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PATHOLOGY OF BRUCELLOSIS IN BISON FROM YELLOWSTONE NATIONAL PARK

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ABSTRACT: Between February 1995 and June 1999, specimens from seven aborted bison (*Bison bison*) fetuses or stillborn calves and their placentas, two additional placentas, three dead neonates, one 2-wk-old calf, and 35 juvenile and adult female bison from Yellowstone National Park (USA) were submitted for bacteriologic and histopathologic examination. One adult animal with a retained placenta had recently aborted. Serum samples from the 35 juvenile and adult bison were tested for *Brucella* spp. antibodies. Twenty-six bison, including the cow with the retained placenta, were seropositive, one was suspect, and eight were seronegative. *Brucella abortus* biovar 1 was isolated from three aborted fetuses and associated placentas, an additional placenta, the 2-wk-old calf, and 11 of the seropositive female bison including the animal that had recently aborted. *Brucella abortus* biovar 2 was isolated from one additional seropositive adult female bison. *Brucella abortus* was recovered from numerous tissue sites from the aborted fetuses, placentas and 2-wk-old calf. In the juvenile and adult bison, the organism was more frequently isolated from supramammary (83%), retropharyngeal (67%), and iliac (58%) lymph nodes than from other tissues cultured. Cultures from the seronegative and suspect bison were negative for *B. abortus.* Lesions in the *B. abortus*-infected, aborted placentas and fetuses consisted of necropurulent placentitis and mild bronchointerstitial pneumonia. The infected 2-wk-old calf had bronchointerstitial pneumonia, focal splenic infarction, and purulent nephritis. The recently-aborting bison cow had purulent endometritis and necropurulent placentitis. Immunohistochemical staining of tissues from the culture-positive aborted fetuses, placentas, 2-wk-old calf, and recently-aborting cow disclosed large numbers of *B. abortus* in placental trophoblasts and exudate, and fetal and calf lung. A similar study with the same tissue collection and culture protocol was done using six seropositive cattle from a *B. abortus*-infected herd in July and August, 1997. Results of the bison and cattle studies were similar.

Key words: Bison, *Bison bison*, cattle, *Brucella abortus*, brucellosis, abortion, pathology, bacteriology, disease.

INTRODUCTION

Evidence of brucellosis in bison from Yellowstone National Park (YNP) (USA) was first discovered in 1917 when serum agglutination tests were positive for *Brucella* spp. antibodies on specimens from two cows that had recently aborted (Mohler, 1917). Subsequently, the disease was found in bison from the National Bison Range in Moiese (Montana, USA) (Creech, 1930), Elk Island National Park (Alberta, Canada) (Corner and Connell, 1958), Wood Buffalo National Park (Canada) (Choquette et al., 1978), Teton County (Wyoming, USA) (Williams et al., 1993), and private herds in the USA (Flagg, 1983; Rhyan et al., 1997b). Abortions or retained placentas in bison from brucellosis infected herds have been reported (Creech, 1930; Corner and Connell, 1958), and have been observed in experimental infections (Davis et al., 1990, 1991); however, only two naturally-occurring abortions with laboratory confirmation of *B. abortus* as the causative agent have been reported (Williams et al., 1993; Rhyan et al., 1994). Little additional

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information is published on the pathogenesis of *B. abortus* infection in female bison, and some investigators have speculated that the disease in YNP bison is markedly different from that in cattle (Meyer and Meagher, 1995). The objectives of this study were to determine the sites of tissue colonization of *B. abortus* in aborted bison fetuses and in juvenile and adult female bison from YNP, to determine the uterine and fetal pathology associated with brucellosis in YNP bison, and to compare the results with those from cattle. To augment the available cattle information, a limited study was done in six seropositive cows from a chronically infected beef herd. Preliminary results (Rhyan et al., 1997a) age data, and serologic results (Roffe et al., 1999) of the bison study have been reported.

MATERIALS AND METHODS

Between April 1996 and June 1999, specimens were collected from seven aborted fetuses or stillborn calves, three neonates, and one calf estimated to be 2 wk of age. Placentas were collected with five of the fetuses or neonates, and two additional placentas without associated fetuses were collected. Aborted fetuses, calves, and placentas were opportunistically collected inside Yellowstone National Park (YNP) (44°8′N to 45°7′N; 110°0′W to 111°4′W).

