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

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

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ABSTRACT: Two groups of six, non-brucellosis vaccinated, brucellosis seronegative pregnant American bison (Bison bison) were individually challenged with $1 \times 10^7$ 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, brucellosus 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 $1 \times 10^7$ 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 immunosay (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 × 10⁶ 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 venipuncture 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.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Weeks postexposure</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Head</td>
<td></td>
</tr>
<tr>
<td>Parotid lymph nodes (right and left)</td>
<td>-</td>
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<tr>
<td>Mandibular lymph nodes (right and left)</td>
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<tr>
<td>Atlantal lymph nodes (right and left)</td>
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<tr>
<td>Suprapharyngeal lymph nodes (right and left)</td>
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<tr>
<td>Thorax</td>
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<tr>
<td>Prescapular lymph nodes (right and left)</td>
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<tr>
<td>Lung</td>
<td>-</td>
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<tr>
<td>Thymus</td>
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<tr>
<td>Mediastinal lymph nodes</td>
<td>-</td>
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<tr>
<td>Abdomen</td>
<td></td>
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<tr>
<td>Mesenteric lymph nodes</td>
<td>-</td>
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<tr>
<td>Hepatic lymph nodes</td>
<td>-</td>
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<tr>
<td>Liver</td>
<td>-</td>
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<tr>
<td>Spleen</td>
<td>-</td>
</tr>
<tr>
<td>Abomasum</td>
<td>-</td>
</tr>
<tr>
<td>Supramammary lymph nodes</td>
<td>-</td>
</tr>
<tr>
<td>Mammary glands (four quarters)</td>
<td>-</td>
</tr>
<tr>
<td>Uterine horn (right and left)</td>
<td>-</td>
</tr>
<tr>
<td>Placenta (placentome and membranes)</td>
<td>-</td>
</tr>
<tr>
<td>Internal iliac lymph nodes (right and left)</td>
<td>-</td>
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<tr>
<td>Prefemoral lymph nodes</td>
<td>-</td>
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<tr>
<td>Fetus</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
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<tr>
<td>Abomasal content</td>
<td>-</td>
</tr>
</tbody>
</table>

* - , 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 necropsed. 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 spatially 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>
<thead>
<tr>
<th>Bison no.</th>
<th>Termination of pregnancy (TOP)</th>
<th>Post-exposure (PE) week</th>
<th>Brucella spp. culture status (TOP)</th>
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<tr>
<td></td>
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<tr>
<td>572</td>
<td>Abortion 48 days PE</td>
<td>All negative</td>
<td>RIV 25&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
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<td>CCFT 20&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>BisELISA 0.868&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>HIG 10</td>
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<td>573</td>
<td>Abortion 38 days PE</td>
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<td>Card (+)</td>
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<td>RIV 100</td>
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<td>CCFT 160</td>
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<td>BisELISA 1.013</td>
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<td>HIG 11</td>
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<tr>
<td>574</td>
<td>Live calf 69 days PE</td>
<td>BisELISA 1.284</td>
<td>Card (+)</td>
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<td>SAT 100</td>
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<td>Live calf 60 days PE</td>
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<td>Card (+)</td>
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<td>Card (+)</td>
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<td>CCFT 160</td>
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<td>SAT 100</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Antibody test; <sup>+</sup> = Positive
<table>
<thead>
<tr>
<th>Bison no</th>
<th>Terminator of pregnancy (TOP)</th>
<th>Post-exposure (PE) week</th>
<th>Brucella spp. culture status (TOP)</th>
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<tbody>
<tr>
<td>2076</td>
<td>Live calf 43 days PE</td>
<td>Card (+) RIV 100</td>
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<td>Card (+) CCFT 160</td>
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<td>SAT 100</td>
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<td></td>
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<td>HIG 10</td>
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<td>Card (+) RIV 400</td>
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<td>Card (+) CCFT 160</td>
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<td>BiELISA 0.752</td>
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<td></td>
<td>HIG 12</td>
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<tr>
<td>2102</td>
<td>Live calf 50 days PE</td>
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<td>HIG 11</td>
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</tbody>
</table>

- RIV 25 = rivaled precipitation test reaction at a serum dilution of 1:25.
- CCFT 20 = cold complement fixation test reaction at a serum dilution of 1:20.
- HIG 9 = hemolysis-in-gel test reaction at a zone of 9 mm.
- BiELISA 0.868 = bison conjugated enzyme linked immunoassay with an optical density reading of 0.868.
- Card (+) = card test agglutination reaction.
- 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 sinuses 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 deep 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 (χ² = 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 (χ² = 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 (χ² = 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>
<thead>
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<th>Brucella spp. culture status</th>
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* Card (+) = card positive reaction.
* HIG 9 = hemolysis-in-gel test reaction at a zone of 9 mm.
* RIV 25 = rivanol precipitation test reaction at a serum dilution of 1:25.
* CF 40 = cold complement fixation tube test reaction at a serum dilution of 1:40.
* SAT 100 = standard agglutination tube test reaction at a serum dilution of 1:100.
* BovELISA 0.978 = bison conjugated enzyme linked immunoassay with an optical density reading of 0.978.
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.

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LITERATURE CITED


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