected with *Brucella abortus* biovar 4 containing multifocal pyogranulomas and caseous abscesses of the epididymis.

neutrophils and epithelioid macrophages surrounding extensive areas of necrosis with lakes of partially mineralized cellular debris.

These findings prompted an investigation of the serologic status of all bighorn sheep. The bighorn sheep were immobilized as described above when they came into feed and blood tested for *Brucella.* Testing of several bighorn sheep was delayed because they either did not come into feed or ran away as soon as they sighted WGFD personnel. In January 2002, an adult ewe tested positive for *Brucella* on five serologic tests. She was euthanized and submitted for necropsy. There were no significant gross lesions; the ewe was pregnant with a single male fetus. Routine tissues were collected, fixed in 10% neutral buffered formalin, and processed for microscopic examination as described above. Significant lesions were confined to the placenta and several lymph nodes draining the reproductive tract (pelvic and inguinal nodes). In the placenta, multifocal placental necrosis was observed in cotyledons associated with light infiltrates of neutrophils and macrophages. Affected lymph nodes contained multifocal small nodular aggregates of neutrophils, epithelioid macrophages, and occasional multinucleate giant cells in subcapsular and medullary sinuses. The uterus, ovary, placenta, mammary gland, and supramammary lymph nodes were culture positive for *B. abortus* biovar 4.

In February 2002, another mature ram was found dead in the enclosure. The testes were of normal size, but the epididymides of both were swollen, firm, and contained yellow caseous abscesses. No cause of death was determined at necropsy, but gross and microscopic lesions in the testes were similar to those described above for the first ram affected, and both testes and epididymides were culture positive for *B. abortus* biovar 4. In March, many of the bighorn sheep were lured into a holding area where they could be immobilized, blood tested, and held until serology results were known. Any bighorn sheep testing positive for *Brucella* on any serologic test was euthanized and necropsied (Table 1). In April, a ewe was seen aborting a fetus in this holding area. The ewe was euthanized and she and her fetus were submitted for examination. Grossly, the ewe and fetus were in excellent body condition. Her uterus and cervix were hyperemic and edematous, but there were no gross lesions suggestive of disease in either the ewe or fetus. Several tissues (uterus, ovary, cervix, placenta, mammary gland, mammary/iliac/retropharyngeal lymph nodes) from the ewe and her fetus (abomasum, amniotic fluid) were culture positive for *B. abortus* biovar 4. Maternal and fetal tissues were processed and examined by microscopic examination as described above, and lesions in this ewe were similar to those in the first female examined, including multifocal necrosis of cotyledonary placenta with associated mild pyogranulomatous inflammation and multifocal mild pyogranulomatous lymphadenitis of nodes draining the reproductive tract. No significant gross or microscopic lesions were observed in the fetus.

This is the first report of brucellosis caused by *B. abortus* in Rocky Mountain

TABLE 1. Serologic history and tissue culture results for captive bighorn sheep.

Sex	Date submitted	Serology history[a]	Tissue culture[b]	Comments
M	12/01	None	Testes	Index case
F	1/02	+ (1/2)	Mammary gland, placenta	Euthanized
M	2/02	None	Testes	Found dead
F	3/02	+ (3/02)	Mammary gland	Euthanized
F	4/02	− (1/02); − (3/02); + (4/02)	Several, including fetus	Aborted, euthanized
F	6/02	+ (3/02)	Reproductive lymph nodes	Euthanized
M	6/02	+ (4/02)	Testes	Euthanized
M	6/02	− (3/02); + (5/02)	Reproductive lymph nodes	Euthanized
M	6/02	+ (5/02)	Epididymis	Euthanized

[a] Results of serologic tests indicating month and year when animal tested negative (−) or positive (+).
[b] Tissues from which *Brucella abortus* biovar 4 was cultured.

bighorn sheep. It also provides evidence that bighorn sheep develop many of the manifestations ascribed to this disease, including abortion and orchitis and epididymitis, and that infection can occur from natural exposure to an aborted fetus from another species. All the isolates from bighorn sheep were *B. abortus* biovar 4, which occurs in infected elk and bison in the GYA (Edwards, unpubl. data). This biovar was not used in any research at any time, so the elk that aborted must have been infected prior to capture. The inability of several serologic tests to detect latent *Brucella* infection is a well-known phenomenon (Lapraik and Moffat, 1982).

It is speculative how the bighorn sheep were exposed to the aborted elk fetus, other than that the elk fetus was found next to the fence line adjacent to where the bighorn sheep were being fed. It is possible that one or more bighorn sheep came in contact with either the fetus or infected birth fluids from the dam and subsequently spread the disease throughout the flock. Another possibility was that the disease was spread by birds (Angus et al., 1971), particularly black-billed magpies (*Pica hudsonia*) that could have scavenged the fetus as well as frequented the food bunks of the bighorn sheep. Although evidence of brucellosis in bighorn sheep was not observed until spring of 2001, bighorn sheep might have developed clinical disease soon after exposure in 2000. Bighorn sheep could have aborted in spring 2000, but this was not observed because they lambed on the hillsides in relative seclusion. The disease could have spread during this period because other bighorn sheep were exposed to one or more aborted lambs. It wasn't until the following year when the bighorn sheep were brought in for winter feeding that the ram with orchitis was observed.

Exposure to *Brucella* by bighorn sheep inhabiting the GYA has not been documented (Cheville et al., 1998), and it remains likely that such exposure is a rare event. Likewise, *B. abortus* infection in domestic sheep is uncommon, but seropositivity (Luchsinger and Anderson, 1979), infection (Shaw, 1976), abortion (Allsup, 1969), and isolation of the bacterium (Stoenner, 1951) have been reported. It was of interest and concern, however, that two rams in good flesh died inexplicably with no lesions other than those referable to *Brucella* infection. Such observations lend support to the hypothesis that serologic evidence of brucellosis in bighorn sheep is lacking because they might not survive the disease. Nonetheless, thousands of infected elk and bison share habitat with wild bighorn sheep, and the observations reported herein should raise concern that brucellosis in elk and bison could pose a heretofore unknown risk to bighorn sheep. If true, these observations add support to the state and federal mul-

tiagency effort to eradicate brucellosis from the GYA (Cheville et al., 1998).

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