BRUCELLA ABORTUS IN CAPTIVE BISON. I. SEROLOGY, BACTERIOLOGY, PATHOGENESIS, AND TRANSMISSION TO CATTLE

Authors: Davis, Donald S., Templeton, Joe W., Ficht, Thomas A., Williams, John D., Kopec, John D., et al.

Source: Journal of Wildlife Diseases, 26(3) : 360-371

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-26.3.360

Journal of Wildlife Diseases, 26(3), 1990, pp. 360–371

BRUCELLA ABORTUS IN CAPTIVE BISON. I. SEROLOGY, BACTERIOLOGY, PATHOGENESIS, AND TRANSMISSION TO CATTLE

Donald S. Davis,[1] Joe W. Templeton,[1] Thomas A. Ficht,[2] John D. Williams,[2] John D. Kopec,[3] and L. Garry Adams[1]

[1] Department of Veterinary Pathology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA
[2] Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA
[3] USDA/APHIS, Veterinary Services, Federal Building, Hyattsville, Maryland 20782, USA

ABSTRACT: Two groups of six, non-brucellosis vaccinated, brucellosis seronegative pregnant American bison (*Bison bison*) were individually challenged with 1×10^7 colony forming units (CFU) of *Brucella abortus* strain 2308. Three days after challenge, each bison group was placed in a common paddock with six non-vaccinated, brucellosis susceptible, pregnant domestic heifers. In a parallel study, two groups of six susceptible, pregnant cattle were simultaneously challenged with the identical dose as the bison and each group was placed with six susceptible cattle in order to compare bison to cattle transmission to that observed in cattle to cattle transmission. Blood samples were collected from bison and cattle weekly for at least 1 mo prior to exposure to *B. abortus* and for 180 days post-exposure (PE). Sera from the bison and cattle were evaluated by the Card, rivanol precipitation, standard plate agglutination, standard tube agglutination, cold complement fixation tube, warm complement fixation tube, buffered acidified plate antigen, rapid screening, bovine conjugated enzyme linked immunosorbent assay, bison or bovine conjugated enzyme linked immunosorbent assay, and the hemolysis-in-gel techniques for the presence of antibodies to *Brucella* spp. At the termination of pregnancy by abortion or birth of a live-calf, quarter milk samples, vaginal swabs, and placenta were collected from the dam. Rectal swabs were collected from live calves, and mediastinal lymph nodes, abomasal contents and lung were taken at necropsy from aborted fetuses for culture of *Brucella* spp. These tissues and swabs were cultured on restrictive media for the isolation and identification of *Brucella* spp. Pathogenesis of brucellosis in bison was studied in an additional group of six pregnant bison which were challenged with 1×10^7 CFU of *B. abortus* strain 2308. One animal was euthanatized each week PE. Tissues were collected at necropsy and later examined bacteriologically and histologically. Lesions of brucellosis in bison did not significantly differ grossly or histologically from those in cattle. There were six abortions and two nonviable calves in the bison group, as compared to nine abortions in the 12 similarly inoculated cattle. As determined by bacterial isolations, transmission of *B. abortus* from bison to cattle (five of 12 susceptible cattle became infected) did not differ statistically from cattle to cattle transmission (six of 12 susceptible cattle became infected) under identical conditions. No single serologic test was consistently reliable in diagnosing *B. abortus* infected bison for 8 wk PE. Multiple testing periods in which the Card test was used in combination with the bison conjugated enzyme linked immunosorbent assay and the hemolysis-in-gel proved to be a useful battery of serologic techniques to diagnose brucellosis in bison after the initial 8 wk PE.

Key words: Bison, *Bison bison*, brucellosis, serologic tests, pathogenicity, interspecific transmission, *Brucella abortus*, experimental study.

INTRODUCTION

The occurrence of *Brucella abortus* in American bison (*Bison bison*) was first documented in 1930 when the bacterium was isolated from the testicle of a bison killed on the National Bison Range, Moiese, Montana (Creech, 1930). Serologic evidence of brucellosis was reported earlier by Mohler (1917) as positive agglutination reactions in sera from three bison cows from Yellowstone National Park, Wyoming, two of which had aborted. In 1930, Rush (1932) found that three of five bison serum samples from the Yellowstone National Park herd were seroreactive for brucellosis. Later testing by Tunnicliff and Marsh (1935) on bison sera collected over several years from the National Bison Range and Yellowstone National Park indicated >60% (305/484) reactor rate. Sera

from 350 bison collected in 1956 from Elk Island National Park, Alberta, Canada reacted to a *Brucella* spp. agglutination test at a rate of 42% (Corner and Connell, 1958). Six of six bison from Utah contained agglutinins reacting with *B. abortus* tube agglutination antigen at titers of ≥20 (Thorpe et al., 1965). Serum samples from 2,365 free-ranging bison in Wood Buffalo National Park, Alberta and Northwest Territories, Canada during 1959 to 1974 were tested for brucellosis and 31% reacted positively to the tube agglutination test (Choquette et al., 1978).

