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THE PATHOGENICITY OF *BRUCELLA SUIS* BIOVAR 4 FOR BISON

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ABSTRACT: The pathogenicity of *Brucella suis* biovar 4 for bison (*Bison bison*) was evaluated by inoculation of 2.1×10^7 colony forming units (CFU) in 0.1 ml saline into the conjunctival sac of six pregnant cows. Six pregnant bison were inoculated with 1.27×10^7 CFU of *Brucella abortus* strain 2308 as a positive control. Bison were inoculated on 23 January 1992, and observed until calving or abortion after which they were euthanized, and necropsied. Bacteriological and histological examinations were conducted on lymph nodes, reproductive tract, mammary gland, and internal organs. Terminal serum samples from calves and cows were evaluated by card, rivanol precipitation, standard tube agglutination, cold complement fixation tube, indirect bison conjugated enzyme linked immunosorbent assay (ELISA), competitive ELISA, and particle-concentration fluorescence immunoassay. No clinical signs of brucellosis were seen in bison inoculated with *B. suis* biovar 4, and infection was found only in lymph nodes of two animals. There was no evidence of metastasis of this organism to the mammary gland or the reproductive tract. There were no detectable levels of antibodies to *Brucella* spp. in terminal blood samples taken from *B. suis* biovar 4-challenged bison. *Brucella abortus* was isolated from several tissues in all control bison. All *B. abortus*-challenged animals developed uterine infection and five developed mammary gland infection. Reproductive disease resulted in abortions in five *B. abortus*-challenged bison and neonatal death in the remaining calf. *Brucella suis* biovar 4 does not appear to be pathogenic for bison.

Key words: *Brucella suis* biovar 4, brucellosis, bison, *Bison bison*, pathogenesis.

INTRODUCTION

Brucellosis in reindeer and caribou (*Rangifer* spp.) is caused by *Brucella suis* biovar 4; in these animals, the disease causes late-term abortion in females, and the birth of weak calves that have a poor survival rate (Davidov, 1961). Additional symptoms commonly seen are arthritis and bursitis with associated lameness and testicular infection (Golosov and Zabrodin, 1959). Brucellosis is enzootic in most herds of reindeer and caribou located in Alaskan arctic regions and coastal areas (Meyer, 1966). Prevalences of brucellosis range from less than 1% to 30% or more in some herds (Dieterich, 1981).

Brucella suis biovar 4 has been isolated from a wild muskox (*Ovibos moschatus* moschatus) with bursitis (Gates et al., 1984). One moose (*Alces alces*) experimentally infected developed a generalized infection (Dieterich et al., 1991). *Brucella suis* biovar 4 can be experimentally transmitted to cattle via contact with infected reindeer (Forbes and Tessaro, 1993). Little is known about the pathogenicity of *B. suis* biovar 4 for ruminants other than cattle and reindeer. Interest in intensive game farming of reindeer near Delta Junction, Alaska (USA), has raised concerns about introduction of *B. suis* biovar 4 into a free ranging bison herd present in that area.

Bison (*Bison bison*) are susceptible to infection with *Brucella abortus*, the species of *Brucella* which usually infects cattle (Davis et al., 1990). *Brucella abortus* can cause abortion or the birth of weak calves in bison. The organism has been maintained in herds of bison independent of contact with infected cattle (Tessaro, 1986).

In response to the public concern over the perceived threat of brucellosis to the Delta Junction bison herd in Interior Alaska, we evaluated the pathogenicity of *B. suis* biovar 4 infection in bison. Our objective was to determine whether bison could be infected with *B. suis* 4, and if so,

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if they would be adversely affected or would transmit the bacteria to other bison.