Between February 1995 and January 1997, specimens were collected from 35 adult and two juvenile (<2-yr-old) female bison. Thirty-six of the bison were killed after leaving YNP and one animal (No. 105) was killed because it had a retained placenta and was in close proximity to the northern YNP border. The animal had recently aborted as evidenced by the lack of mammary development and the early spring date (10 March 1995). No fetus was found.

Whole or heparinized blood samples collected from the juvenile and adult female bison immediately after death were centrifuged and serum or plasma, respectively, was tested for *Brucella* spp. antibodies using the standard card test. When present, card test-positive animals were selected for necropsy and specimen collection. Serum samples from all animals necropsied were sent to the National Veterinary Services Laboratories (NVSL, Ames, Iowa, USA) for a panel of serologic tests as reported (Roffe et al., 1999).

Tissue specimens for bacteriologic examination were collected from the necropsied animals based on guidelines developed by the Greater Yellowstone Interagency Brucellosis Committee, Boise, Idaho, USA, (a state and Federal interagency committee established to facilitate the development and implementation of brucellosis management plans for elk and bison in the greater Yellowstone area). In brief, specimens from juveniles and adult cows consisted of vaginal, rectal, and uterine swabs; samples of mammary gland, uterus, spleen, blood, synovial fluid, liver, kidney, ileum, and bone marrow; and the following lymph nodes: medial and lateral retropharyngeal, tracheobronchial, mediastinal, hepatic, mesenteric, lumbar, medial and lateral iliac, superficial inguinal (supramammary and scrotal), superficial cervical, prefemoral, popliteal, mandibular, and parotid. For convenience, medial and lateral retropharyngeal lymph nodes and medial and lateral iliac lymph nodes were collectively referred to as retropharyngeal lymph nodes and iliac lymph nodes, respectively. From fetuses, stillborns, neonates, and the 2-wk-old calf, bacteriologic specimens consisted of heart blood, cerebrospinal fluid, lung, rectal swab, spleen, and mandibular, mesenteric, and parotid lymph nodes. In addition, placentas, if present, and abomasal contents were collected from fetuses and stillborn calves. Tissues were frozen and shipped on dry ice to the NVSL where they were processed and cultured using the methods of Alton et al. (1988) with modifications. In brief, whole lymph nodes or samples of organs were individually minced, placed in an equal volume of phosphate buffered saline (PBS), and macerated in a Colworth stomacher (Tekmar Company, Cincinnati, Ohio, USA). Each specimen was then processed in a Tenbroeck tissue grinder (Corning Glass Works, Corning, New York, USA) and the entire suspension was poured in aliquots onto the following media: tryptose agar with 5% bovine serum and antibiotics (TSA), TSA with ethyl violet, Ewalt's medium (Ewalt et al., 1983), and Farrell's medium (Farrell, 1974). Cultures were incubated for 1 wk. *Brucella* spp. isolates were identified to species and biovar using the methods of Alton et al. (1988).

Tissues for histologic examination were collected from the aborted fetuses, placentas, and calves found inside YNP and from 15 of the adult bison cows and their fetuses. Specimens consisted of uterus with placentome, cotyledon and intercotyledonary placenta, and fetal lung, liver, kidney, and spleen. In addition, brains from four aborted fetuses and portions of ovary, mammary gland, liver, kidney, spleen, and supramammary and medial iliac lymph nodes from the cow with the retained placenta were

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collected for histopathology. Specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Selected tissues were also stained using Gram's and Giemsa techniques and an immunohistochemical technique (Rhyan et al., 1997b) that employs a polyconal antibody developed against *B. abortus.*

For comparative purposes, a bacteriologic study was done on a small sample of adult female cattle from a brucellosis infected herd in July and August 1997. Six seropositive, nonvaccinated, 3- to 6-yr-old, beef cattle from a chronically infected herd in Texas, USA, were transported to the NVSL, clinically and serologically monitored for several weeks, then euthanized and necropsied. Serum samples collected immediately before euthanasia were tested at the NVSL using the same tests that were used on the bison sera (Roffe et al., 1999). Specimens for bacteriology were collected, processed, and cultured following the same protocol as was used in the bison study. Histopathology was not done.