Private ownership of bison has become widespread in the United States and Canada. The American Bison Association and The National Buffalo Association report that 80,000–100,000 bison are privately owned and their popularity is increasing due to the favorable market for bison meat and by-products. Many federal and state parks annually auction excess bison to private owners. All privately owned bison in the United States are presently under the same United States Department of Agriculture/Animal and Plant Health Inspection Service/Veterinary Services (USDA/APHIS/VS) regulations for *Brucella* spp. testing prior to movement as those for domestic cattle and are listed in the Uniform Method and Rules, Brucellosis Eradication 1984. Although brucellosis in cattle has been extensively studied, prior to this study no documentation existed on the susceptibility, precise host response, or transmission potential of *B. abortus* from bison under controlled experimental conditions. The objectives of the study were to: (1) document the serologic response to non-brucellosis vaccinated, pregnant bison after challenge with a standard bovine infective dose of *B. abortus* strain 2308 by 11 diagnostic techniques, (2) compare the susceptibility of bison and cattle to *B. abortus* infection, (3) determine the pathogenesis of *B. abortus* in bison, and (4) determine the potential for transmission of *B. abortus* infected pregnant bison to susceptible pregnant cattle as compared with *B. abortus* between cattle under identical experimental conditions.

MATERIALS AND METHODS

The 18 bison and 36 cattle utilized in the experiments were all non-brucellosis vaccinated and seronegative for *Brucella* spp. for at least 30 days prior to challenge as determined by the buffered *Brucella* spp. antigen (Card), rivanol precipitation (RIV), standard tube agglutination (STA) (National Animal Disease Laboratory, Diagnostic Reagents Manual 65 E and F, Ames, Iowa 50010, USA); cold complement fixation tube (CCFT) (Jones et al., 1963); and the hemolysis-in-gel (Nielsen et al., 1983). In addition, the cattle and bison were tested with a bovine conjugated enzyme linked immunosorbent assay (BovELISA) (Heck et al., 1980) and a bison conjugated enzyme linked immunoassay (BisELISA), respectively. The BisELISA was identical to the BovELISA procedure with the exception that bison antisera were substituted in place of cattle antisera. All were in the second trimester of pregnancy, all the cattle were first calf heifers, and originated from brucellosis-free herds. On 4 February 1986, six bison and six cattle were exposed to a standard bovine challenge dose (Davies et al., 1973) of 1×10^7 colony forming units (CFU) of *B. abortus* strain 2308 by bilateral conjunctival inoculation. After 3 days, the six inoculated bison were placed in a 1.1 ha paddock with six *brucellosis* susceptible cattle, and the six *B. abortus* inoculated cattle were placed in a 1.2 ha paddock with an additional six brucellosis susceptible cattle. On 19 February 1987, the entire *B. abortus* 2308 exposure was repeated in additional bison and cattle. Blood samples were collected via jugular venapuncture from all bison and cattle on the day of inoculation and weekly thereafter for 180 days, and from the dam at the termination of pregnancy by abortion or live birth. Live calves were also bled at birth.

Sera were harvested from blood samples and stored at −70 C. Subsequently, sera were thawed and evaluated for the presence of *Brucella* spp. specific antibodies by the Card, RIV, STA, HIG, standard plate agglutination (SPA), buffered acidified plate antigen (BAPA), rapid screening (RS) (National Animal Disease Laboratory, undated), CCFT, warm complement fixation tube (WCFT) (Jones et al., 1963), and BovELISA or BisELISA. Minimum criteria for a diagnostically positive serologic reaction for cattle to the various tests are listed in the United States Department of Agriculture/Animal and Plant Health Inspection Service/Veterinary Service (1984). There are no such established diagnostic criteria for bison.

TABLE 1. Tissues collected from adult female bison exposed to *Brucella abortus* via bilateral conjunctival inoculation.