MATERIALS AND METHODS

Twelve second trimester pregnant bison were obtained from a privately owned brucellosis-free herd located near Gillette, Wyoming (USA) (43°40′ to 43°41N, 105°28′ to 105°29′W). All bison were seronegative for brucellosis by card test (Alton et al., 1975) and had not been vaccinated with *B. abortus* strain 19. These bison were shipped via truck to the *Brucella* spp. research facility at the Texas Veterinary Medical Park, Texas A&M University (College Station, Texas, USA). All bison were given hay and water ad libitum and fed a balanced ration at 2 to 3% of body weight (TAMU Mix Number 1; Producer's Cooperative Association, College Station, Texas). Experimental protocols for the studies were approved by an independent campus-wide animal welfare committee.

Six bison were moved into an animal facility that met published Bio Safety Level 3 requirements (Richardson and Barkley, 1988). Bison were housed in individual pens within this facility. These animals were challenged on 23 January 1992 with 2.1×10^7 colony forming units (CFU) *B. suis* biovar 4 in a 0.1 ml physiological saline suspension placed in the conjunctival sac. The *B. suis* biovar 4 culture was originally isolated from the carpus of a reindeer shot near Nome, Alaska in 1977 and had been passed once through lemmings (*Lemmus sibiricus*) to assure virulence. This culture and challenge protocol had been employed successfully in similar challenge experiments in reindeer at the University of Alaska, Fairbanks, Alaska (Dieterich et al., 1981). Two lemmings and a guinea pig (*Cavia porcellus*) were inoculated with the challenge suspension, as an additional virulence check. The remaining six pregnant bison were challenged with 1.27×10^7 CFU of *B. abortus* strain 2308 as positive controls. These positive control bison remained in outdoor biocontainment pens at the *Brucella* spp. research facility.

All bison were observed until the time of abortion or calving, after which they were euthanized with an overdose of 19.5 g pentobarbital sodium and 2.5 g phenytoin sodium (Beuthanasia-D, Shering-Plough Animal Health Corp. Phoenix, Arizona, USA) and necropsied from 21 February to 14 May 1992. Samples taken at the time of necropsy included blood for serology and 49 tissues for bacterial culture. These tissues included: right and left atlantal, axillary, internal iliac, mandibular, parotid, popliteal, prefemoral, prescapular, renal, supramammary, and suprapharangeal lymph nodes; samples of hepatic, mediastinal and mesenteric lymph nodes; tissue samples and milk swabs from each mammary gland quarter; and samples of kidney, liver, lungs, both adrenal glands, spleen, tonsil, both uterine horns, vagina, and cervix. Tissues from *B. suis* biovar 4-challenged bison were frozen at −70 C and later thawed and inoculated onto trypticase soy agar (Baltimore Biological Laboratories, Cockeysville, Maryland, USA) and a more selective media containing per liter: 25g tryptose broth (Difco Laboratories, Detroit, Michigan, USA), 20 g agar (Bacto agar, Difco Laboratories), 0.15 ml Tergitol 7 (Sigma Chemical Co., St. Louis, Missouri, USA), 25 ml Tween 40 (Sigma Chemical Co.), 1.4 mg ethyl violet (Sigma Chemical Co.), 1.44 g sodium lauryl sulfate (Sigma Chemical Co.), 1 vial of CNV (Cholistin sulfate, 7,500 mcg, nystatin, 12,500 units, vancomycin, 3,000 mcg, Difco Laboratories) and 500 mg of cycloheximide (Actidione, Sigma Chemical Co.) Tissues from *B. abortus*-challenged bison were frozen at −70 C and later thawed and plated on Farrell's restrictive media (Farrell, 1974). Fetuses were necropsied within 12 hr of abortion. Healthy calves were bled, euthanized, and necropsied at the time of adult necropsy. Tissues taken for bacterial culture from calves and fetuses included liver, kidney, spleen, lung, abomasal and rectal swabs, and mediastinal lymph nodes. Serum samples were not taken from bison between the time of challenge and necropsy because of the risk of inducing stress-related abortions.

Serum samples from calves and cows were evaluated by card (Alton et al., 1975), rivanol precipitation (Alton et al., 1975), standard tube agglutination (Alton et al., 1975), cold complement fixation tube (Alton et al., 1975), indirect bison conjugated enzyme linked immunosorbent assay (ELISA) (Davis et al., 1990), competitive ELISA (Adams and Mia, 1992), and particle concentration fluorescence immunoassay (PCFIA) (Reynolds, 1987). Minimum criteria for diagnostically positive reactions for bison on these tests are listed in Animal and Plant Health Inspection Service (1992).