RESULTS

Bacteriology

Brucella abortus biovar 1 was isolated from three aborted fetuses, the 2-week-old calf (Table 1), and a placenta expelled from a cow observed without a calf in February 1999. Cultures from fetal and calf tissues and placental specimens generally yielded heavy growth of *B. abortus.*

Two of the 37 juvenile and adult bison sampled were excluded from the study due to severe bacterial contamination of the collected tissues. On serologic tests, 26 of the remaining 35 bison, including the cow with the retained placenta (No. 105), were considered seropositive, one had low antibody titers and was considered a suspect, and eight had negative titers to *B. abortus* (Roffe et al., 1999). *Brucella abortus* biovar 1 was cultured from 11 of the 26 seropositive juvenile and adult bison, and biovar 2 from one animal (Table 2). Cultures from numerous specimens from the recently-aborting cow and one juvenile bison yielded heavy growth of *B. abortus*; cultures from specimens of other animals generally contained few or moderate numbers of *B. abortus* colonies. Brucellae were

TABLE 1. *Brucella abortus* culture results from specimens collected from bison fetuses and a 2-wk-old bison calf from Yellowstone National Park.

	Abomasal fluid	Spleen	Lung	Liver	Rectum	Placenta	Lymph nodes[a]					
							RPH	MD	PAR	MES	TBR	MED
Three late term fetuses[b,c]	3/3	3/3	3/3	1/1	3/3	3/3	3/3	3/3	3/3	3/3	2/2	1/1
2-wk-old calf[d,e]	ND[f]	+	+++	+++	+++	ND	+++	+++	+++	+++	+++	+++

[a] Lymph nodes abbreviated include RPH = retropharyngeal; MD = mandibular; PAR = parotid; MES = mesenteric; TBR = tracheobronchial; MED = mediastinal.
[b] Results recorded: Number animals positive/number animals collected.
[c] *Brucella abortus* also isolated from uterus, kidney, cerebrospinal fluid, hepatic lymph node, conjunctival swabs, and nasal swabs.
[d] Results recorded: + = few colonies, ++ = moderate growth, +++ = heavy growth.
[e] *Brucella abortus* also isolated from synovial fluid and hepatic, iliac, and popliteral lymph nodes.
[f] No data.

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TABLE 2. *Brucella abortus* culture results[a] from specimens[b] collected from 12 culture-positive female bison from Yellowstone National Park and three culture-positive female cattle.

Animal groups	Vagina	Uterus	Plac[d]	Ileum	Rect[e]	SF[f]	MG[g]	Lymph nodes[c]										
								SM	RPH	IL	PF	PAR	MD	SC	TBR	HEP	MES	POP
Pregnant bison (n = 6)	0/5	0/4	0/4	0/3	0/6	0/6	0/6	4/6	3/6	1/6	2/5	2/6	0/5	2/5	0/6	1/6	0/5	0/6
Nonpregnant bison[h] (n = 6)	1/5	1/6	1/1	1/5	1/5	1/5	0/6	6/6	5/6	6/6	2/6	2/6	4/6	1/6	4/6	1/6	1/5	3/6
Pregnant cattle (n = 1)	0/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Nonpregnant cattle (n = 2)	0/1	2/2	0/0	0/2	0/2	0/2	2/2	1/2	2/2	1/2	0/2	0/2	0/2	0/2	1/2	2/2	0/2	0/2

[a] Results expressed: Number animals positive/number animals collected (heavily contaminated cultures excluded).
[b] Specimens that were culture-positive in one or more animals are listed.
[c] Lymph nodes abbreiviated: SM = supramammary; RPH = retropharyngeal; IL = iliac (includes lumbar LNs); PF = prefemoral; PAR = parotid; MD = mandibular; SC = superficial cervical; TBR = tracheobronchial; HEP = hepatic; MES = mesenteric; POP = popliteal.
[d] Placenta.
[e] Rectum.
[f] Synovial fluid.
[g] Mammary gland.
[h] Group includes recently-aborting animal with retained placenta.

not cultured from tissues from the seronegative bison nor from the serologic suspect animal.