Tissue	Weeks postexposure					
	1	2	3	4	5	6
Head						
Parotid lymph nodes (right and left)	−[a]	+	+	+	+	+
Mandibular lymph nodes (right and left)	−	−	+	+	+	−
Atlantal lymph nodes (right and left)	−	−	+	+	+	+
Suprapharyngeal lymph nodes (right and left)	−	−	+	+	+	+
Thorax						
Prescapular lymph nodes (right and left)	−	−	+	+	+	+
Lung	−	−	−	−	−	+
Thymus	−	−	−	−	−	−
Mediastinal lymph nodes	−	−	−	+	+	−
Abdomen						
Mesenteric lymph nodes	−	−	−	−	−	+
Hepatic lymph nodes	−	−	+	+	−	+
Liver	−	−	−	−	−	+
Spleen	−	−	−	+	+	+
Abomasum	−	−	−	−	−	−
Supramammary lymph nodes	−	+	−	+	+	−
Mammary glands (four quarters)	−	−	+	+	+	+
Uterine horn (right and left)	−	−	+	+	+	+
Placenta (placentome and membranes)	−	−	−	+	+	+
Internal iliac lymph nodes (right and left)	−	−	+	+	+	+
Prefemoral lymph nodes	−	−	+	+	+	+
Fetus						
Lung	−	−	+	+	+	+
Abomasal content	−	−	+	+	+	+

[a] −, negative; +, positive.

Fetuses were collected within 12 hr of abortion and tissue from lungs, abomasum, mediastinal lymph nodes and rectal and abomasal content swabs were taken at necropsy. Calves were ear tagged and rectal swabs were collected from live calves within 12 hr of birth. On the day of parturition, placenta, uterine swabs, and quarter milk samples were collected. Tissues and swabs were stored at −70 C until thawed and plated on Farrell's restrictive media (Farrell, 1974) and blood agar for bacteriologic isolation and identification. Sub-cultures of the *B. abortus* isolates were sent to the National Veterinary Services Laboratory (NVSL, Ames, Iowa 50010, USA) for confirmation of identification.

Pathogenesis of *B. abortus* in female bison was studied in six additional non-brucellosis vaccinated, brucellosis seronegative pregnant bison which were exposed on 19 February 1987 via conjunctival inoculation with the same challenge dose. One bison per week beginning 1 wk postexposure (PE) was randomly selected, euthanatized, and necropsied. Thirty-two tissues were collected from each bison (Table 1) and frozen at −70 C. Samples were thawed and cultured as above. An identical set of tissues were fixed in 10% buffered formalin, paraffin embedded, stained with eosin and hematoxylin and sectioned at 4 μm for histologic examination.

During the experiment bison and cattle were fed a commercially available complete bulk ration (TAMU Mix Number 1; Producer's Cooperative Association, College Station, Texas 77802, USA) containing 11% protein at the rate of approximately 3% body weight per day and *ad libitum* round baled grass hay. Each portion of the study was conducted in separate spacially isolated paddocks. Each paddock was provided with separate feed troughs, water and animal handling facilities. Animal caretakers and researchers were required to wear rubber boots, gloves and coveralls before entering the bison/cattle paddocks which were padlocked and clearly marked with biohazard signs.

RESULTS

Serology

All bison (Table 2) and cattle (Table 3) experimentally exposed to *B. abortus* strain 2308 via conjunctival inoculation reacted to at least one serologic test PE. The SPA, RST, and BAPA test results were not found to differ significantly from the Card and therefore are not included in Table 2 or Table 3. Similarly the WCFT results were excluded due to their duplication of the CCFT. Bison #574 (culture negative for *Brucella* spp.) had a BisELISA reaction of 1.284 at 2 wk PE, had lower reactions on the BisELISA and CCFT at 3 to 6 wk PE, and then remained negative by all 11 serologic tests for the remainder of the experiment. Bison #578 (culture negative for *Brucella* spp.) had a low CCFT at 8 wk PE, a high HIG reaction at 8 to 12 wk PE, a BisELISA reaction at 10 to 12 wk PE, and then remained negative to all 11 serologic tests utilized for the remainder of the experiment. All other inoculated bison and cattle were reactive at some level to most serologic tests by 6 wk PE. The antibody response of bison to *B. abortus* challenge, as measured by all serologic tests, lagged approximately 2 to 3 wk behind that seen in cattle exposed at the same time. No individual serologic test was entirely accurate and reliable in diagnosing brucellosis in the bison for 6 to 8 wk PE. Seven of 12 susceptible heifers in paddocks with *B. abortus* inoculated bison became brucellosis seroreactive, while 5 of 12 susceptible heifers with the *B. abortus* inoculated cattle became brucellosis seroreactive.