RESULTS

Two of the bison infected with *B. suis* biovar 4 aborted, but in neither case was this attributable to brucellosis. One bison aborted 3 wk post-challenge; no pathogens were isolated from the fetus. *Actinomyces pyogenes* was isolated from the fetus and uterus of the second bison that aborted, 2 mo post-challenge. All fetal tissues contained this pathogen. Both bison cows also

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TABLE 1. Pre-inoculation and terminal serology in bison challenged with *Brucella abortus* and *Brucella suis* biovar 4.

	Indirect ELISA[a]		PCFIA[b]		Competitive ELISA[c]	
	B. abortus (n = 6)	*B. suis* 4 (n = 6)	*B. abortus*	*B. suis* 4	*B. abortus*	*B. suis* 4
Pre-inoculation	0.194 ± 0.068[d]	0.280 ± 0.123	0.601 ± 0.000	0.604 ± 0.008	4.58 ± 4.98	7.22 ± 1.76
Terminal	0.975 ± 0.233	0.217 ± 0.167	0.152 ± 0.046	0.601 ± 0.000	71.65 ± 23.02	6.24 ± 2.30
Calf	0.428[e]	0.125 ± 0.111[f]	0.64	0.601 ± 0.000	2.72	4.14 ± 0.98

[a] Positive results are >1.000 optical density (OD) units.
[b] Particle concentration fluorescence immunoassay. Positive results are <0.25 channel counts.
[c] Competitive ELISA. Positive results are >70% inhibition. Bison are considered suspect at >40% inhibition.
[d] Mean ± standard deviation.
[e] n = 1.
[f] n = 4.

were infected with *B. suis* biovar 4, which was isolated from a mandibular lymph node of the first cow and from both mandibular lymph nodes, a parotid lymph node and a popliteal lymph node of the second cow. There was no evidence of *Brucella* spp. infection in the reproductive tracts or mammary glands. The four remaining bison challenged with *B. suis* biovar 4 bore healthy calves at term, approximately 4 mo post-challenge. These four cows and their calves were negative for brucellosis when cultured. No signs of brucellosis were observed in any of the bison challenged with *B. suis* biovar 4.

All six bison challenged with *B. suis* biovar 4 were seronegative by all tests at the time of necropsy, including card, rivanol precipitation, standard tube agglutination and cold complement fixation tests. Results for Indirect ELISA, PCFIA and competitive ELISA are given in Table 1. Similarly, all surviving calves were seronegative by all tests at the time of necropsy (Table 1).

By comparison, five of six bison challenged with *B. abortus* aborted 33 days to 50 days post-challenge, and one bore a weak calf that died shortly after birth. Four of these bison had retained placentas ≥24 hr following parturition. Vaginal swabs taken from each animal shortly after calving or abortion yielded cultures of *B. abortus*. Cultures were also obtained from all eight tissues sampled in the calf and fetuses (Table 2). Cultures were obtained from the uterus of all six cows and from the mammary glands of five of these animals (Table 2). Many tissues in each of the bison challenged contained *B. abortus* (Table 2). All bison infected with *B. abortus* developed antibodies to the disease and were strongly positive on more than one serologic test (Table 1).

DISCUSSION

Based on a sample size of six animals, *B. suis* biovar 4, at a dose of 2×10^7 CFU, was not pathogenic for bison. Although bison may become infected with *B. suis* biovar 4, these preliminary results are evidence that the infection was sub-clinical, with infection localized in regional or peripheral lymph nodes. In the one bison which aborted, lack of any significant pathogens in the fetus and uterus was evidence that this abortion was stress-related. There was no evidence of metastasis of *B. suis* 4 to the mammary gland or the reproductive tract in any of the experimentally infected bison, and therefore, it is unlikely that *B. suis* biovar 4 would be shed by these animals.