Gross observations

One of the *Brucella* spp.-negative aborted fetuses was markedly autolyzed. Gross lesions in a culture-negative neonate were indicative of trauma (fractured ribs with marked thoracic and abdominal hemorrhage). In the 2-wk-old calf, caudal lung lobes were heavy, wet, and dark red. Mediastinal and tracheobronchial lymph nodes were dark red and enlarged. The coronary groove contained numerous petechia and few ecchymoses. The parietal surface of the liver had a 3 cm fracture of the hepatic capsule with adherent clotted blood. Gross lesions were not observed in the remaining fetuses and neonates. Gross lesions were observed in two of the adult bison: the cow with the retained placenta (No. 105), and a 12-yr-old seronegative cow with a 6 cm. liver abscess containing white, viscous, foul-smelling exudate. Cow No. 105 weighed 290 kg and was in poor physical condition with scant subcutaneous adipose tissue and small quantities of perirenal and coronary groove adipose tissue. The uterus was partially involuted and placental membranes were firmly attached to caruncles. There was abundant, yellow, viscous exudate present in the uterine lumen and adherent to the placenta. Similar placentitis was observed grossly in the aborted *Brucella* spp.-positive placentas.

Histopathology

Examination of tissue sections from *Brucella* spp.-positive aborted fetuses and placentas revealed a severe necropurulent placentitis and mild bronchointerstitial pneumonia. Lesions in the placentas were characterized by edema of the chorionic stroma and multifocal necrosis of the allantochorion and placental trophoblasts accompanied by large accumulations of neutrophils and degenerate leukocytes. Severe well-demarcated necrosis of cotyledonary villi was characteristic. Large numbers of tiny Gram-positive coccobacilli were present in some trophoblasts. Immunohistochemical (IHC) staining of placental sections demonstrated numerous labelled bacteria in trophoblasts and adherent exudate (Fig. 1). Lung sections from the three *B. abortus*-positive aborted fetuses had mild hypercellularity of interalveolar septae and occasional large macrophages, amnionic debris, few neutrophils, and occasional or rare binucleated or multinucleated cells in airways. There was particulate IHC labelling of scattered macrophages, amnionic debris, and rare neutrophils in airways. In one fetus, a small irregular area of caseous necrosis in the splenic red pulp contained numerous IHC-positive coccobacilli. Lesions in *Brucella* spp.-negative fetuses and neonates consisted of mild purulent bronchointerstitial pneumonia in one neonate, and mild

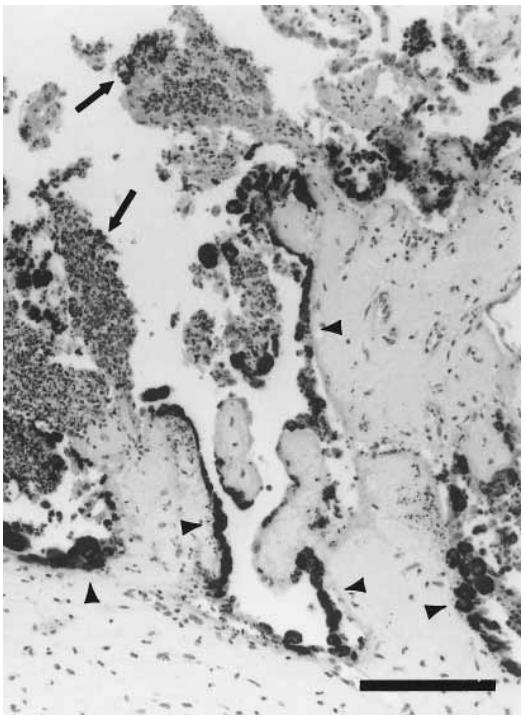

FIGURE 1. Placenta of aborted bison fetus with marked villus necrosis and immunohistochemical stain labeling *Brucella abortus* antigen in trophoblasts (arrowheads) and in exudate (arrows). Bar = 100 μm.