Bacteriology

Brucella abortus strain 2308 was recovered at or subsequent to the termination of pregnancy from 10 of 12 (83%) bison and from 11 of 12 (91%) cattle which had been primarily inoculated with the organism. The same organism was recovered at the termination of pregnancy from five of 12 of the cattle or their calves placed with the inoculated bison, and from six of 12 heifers or their calves placed with the inoculated cattle. All 11 culture positive secondarily infected cattle aborted their calves or had non-viable calves. Subcultures of the *B. abortus* isolates were sent to the NVSL for confirmation of identification.

Pathogenesis

Brucella abortus strain 2308 was isolated from many bison tissues (Table 1). Most isolations were at ≥3 wk PE.

Transmission

As stated above, serologic reactions in the secondarily exposed cattle indicated that the transmission of brucellosis from inoculated animals to susceptible animals occurred. Transmission of brucellosis from bison to cattle as determined by serologic response in the cattle (seven of 12) was not statistically different from the transmission rate (six of 12) from infected to susceptible cattle. As determined by bacterial isolations, transmission of *B. abortus* from bison to cattle (five of 12 susceptible cattle became infected) did not differ statistically from cattle to cattle transmission (six of 12 susceptible cattle became infected) under identical conditions.

Pathology

Gross lesions were not observed in bison cows or their fetuses. Microscopic lesions were confined to the lymphoreticular and reproductive systems of cows and the lung and placenta of the fetuses. By 7 days PE, the mandibular, suprapharyngeal, parotid and atlantal lymph nodes bilaterally had expansion of the B cell compartment, including primary and secondary germinal centers as well as mild histiocytic macrophage hyperplasia in the marginal and medullary sinuses. The marginal and medullary sinuses also contained focal accumulations of neutrophils and eosinophils. These lesions were most pronounced in the parotid and suprapharyngeal lymph nodes. By 14 days post-challenge, the expansion

TABLE 2. Serologic responses of bison after exposure to *Brucella abortus* strain 2308 via conjunctival inoculation.

Bison no.	Termination of pregnancy (TOP)	Post-exposure (PE) week					*Brucella* spp. culture status (TOP)
		2	3	4	6	8	
572	Abortion 48 days PE	All negative	All negative	RIV 25[a] CCFT 20[b] HIG 9[c]	RIV 25 CCFT 40 BisELISA 0.868[d] HIG 10	Card (+)[e] RIV 100 CCFT 160 BisELISA 1.013 HIG 11	Positive (*B. abortus* 2308)
573	Abortion 38 days PE	All negative	All negative	CCFT 40 HIG 10	Card (+) RIV 400 CCFT 160 SAT 100[f] BisELISA 0.978 HIG 11	Card (+) RIV 400 CCFT 160 SAT 400 BisELISA 1.016 HIG 12	Positive (*B. abortus* 2308)
574	Live calf 69 days PE	BisELISA 1.284	BisELISA 0.879 CCFT 10	BisELISA 0.653 CCFT 10	BisELISA 0.544 CCFT 10	All negative	Negative
575	Live calf 60 days PE	All negative	All negative	CCFT 10	Card (+) RIV 50 CCFT 40 BisELISA 1.011	Card (+) RIV 200 CCFT 160 SAT 400 BisELISA 1.245	Positive (*B. abortus* 2308)
576	Abortion 40 days PE	All negative	All negative	All negative	RIV 25 CCFT 40	Card (+) CCFT 160 SAT 400 BisELISA 0.612 HIG 11	Positive
578	Live calf 108 days PE	All negative	All negative	All negative	All negative	CCFT 20 HIG 9	Negative
2034	Abortion 39 days PE	All negative	SAT 50 HIG 9	RIV 25 CCFT 20 SAT 100 HIG 9	RIV 25 CCFT 20 SAT 100 BisELISA 0.801 HIG 10	Card (+) RIV 400 CCFT 160 SAT 400 BisELISA 0.718 HIG 12	Positive (*B. abortus* 2308)

TABLE 2. Continued.