The experimental dose of *B. suis* biovar 4 should have been a sufficient challenge dose. A dose of 1.4×10^3 CFU *B. abortus*

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TABLE 2. Tissues from which *Brucella* spp. was isolated in bison challenged with *B. abortus*.

Tissues and organs	Animal					
	808	810	812	816	817	819
Head lymph nodes[a]	3/8	8/8	6/8	5/8	4/8	5/8
Organ lymph nodes[b]	0/6	3/6	3/6	4/6	2/6	0/6
Other peripheral lymph nodes[c]	4/10	9/10	8/10	8/10	4/10	4/10
Supramammary lymph nodes	2/2	2/2	2/2	2/2	1/2	2/2
Mammary gland and swabs[d]	2/8	5/8	2/8	0/8	5/8	1/8
Reproductive organs[e]	1/4	2/4	1/4	1/4	1/4	1/4
Other organs[f]	0/4	3/4	1/4	0/4	0/4	0/4
Tonsil	1/1	1/1	0/1	0/1	1/1	0/1
Adrenal glands	0/2	0/2	0/2	0/2	0/2	0/2
Calf or fetal tissues[g]	4/8	8/8	8/8	2/8	7/8	8/8

[a] Right and left atlantal, mandibular, parotid, and suprapharyngeal lymph nodes.
[b] Bronchial, hepatic, mediastinal, mesenteric, and right and left adrenal lymph nodes.
[c] Right and left axillary, prescapular, prefemoral, popliteal, and internal iliac lymph nodes.
[d] Tissue from each mammary quarter with corresponding swabs.
[e] Right and left uterine horns, cervix, and vagina.
[f] Liver, lung, spleen, and kidney.
[g] Calf (808) or fetal (all others) liver, lung, spleen, kidney, abomasum, abomasal swab, rectal swab, and mediastinal lymph node.

is sufficient to infect a cow (McEwen et al., 1939). Tests of vaccine efficacy other species have used 10^6 or 10^7 CFU of *Brucella* spp. as challenge doses (Plommet, 1990). If bison were susceptible to naturally-occurring *B. suis* biovar 4 infection, the challenge of 10^7 CFU should have been sufficient to produce infection.

If these results of an experimental *B. suis* biovar 4 infection in six bison reflected the susceptibility of the species, and if *B. suis* biovar 4 was not shed by infected animals, then it is possible to extrapolate and state that *B. suis* biovar 4 is unlikely to be maintained in a bison herd independent of another source of infection. This is clearly unlike *B. abortus* infection in bison. *Brucella abortus* causes both reproductive disease and mammary gland infections in bison, and the disease has been maintained in populations of wild bison in North America independent of contact with infected cattle (Tessaro, 1986; Meagher, 1973).

The bison in this study were not tested for *Brucella* spp.-specific antibody production between the time of challenge and the time of necropsy. It is possible that bison challenged with *B. suis* biovar 4 developed temporary antibody responses which were not detected by our experimental protocol. If indeed these were produced, they are probably of little diagnostic significance. Antibodies are usually produced in detectable quantities for several months or more in response to active *Brucella* spp. infections in other species. The lack of detectable levels of *Brucella* spp.-specific antibodies at the time of necropsy in *B. suis* biovar 4 -challenged bison is evidence of reduced immunoreactivity to this organism in this species. Elevated antibody responses in wild bison are unlikely to be detected in yearly samplings, particularly if animals are sampled in the fall at hunting season.

Although the *B. suis* biovar 4 organism did not appear to be pathogenic for bison, we are recommending that precautions be taken when reindeer are shipped from the Seward Peninsula to Alaska's interior. Such precautions include adequate fencing for separation of bison and reindeer and quarantine and testing of shipped reindeer (Stahmann, 1991).

In summary, based on the experimental results from six bison, *B. suis* biovar 4 causes only sub-clinical infections in bison.

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Imported reindeer which may carry brucellosis are not expected to present a brucellosis disease risk to interior bison herds of Alaska.

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