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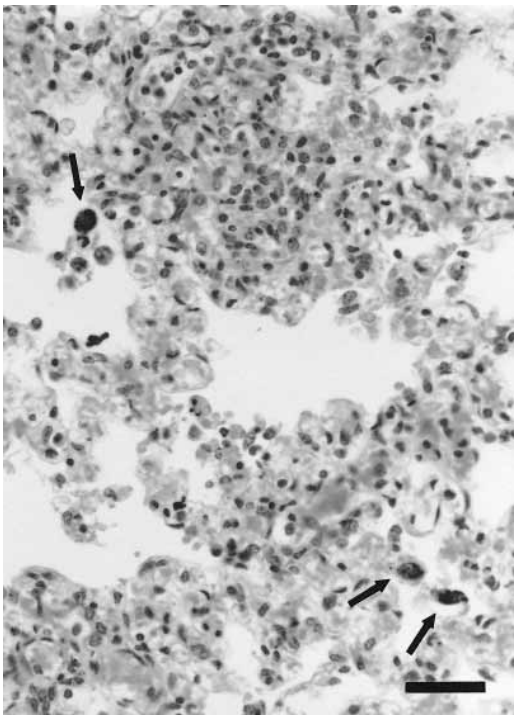

FIGURE 2. Lung from 2-wk-old bison calf with bronchointerstitial pneumonia and immunohistochemical stain labeling *Brucella abortus* antigen in macrophages (arrows). Bar = 50 μm.

pneumonia with purulent placentitis of unknown etiology in one aborted fetus.

Histopathologic lesions in the 2-wk-old calf consisted of bronchointerstitial pneumonia with moderate numbers of macrophages and neutrophils in airways, marked purulent nephritis, focal splenic infarction and moderate lymphocyte depletion of the splenic white pulp and lymph node cortices. Immunohistochemical staining for *B. abortus* resulted in marked particulate labeling in pulmonary macrophages (Fig. 2) and in scattered cells in lymph node follicles.

In the adults, histologic lesions of the uterus were limited to two animals (No. 105 and 130). Examination of uterus and placenta from No. 105 revealed a marked endometritis and placentitis characterized by moderate endometrial infiltrates of lymphocytes, plasma cells, and neutrophils; focal uterine epithelial necrosis; adherence of neutrophils, macrophages, degenerate leukocytes, and fibrin to the endometrium; and marked necrosis of placental trophoblasts with diffuse infiltrates of neutrophils in the chorionic stroma. Special stains and IHC staining of selected specimens for *B. abortus* showed labeling of large numbers of coccobacilli in trophoblasts, in exudate adherent to the placenta and endometrium, and within the placental chorion. Cow No. 130, a seronegative animal, had a mild, diffuse, lymphoplasmacytic infiltrate of the endometrum with several, large, focal, endometrial accumulations of lymphocytes. Special stains for bacteria and IHC staining for *B. abortus* were negative. The cause of the endometritis was undetermined. Lesions were not observed in fetal tissues and other placentas collected from the bison cows. Microscopically, the liver abscess consisted of a necropurulent center containing large numbers of Gram-positive filamentous bacteria surrounded by marked fibrosis and lymphoplasmacytic infiltrates. The lesion was considered unrelated to *B. abortus* infection.

Cattle

Serologic results on sera collected from the six Texas cattle revealed five with high titers to *B. abortus* and one with low to moderate titers. Gross lesions were not observed in the cattle at necropsy. *Brucella abortus* biovar 1 was isolated from tissues from three of the six cattle (Table 2).

DISCUSSION

Lesions and bacteriologic findings in the *Brucella* spp.-positive aborted fetuses and placentas are similar to those in experimental infections of *B. abortus* in bison (Davis et al., 1990) and cattle (Payne, 1959), and are consistent with the laboratory results in the previously reported brucellosis abortion in a YNP bison (Rhyan et al., 1994). The lesions and bacteriologic findings in the 2-wk-old calf indicate multiorgan involvement with likely septicemia. Similar deaths have occurred in young bi-

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son calves following experimental infections. (D. S. Davis, pers. comm.)

In this sample of culture positive, juvenile and adult, female bison from YNP, *B. abortus* was more frequently isolated from supramammary (83%), retropharyngeal (67%), and iliac (58%) lymph nodes than from other tissues cultured. Culture of these sites and the superficial cervical lymph nodes would have detected all culture positive bison in the sample. These findings are consistent with those of Williams et al. (1993) who isolated the organism from vaginal discharge, uterine contents, uterus, and supramammary lymph node from a bison that had recently aborted and from iliac lymph nodes from two other naturally-infected female bison.