Bison no.	Termination of pregnancy (TOP)	Post-exposure (PE) week 2	3	4	6	8	*Brucella* spp. culture status (TOP)
2076	Live calf 43 days PE	All negative	SAT 50	SAT 50	Card (+) RIV 100 CCFT 20 SAT 100 HIG 10	Card (+) RIV 400 CCFT 160 SAT 400 BisELISA 0.674 HIG 10	Positive (*B. abortus* 2308)
2080	Abortion 65 days PE	All negative	All negative	All negative	Card (+) SAT 50 HIG 8	Card (+) RIV 400 CCFT 160 SAT 400 BisELISA 0.289 HIG 10	Positive (*B. abortus* 2308)
2091	Abortion 39 days PE	All negative	All negative	All negative	CCFT 20 SAT 100 HIG 10	Card (+) RIV 400 CCFT 160 SAT 400 BisELISA 0.752 HIG 12	Positive (*B. abortus* 2308)
2101	Live calf 53 days PE	All negative	All negative	All negative	HIG 10	Card (+) RIV 400 CCFT 160 SAT 200 BisELISA 0.939 HIG 11	Positive (*B. abortus* 2308)
2102	Live calf 50 days PE	All negative	All negative	All negative	SAT 50 HIG 8	RIV 50 CCFT 10 SAT 50 HIG 10	Positive (*B. abortus* 2308)

[a] RIV 25 = rivanol precipitation test reaction at a serum dilution of 1:25.
[b] CCFT 20 = cold complement fixation tube test reaction at a serum dilution of 1:20.
[c] HIG 9 = hemolysis-in-gel test reaction at a zone of 9 mm.
[d] BisELISA 0.868 = bison conjugated enzyme linked immunoassay with an optical density reading of 0.868.
[e] Card (+) = card test agglutination reaction.
[f] SAT 100 = standard agglutination tube test reaction at a serum dilution of 1:100.

of the B cell compartment and sinus histiocytic macrophage hyperplasia were much more pronounced with prominent expansion of the mantles of secondary germinal centers. Large populations of plasma cells and focal aggregations of neutrophils and eosinophils were evident in the outer cortex and expanding into the medullary sinusoids in the regional lymph nodes of the head. Similar but less pronounced lesions were evident in supramammary, internal iliac and hepatic lymph nodes. From 21 through 42 days PE, the proliferative lesions of the B cells and histiocytic macrophages became increasingly prominent accompanied by expansion of the T cell compartment in the paracortex and massive increases in plasma cell populations, particularly in inner cortex and medullary sinuses. Neutrophils and eosinophils were less prominent. During this time, most lymph nodes had similar lesions, but lesions in the lymph nodes regional to the head and reproductive tract were much more pronounced.

No microscopic lesions were observed in the fetus or placenta until 35 days PE when edema of the inter-cotyledonary chorionic membranes occurred in association with sparse focal accumulations of neutrophils and mononuclear leukocytes. By 42 days PE, the chorionic trophoblastic epithelial cells contained dense intracytoplasmic accumulations of Gram negative bacteria, often resulting in cellular necrosis. The zones of necrosis of the chorionic epithelial cells were infiltrated with diffuse dense infiltrations of neutrophils accompanied by histiocytic macrophages. Inflammatory cells frequently were degenerative or necrotic which resulted in desquamation of necrotic cellular debris and bacteria into the uterochorionic spaces. These lesions extended deeply into the intervillous placental arcades which contained areas of necrosis, neutrophilic infiltration associated with zones of chorionic epithelial cells containing intracytoplasmic bacteria were interpreted to be *B. abortus*. These zones eroded as exudates into the space between the arcades and the epithelium covering of the maternal villi. Fetal pulmonary lesions were only observed 42 days PE as predominantly immature neutrophilic and some mononuclear leukocytic accumulation in bronchioles and bronchi; this constituted a purulent bronchiolitis and bronchitis with expansion of the inflammatory exudate into the peribronchial alveoli resulting in purulent bronchopneumonia.

DISCUSSION

The bacteriologic and serologic results indicate that bison are as susceptible to *B. abortus* infections as domestic cattle under our experimental conditions. While all bison and cattle inoculated with *B. abortus* strain 2308 seroconverted, 83% (10 of 12) of bison and 91% (11 of 12) of cattle became infected as determined by bacterial isolations; these infection rates do not differ statistically ($\chi^2 = 0.38$, $P > 0.50$). The histologic observations also indicate that brucellosis in bison does not differ significantly from that previously described in cattle (Payne, 1959). Transmission of brucellosis from bison to cattle as determined by serologic response in the cattle (seven of 12) was not statistically different ($\chi^2 = 0.67$, $P > 0.25$) from the transmission rate (five of 12) from infected to susceptible cattle. Similarly, isolations of *B. abortus* from the cattle (five of 12) housed with the inoculated bison did not differ statistically ($\chi^2 = 0.17$, $P > 0.50$) from that observed in the cattle to cattle transmission (six of 12). These data indicate that under controlled conditions, transmission of *B. abortus* from bison to cattle can occur as readily as cattle to cattle transmission.

