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Authors: Williams, E. S., Thorne, E. T., Anderson, S. L., and Herriges, J. D.

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Brucellosis in Free-ranging Bison (*Bison bison*) from Teton County, Wyoming

E. S. Williams,[1] E. T. Thorne,[2] S. L. Anderson,[2] and J. D. Herriges, Jr.,[2] [1] Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070, USA; [2] Wyoming Game and Fish Department, Research Laboratory, Box 3312, University Station, Laramie, Wyoming 82071, USA

ABSTRACT: Brucellosis was studied opportunistically in bison (*Bison bison*) in the free-ranging Jackson herd of approximately 120 in Teton County, Wyoming (USA) in March 1989. Recent abortion was diagnosed in a 2-yr-old cow and *Brucella abortus* biovar 1 was isolated from vaginal discharge, uterine contents, uterus, and supramammary lymph nodes. Endometritis was characterized by lymphoplasmacytic infiltrates in the lamina propria and neutrophils in uterine glands and within necrotic debris and exudate in the uterine lumen. A 5-yr-old bull had diffuse lymphoplasmacytic infiltrates in epididymis and accessory sex glands; *B. abortus* was isolated from seminal vesicle and ampulla. Twenty-seven (77%) of 35 bison tested from 1989 to 1990 were serologically positive or suspect on tests for *Brucella* antibodies. We report the occurrence of abortion due to brucellosis in free-ranging bison in the Jackson herd, suggest that bison in this herd are capable of transmitting brucellosis to other susceptible hosts, and report the first confirmation of brucellosis in this herd.

Key words: Brucellosis, *Brucella abortus* biovar 1, bison, *Bison bison*, Wyoming, serosurvey.

Brucellosis long has been recognized in bison (*Bison bison*) and elk (*Cervus elaphus nelsoni*) in Yellowstone National Park (USA) (Mohler, 1917; Rush, 1932; Tunnicliff and Marsh, 1935; Thorne et al., 1991b) and in elk maintained by winter feeding in northwestern Wyoming (USA), including the National Elk Refuge (Murie, 1951; Thorne et al., 1978). These free-ranging species maintain brucellosis without reinfection from domestic animal sources by intraspecific and, perhaps, interspecific transmission. Wyoming, Montana (USA), and Idaho (USA) are classified as brucellosis-free by the U.S. Department of Agriculture Brucellosis Eradication Program. Captive bison have been shown to experience a high frequency of abortion following experimental infection with *B. abortus* (Davis et al., 1990). Abortions due to brucellosis have been suspected in free-ranging herds based on serologically positive animals and the occurrence of abortions (Rush, 1932; Tunnicliff and Marsh, 1935; Corner and Connell, 1958), but *B. abortus*-induced abortion has not been documented by culture and pathology in free-ranging bison.

The free-ranging bison herd which migrates between Grand Teton National Park and the National Elk Refuge, Teton County, Wyoming (43°30'N, 110°45'W) was started in 1968 when 15 animals escaped from an enclosure in the park where they had been confined for exhibit (National Park Service, 1985). All but three were recaptured or died, but the herd was subsequently permitted to range freely in 1969 and 1970. In 1967, one animal in the herd tested positive for antibodies to *Brucella*, and was removed from the herd; all calves were vaccinated. The herd was considered negative for brucellosis on tests conducted in 1968. The free-ranging herd of approximately 15 bison began wintering on the National Elk Refuge in December 1975 and has since been fed along with approximately 8,500 elk. The population grew to about 120 bison by fall 1988.

Eight female and eight male bison, representing approximately 13% of the herd, were shot during a herd reduction program on the National Elk Refuge on 28 and 29 March 1989. Carcasses were examined for gross lesions and sections of most organs were preserved in 10% buffered formalin, embedded in paraffin and stained with hematoxylin and eosin. Ten lymph node, uterus, placenta, cervix, amnionic fluid, mammary gland, vaginal discharge, testicle, epididymis, seminal vesi-