The isolation of *B. abortus* biovar 2 from YNP bison is unprecedented; however, Tessaro et al. (1990) reported the isolation of biovar 2 from one of 11 culture positive bison from Wood Buffalo National Park (WBNP), Canada. Both YNP and WBNP received bison from a common source herd in the 1890s and early 1900s, respectively (Meagher and Meyer, 1994). However it is unknown when brucellosis was introduced to the bison in both parks.

The isolation of *B. abortus* from multiple sites from the bison that had recently aborted (No. 105) was similar to the findings of Williams et al. (1993), and to results of experimental infections in bison (Davis et al., 1990) and cattle (Payne, 1959). The presence of large numbers of brucellae in the ileum and rectum probably resulted from ingestion of portions of the infected placenta or licking the aborted fetus. A similar finding in cattle has been reported (Fitch et al., 1932).

In the small cattle study reported here, specimens collected and processed using the bison study protocol yielded positive cultures from three of six seropositive cows. Similar to the bison (Roffe et al., 1999), cattle with the highest titers tended to be positive on culture. There was some variation in tissue colonization sites between the bison and cattle. In the cattle, retropharyngeal and hepatic lymph nodes were culture positive in two of three animals, and supramammary, iliac, and tracheobronchial lymph nodes were positive in one animal. *Brucella abortus* was isolated from the uterus of two nonpregnant cattle and from the mammary gland of all three culture-positive animals. These findings are consistant with previous studies in cattle. McCullough et al, (1951) isolated *B. abortus* from 42 of 100 seropositive naturally-infected cattle. Of the 42 cattle, supramammary lymph nodes were most often culture positive (68%) followed by uterus (38%). Mammary glands were not cultured in that study. Experimental infection studies in cattle reflect similar findings. Payne (1959) demonstrated infection of retropharyngeal lymph nodes at one week post infection (PI), pregnant uterus and supramammary lymph nodes at 4 wk PI, and mammary gland at 6 wk PI. Two yr PI, Manthei and Carter (1950) found *B. abortus* present in the mammary gland and supramammary lymph nodes from 13 of 15 culture-positive cattle; iliac lymph nodes were positive in six animals; prescapular lymph nodes in three; and uterus, retropharyngeal lymph nodes and other lymph nodes were positive in two of the cows. Washko et al., (1952) reported recovering *B. abortus* from nine of 18 experimentally infected cows killed from 52 to 248 days PI. The organism was recovered from the mammary gland of eight animals, retropharyngeal lymph nodes of five, iliac lymph nodes of four, supramammary lymph nodes of three, internal inguinal lymph nodes of three, and uterus of two of the cows. Spleen, lung, muscle, bladder, and other lymph nodes were culture positive in one or two of the animals.

Surprisingly, *B. abortus* was not cultured from the mammary gland of any bison and was only isolated from the uterus of the cow that had recently aborted. The bison were sampled at approximately mid-gestation and the cattle in late gestation; however, differences in the stage of gestation do not account for differences in

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mammary gland and uterine culture results between nonpregnant bison and cattle. Since the lymphatics of the mammary gland drain to the supramammary lymph nodes, and since only portions of each mammary gland were collected and cultured, it is likely the high rate of supramammary lymph node infection in the bison is indicative of an actual higher rate of mammary gland infection than is reflected by these results. Two experimental infection studies in pregnant bison have demonstrated *B. abortus* present in mammary gland following abortion or calving (Davis et al., 1990; Bevins et al., 1996). Nevertheless, the discrepencies in demonstrable mammary gland and uterine infection rates between the cattle and bison in this study suggest that some species-related differences in sites of tissue localization of *B. abortus* in chronically-infected animals may occur.

In summary, the occurrence of and laboratory results in five cases of *Brucella* abortion in bison and the isolation of *B. abortus* from specimens collected from 12 of 26 seropositive animals indicate that the disease in bison is, in most aspects, similar to that in cattle. Results also indicate *B. abortus* can be associated with death in bison calves at least 2 wk of age. Results suggest, however, that while similar, some interspecies differences in tissue sites of *B. abortus* colonization may exist. Finally, the identification of supramammary, retropharyngeal, and iliac lymph nodes as the most frequent culture-positive tissues elevates the priority of these sites in any tissue collection protocol for brucellosis in female bison.

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