Evaluation of the 11 serologic techniques demonstrated for that 0 to 8 wk PE no single test consistently identified bison infected with *B. abortus* strain 2308. Therefore, no single test should be relied upon to definitively diagnosis brucellosis in bison. It should be noted that three of six of the aborting bison infected with *B. abortus* were Card test negative at the day

TABLE 3. Serologic response of 12 domestic heifers after exposure to *Brucella abortus* strain 2308 via conjunctival inoculation.

Cow no.	Termination of pregnancy (TOP)	Post-exposure (PE) week					*Brucella* spp. culture status
		2	3	4	6	8	
552	Abortion 55 days PE	All negative	All negative	Card (+)[a] HIG 9[b]	Card (+) RIV 25[c] CCFT 40[d] SAT 100[e] BovELISA 0.978[f] HIG 10	Card (+) RIV 50 CCFT 80 SAT 100 HIG 11	Positive (*B. abortus* 2308)
559	Abortion 36 days PE	All negative	Card (+) RIV 25 CCFT 40 BovELISA 0.916 HIG 9	Card (+) RIV 100 CCFT 80 SAT 200 BovELISA 0.978 HIG 10	Card (+) RIV 100 CCFT 160 SAT 200 BovELISA 1.276 HIG 10	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 1.160 HIG 11	Positive (*B. abortus* 2308)
563	Abortion 80 days PE	All negative	All negative	Card (+) CCFT 20 HIG 9	Card (+) RIV 25 CCFT 40 SAT 200 BovELISA 0.801 HIG 10	Card (+) RIV 200 CCFT 160 SAT 200 BovELISA 0.589 HIG 11	Positive (*B. abortus* 2308)
566	Abortion 55 days PE	All negative	Card (+) CCFT 20	Card (+) CCFT 20 HIG 9	Card (+) RIV 50 CCFT 80 SAT 100 BovELISA 1.029 HIG 10	Card (+) RIV 200 CCFT 160 SAT 400 BovELISA 0.945 HIG 11	Positive (*B. abortus* 2308)
569	Live calf 99 days PE	All negative	All negative	All negative	CCFT 10 HIG 8 BovELISA 0.584	RIV 25 CCFT 40 SAT 100 BovELISA 0.808 HIG 10	Positive (*B. abortus* 2308)

TABLE 3. Continued.

Cow no.	Termination of pregnancy (TOP)	Post-exposure (PE) week 2	3	4	6	8	*Brucella* spp. culture status
570	Abortion 43 days PE	All negative	Card (+) CCFT 20 BovELISA 0.622	Card (+) RIV 50 CCFT 40 BovELISA 0.799 HIG 9	Card (+) RIV 100 CCFT 40 SAT 100 BovELISA 1.036 HIG 10	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 1.157 HIG 10	Positive (*B. abortus* 2308)
2103	Abortion 76 days PE	All negative	Card (+) SAT 50	Card (+) SAT 100	Card (+) RIV 25 CCFT 20 SAT 100 BovELISA 1.340 HIG 10	Card (+) RIV 25 CCFT 20 SAT 100 BovELISA 0.518 HIG 8	Positive (*B. abortus* 2308)
2104	Abortion 60 days PE	SAT 25	All negative	RIV 25 CCFT 10 SAT 50 HIG 9	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 1.500 HIG 11	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 0.902 HIG 10	Positive (*B. abortus* 2308)
2105	Live calf 135 days PE	SAT 25	SAT 25	Card (+) SAT 50	Card (+) SAT 50 HIG 8	HIG 7	Positive (*B. abortus* 2308)
2106	Abortion 13 days PE	All negative	SAT 50	RIV 50 CCFT 20 SAT 100 HIG 9	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 0.723 HIG 11	Card (+) RIV 400 CCFT 160 SAT 400 BovELISA 1.145 HIG 12	Negative
2107	Live calf 43 days PE	All negative	CCFT 10	Card (+) RIV 100 CCFT 20 SAT 100	Card (+) RIV 200 CCFT 40 SAT 200 BovELISA 1.128 HIG 9	Card (+) RIV 400 CCFT 160 SAT 200 BovELISA 0.904 HIG 10	Positive (*B. abortus* 2308)

TABLE 3. Continued.