cle, ampulla, and prostate samples, as well as samples from gross lesions, were collected aseptically, placed in sterile plastic bags, and frozen at −70 C for ≤2 months. Tissues were cultured for *B. abortus* (Thorne et al., 1978), and representative isolates were sent to the National Veterinary Services Laboratory (Ames, Iowa, USA) for confirmation. Blood samples were collected from veins severed during necropsy. Sera were collected within 48 hr and frozen at −20 C until tested by the standard card test, rivanol test, standard plate test (United States Department of Agriculture, undated) and complement fixation test (Thorne et al., 1978). Criteria for serologic reactors were as defined for bison and cattle (United States Department of Agriculture, 1986). Sera also were tested for antibodies against bovine virus diarrhea (BVD) virus, infectious bovine rhinotracheitis (IBR) virus, parainfluenza 3 (PI3) virus, and bovine respiratory syncytial virus (BRSV) using the virus neutralization technique of Carbrey et al. (1971) with the following modifications. Stock virus cultures for BRSV were harvested using sonication. Diluent for the stock viruses contained penicillin and streptomycin. Ninety-six well, sterile, flat bottom microtiter plates with lids (Corning Glass Works, Corning, New York, USA) were used for stock virus titration and serum neutralization tests. Inoculated plates were incubated at 37 C in 5% CO_2 for 66 to 72 hr. The virus suspensions used for virus neutralization tests were made in minimum essential medium and contained 50 to 175 50% tissue culture infectious doses per 0.05 ml. Test sera were diluted twofold rather than fourfold in minimum essential medium. Serum-virus dilutions were incubated for 60 min followed by addition of MDBK cells and incubation as described above. Antibody titers of ≥1:4 for IBR and ≥1:16 for BVD, PI3 and BRSV were considered positive for exposure. Sera also were tested by microscopic agglutination for antibodies against *Leptospira interogans* serovars *hardjo, pomona, canicola, icterohemorrhagiae*, and *grippotyphosa* (National Veterinary Services Laboratory, 1987); agglutination at a dilution of ≥1:100 was considered positive. Animals were aged by tooth replacement (Larson and Taber, 1980) and modified cementum annuli techniques (Low and Cowan, 1963; Novakowski, 1965; Monk and Bozell, 1980).

All animals were in excellent body condition. A yearling and two 2-yr-old cows were not pregnant. One 2-yr-old cow (Number 12, Table 1) had a purulent vaginal discharge and the uterus was dilated with purulent exudate, necrotic placenta, and unidentifiable necrotic debris. One ovary contained a large corpus luteum. Microscopically, the uterine mucosa was characterized by focal loss of superficial epithelium, lymphoplasmacytic infiltrates in the lamina propria, and scattered aggregates of neutrophils within glands and occasionally in the endometrium. The lumen was filled with neutrophils and necrotic debris. Lymphocytes were numerous in the fibromuscular tissue of the cervix. Cells in the corpus luteum were degenerating. A diagnosis of recent abortion with retained placenta and endometritis was made. No gross or microscopic lesions suggestive of brucellosis were observed in the other cows (Table 1).

Diffuse interstitial lymphoplasmacytic infiltrates were present in epididymis, seminal vesicle, and ampulla of a 5-yr-old bull and neutrophils occasionally were present in interstitial tissues and were migrating through tubular and glandular epithelium. Two other bulls had mild seminal vesiculitis and one bull had fibrous adhesions of the testicular capsule to the tunica vaginalis (Table 1).

Brucella abortus biovar 1 was isolated from vaginal discharge, uterine contents, uterus, and supramammary lymph node from the affected cow and from the seminal vesicle and ampulla of the 5-yr-old bull. Internal iliac lymph nodes of two other bison were culture positive for *B. abortus* (Table 1). Most cases of brucellosis in the United States are caused by *B. abortus*

TABLE 1. Bacteriologic and serologic results of tests for *Brucella abortus* and lesions in 16 bison from the Jackson bison herd, Wyoming, 1989.

Bison number	Sex	Age (years)	Serologic results[a]				Serologic diagnosis	Culture results[b]	Lesions
			SPT	Card	Riv	CFT			
1	M	5	I50[c]	+[d]	I50[c]	T10[e]	Reactor	0/10	NSL[f]
2	F	9	+25	−	N25	N10	Negative	0/17	NSL
3	M	4	I50	+	+50	84	Reactor	0/15	Mild seminal vesiculitis
4	F	4	I25	−	N25	N10	Negative	0/10	NSL
5	M	5	N25	+	I50	164	Reactor	0/14	NSL
6	F	3	N25	−	N25	N10	Negative	0/19	NSL
7	M	9	N25	−	N25	N10	Negative	0/17	Fibrous adhesions on testicle
8	F	4	N25	+	+25	44	Reactor	0/20	NSL
9	M	4	+100	+	+50	84	Reactor	0/14	NSL
10	F	2	I25	−	N25	N10	Negative	0/17	NSL
11	M	5	I25	±	I100	164	Reactor	0/15	NSL
12	F	2	+100	−	I200	644	Reactor	5/17	Recent abortion, endometritis, cervicitis
13	M	5	I200	+	I100	84	Reactor	0/17	Mild seminal vesiculitis
14	F	1	N25	+	I200	324	Reactor	1/16	NSL
15	M	5	+200	+	+200	644	Reactor	2/19	Epididymitis, seminal vesiculitis, ampullitis
16	F	>3	+50	+	+200	324	Reactor	2/19	NSL