Cow no.	Termination of pregnancy (TOP)	Post-exposure (PE) week								*Brucella* spp. culture status
		2	3	4		6		8		
2108	Abortion 79 days PE	All negative	Card (+)	Card (+)		Card (+)		Card (+)		Positive (*B. abortus* 2308)
			RIV 25	CCFT 80		RIV 200		RIV 400		
			CCFT 20	SAT 200		CCFT 80		CCFT 160		
			SAT 50	BovELISA 0.866		SAT 200		SAT 400		
			HIG 8	HIG 10		BovELISA 1.500		BovELISA 1.122		
						HIG 11		HIG 11		

[a] Card (+) = card positive reaction.
[b] HIG 9 = hemolysis-in-gel test reaction at a zone of 9 mm.
[c] RIV 25 = rivanol precipitation test reaction at a serum dilution of 1:25.
[d] CF 40 = cold complement fixation tube test reaction at a serum dilution of 1:40.
[e] SAT 100 = standard agglutination tube test reaction at a serum dilution of 1:100.
[f] BovELISA 0.978 = bison conjugated enzyme linked immunoassay with an optical density reading of 0.978.

of abortion. After 10 wk PE, most of the tests utilized detected bison infected with *B. abortus*. Multiple testing utilizing the Card test when combined with the Bis-ELISA or the HIG would be the most effective combination of serologic techniques to reliably diagnose brucellosis in bison after 8 wk PE.

Brucella abortus was cultured from a wide variety of bison tissues following experimental inoculation, as was described in cattle with brucellosis (Davies et al., 1980). Progressive stimulation of regional lymph nodes by *B. abortus* resulted in the expansion of the B cell compartment, histiocytic macrophage hyperplasia and T cell compartment expansion as has been previously described (Meador et al., 1988a, b; and Payne, 1959). The fetal and placental lesions parallel those previously described in cattle (Jubb and Kennedy, 1985), sheep (Morello et al., 1963), and goats (Anderson et al., 1986), and this supports the conclusion that brucellosis in bison does not differ from that observed in other ruminant species.

As brucellosis continues to be successfully controlled or eradicated in domestic cattle, the largest remaining foci of *B. abortus* infections in North America will be in the publicly owned free-ranging elk (*Cervus elaphus nelsoni*) in northwest Wyoming and bison herds in Yellowstone National Park and in western Canada. These migratory herds are heavily infected with *B. abortus* and as such will pose a continuing threat to the brucellosis-free livestock industries and associated human populations (Tessaro, 1986, 1989). Most privately owned bison herds and many publicly owned herds have successfully eliminated brucellosis from their animals through test, removal, and vaccination programs. Governmental brucellosis eradication or control programs will not be effective unless the disease is understood in free-ranging wildlife reservoirs as well as in domestic livestock. As the epidemiologic significance of sylvatic brucellosis increases concurrently with the decrease of

brucellosis in domestic livestock, the need for improved field diagnostic techniques, safe and effective vaccination regimes, and vaccine delivery systems designed specifically for use in free-ranging wildlife will become more critical.

ACKNOWLEDGMENTS

The authors gratefully acknowledge funding through a USDA/APHIS contract with the Texas Agricultural Experiment Station. The authors would like to acknowledge the considerable efforts of Russell Stocks, James Bell and Jim Nowak in the construction of animal handling facilities suitable for bison. W. J. (Bubba) Skrivanek, the manager of the Veterinary Research Park was also invaluable in the construction and maintenance of the bison working facility. A special thanks is in order to Roger Price, Roger Smith III, and Gregg Campbell for providing veterinary assistance in the collection of samples. A large portion of the research was conducted by the technicians, research associates and student workers employed in laboratories of the investigators and the authors offer their sincere thanks for the excellent support. We thank Kay Sanders for assistance in the preparation of this manuscript. The bison brucellosis project could not have been accomplished without the cooperation and hospitality provided by the entire Flocchini family and crew of the Durham Ranch in Wyoming.

LITERATURE CITED

ANDERSON, T. D., V. P. MEADOR, AND N. F. CHEVILLE. 1986. Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*. I. Gross and histologic lesions. Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*. II. Ultrastructural studies. Veterinary Pathology 23: 219–226.