[a] SPT = standard plate agglutination test; Card = standard card test; Riv = rivanol test; CFT = complement fixation test.
[b] Number of tissues positive for *B. abortus*/number of tissues cultured.
[c] I50 = incomplete agglutination at a serum dilution of 1:50; +25 = positive agglutination at a serum dilution of 1:25; N25 = no agglutination at a serum dilution of 1:25.
[d] + = card test positive; − = card test negative.
[e] T10 = trace of complement fixation at a serum dilution of 1:10; N10 = negative complement fixation at a dilution of 1:10; 84 = complement fixation test reaction of 4+ at a serum dilution of 1:80.
[f] NSL = no significant lesions.

biovar 1 (Timoney et al., 1988) and this is the biovar present in elk from the National Elk Refuge (Thorne et al., 1978).

The cow that had recently aborted was seronegative for IBR, BRSV, and leptospirosis, but had antibodies to BVD (1:32) and PI3 (1:4096). All 16 bison tested were seronegative for IBR and leptospirosis; seven of 16 (44%) had antibodies against BRSV (geometric mean titer [GMT] = 39; range 1:16 to 1:128); 12 of 16 (75%) had antibodies against BVD (GMT = 609; range 1:32 to >1:8192); and 16 of 16 (100%) had antibodies against PI3 (GMT = 2435; range 1:512–1:8192).

We believe the affected cow had aborted because no newborn calf was observed with the cow or the herd at the time and March is earlier than the normal calving season for free-ranging bison in this region (Meagher, 1973). Because the cow was 2 yr old, this would have been her first pregnancy following infection with *B. abortus* which is when abortions normally occur in domestic cattle (Timoney et al., 1988). Isolation of *B. abortus* from the placenta and uterine fluids is considered presumptive evidence of brucellosis as the cause of abortion (Nicoletti, 1990). And the serologic results and lesions were not suggestive of abortion due to other infectious agents (Kirkbride, 1990) or of a normal birth.

Abortion due to brucellosis in free-rang-

ing bison is not unexpected, based on reported high abortion rates in experimentally infected captive bison (Davis et al., 1990). The cow bison was shedding *B. abortus* in vaginal exudates at the time of necropsy. These fluids could serve as a source of the pathogen for susceptible animals coming in contact with these exudates.

Eleven (69%) of the 16 bison examined in March 1989 had antibodies against *Brucella*. Antibodies detected by the rivanol and complement fixation tests were highest in animals from which *B. abortus* was isolated (Table 1). Results from these bacteriologically positive animals were variable on the card and standard plate tests. Four of 11 (36%) serologic reactors were culture positive. Tessaro et al. (1990) reported isolation of *B. abortus* from 11 of 18 (61%) serologically positive bison from Wood Buffalo National Park, Canada. They also found high antibody titers in bison that were culture positive.

Between November 1989 and October 1990 an additional 19 (11 male, 8 female) bison from this herd were shot by hunters or agency personnel; blood samples, but not tissues, were collected. Fourteen (74%) tested positive and two (11%) were classified as suspects for *Brucella* antibodies. After combining these serologic results with the 16 animals tested in 1988, 28 (76%) of 35 bison from this herd were serologically positive or suspect; a 95% confidence interval for seroprevalence ranged from 64 to 88%.

This study is the first to document abortion due to brucellosis in a free-ranging bison and to establish the presence of the disease in the Jackson bison herd; however, the disease was assumed to be present because of association with known infected elk (Thorne et al., 1991a). The source of *B. abortus* in this herd was not determined. There was evidence for a high prevalence of exposure in the bison. Elk also are known to be infected and to abort due to brucellosis on the National Elk Refuge (Thorne et al., 1978) and both intraspecific and interspecific transmission of brucellosis likely occurs. The presence of brucellosis in large populations of free-ranging ruminants in northwestern Wyoming is a major state and federal management problem (Thorne et al., 1991a, b). The abortions in bison due to *B. abortus* suggest to us that under appropriate circumstances transmission from members of the Jackson bison herd to domestic cattle might occur.

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