CHOQUETTE, L. P., E. BROUGHTON, J. G. COUSINEAU, AND N. S. NOVAKOWSKI. 1978. Parasites and diseases of bison in Canada. IV. Serologic survey for brucellosis in bison in northern Canada. Journal of Wildlife Diseases 14: 329–332.

CORNER, A. H., AND R. CONNELL. 1958. Brucellosis in bison, elk and moose in Elk Island National Park, Alberta, Canada. Journal of Comparative Medicine 22: 9–21.

CREECH, G. T. 1930. *Brucella abortus* infection in a male bison. North American Veterinarian 11: 35–36.

DAVIES, G., E. COOKS, AND N. HERBERT. 1980. *Brucella abortus* (strain 19) vaccine: (a) Determination of the minimum protective dose in cattle; the effect of vaccinating calves previously inoculated with anti-*Brucella* serum. Journal of Biological Standards 8: 165–175.

———, N. Herbert, and A. Casey. 1973. The preservation of *Brucella abortus* (strain 554) in liquid nitrogen and its virulence when subsequently used as a challenge. Journal of Biological Standards 1: 165–170.

FARRELL, I. D. 1974. The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. Research in Veterinary Science 16: 280–286.

HECK, F. C., J. D. WILLIAMS, AND J. PRUETT. 1980. Interpretation of spectrophotometric absorbance values to define results of enzyme-linked immunosorbent assays. Journal Clinical Microbiology 11: 398–401.

JONES, L. M., J. R. HENDRICKS, AND D. T. BERMAN. 1963. The standardization and use of the complement-fixation test for the diagnosis of bovine brucellosis, with a review of the literature. American Journal of Veterinary Research 24: 1143–1151.

JUBB, K. V. F., AND P. C. KENNEDY. 1985. Pathology of domestic animals, 3rd ed., Vol. 3. Academic Press, New York, New York, 527 pp.

MEADOR, V. P., W. A. HAGEMOSER, AND B. L. DEYOE. 1988a. Histopathologic findings in *Brucella abortus*-infected, pregnant goats. American Journal of Veterinary Research 49: 274–280.

———, D. P. WARNER, AND B. L. DEYOE. 1988b. Distribution of *Brucella abortus* organisms in calves after conjunctival exposure. American Journal of Veterinary Research 49: 2015–2017.

MOHLER, J. F. 1917. Annual report of the United States Bureau of Animal Industry, 105–106.

MOLELLO, J. A., R. JENSEN, J. R. COLLIER, AND J. C. FLINT. 1963. Placental pathology. III. Placental lesions of sheep experimentally infected with *Brucella abortus*. American Journal of Veterinary Research 24: 915–921.

NIELSEN, K. H., F. C. HECK, J. STILLER, AND B. ROSENBAUM. 1983. Interaction of specifically purified isotypes of bovine antibody to *Brucella abortus* in the haemolysis in gel test and enzyme-linked immunosorbent assay. Research in Veterinary Science 35: 14–18.

PAYNE, J. M. 1959. The pathogenesis of experimental brucellosis in the pregnant cow. Journal of Pathology and Bacteriology 78: 447–463.

RUSH, W. M. 1932. Bang's disease in the Yellowstone National Park buffalo and elk herds. Journal of Mammalogy 13: 371–372.

TESSARO, S. V. 1986. The existing and potential importance of brucellosis in Canadian wildlife: A review. Canadian Veterinary Journal 27: 119–124.

———. 1989. Review of the diseases, parasites and miscellaneous pathological conditions of North American bison. Canadian Veterinary Journal 30: 416–422.

THORPE, D., R. W. SIDWELL, J. B. BUSHMAN, K. L. SMART, AND R. MOYES. 1965. Brucellosis in wildlife and livestock of west central Utah. Journal of the American Veterinary Medical Association 146: 225–232.

TUNNICLIFF, E. A., AND H. MARSH. 1935. Bang's disease in bison and elk in the Yellowstone National Park and on the National Bison Range. Journal of the American Veterinary Medical Association 86: 745–752.

UNITED STATES DEPARTMENT OF AGRICULTURE/ANIMAL AND PLANT HEALTH INSPECTION SERVICE/VETERINARY SERVICE. 1984. Brucellosis eradication. Uniform methods and rules. U.S. Government Printing Office, Washington, D.C., 421-227/10102: 107.

Received for publication 28 March 1989.