Comparison of Abortion and Infection after Experimental Challenge of Pregnant Bison and Cattle with *Brucella abortus* Strain 2308™

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A comparative study was conducted using data from naive bison (*n = 45*) and cattle (*n = 46*) from 8 and 6 studies, respectively, in which a standardized *Brucella abortus* strain 2308™ experimental challenge was administered during midgestation. The incidence of abortion, fetal infection, uterine or mammary infection, or infection in maternal tissues after experimental challenge was greater (*P < 0.05*) in bison than in cattle. In animals that did abort, the time between experimental challenge and abortion was shorter (*P < 0.05*) for bison than for cattle. *Brucella* colonization of four target tissues and serologic responses on the standard tube agglutination test at the time of abortion did not differ (*P > 0.05*) between cattle and bison. The results of our study suggest that naive bison and cattle have similarities and differences after experimental exposure to a virulent *B. abortus* strain. Although our data suggest that bison may be more susceptible to infection with *Brucella*, some pathogenic characteristics of brucellosis were similar between bison and cattle.

The United States achieved a significant milestone in 2007 in that all states were declared free of brucellosis in cattle. Although several infected herds were subsequently found and this first brucellosis-free period was only transient, actions by state and federal regulatory personnel have allowed all states to regain and maintain brucellosis-free status since July 2009. This accomplishment is the culmination of eradication activities that were initiated in 1934 as part of a drought recovery program by removing brucellosis-infected animals as a means to reduce cattle populations (22).

Although brucellosis has been essentially eliminated from domestic livestock, wildlife reservoirs remain a threat for re-introduction. One of the most publically visible wildlife reservoirs of *Brucella abortus* is plains bison (*Bison bison*) within Yellowstone National Park. Primarily during winter months, bison may exit the park by migrating to lower elevations in search of forage. In studies conducted over a number of years, the bison herd in Yellowstone National Park has maintained an average seroprevalence of 50 to 60% (3, 21, 26). Although hazing and removal operations have prevented close associations with cattle and the opportunity for disease transmission to occur, these operations are controversial and not readily accepted by all members of the public.

In an effort to develop management tools to address the disease in bison, various studies have been conducted in bison characterizing the pathophysiology of brucellosis, immunologic responses to brucellosis vaccination, and the efficacy of brucellosis vaccines (12, 23, 24). The data have suggested some differences between bison and cattle in their susceptibility and immunologic responses to infection with *B. abortus* field strains or inoculation with vaccine strains (17, 18, 20). In this study, we compare data across a number of studies comparing abortion and infection rates in naive bison and cattle after experimental challenge using a standardized brucellosis challenge model for large ruminants.

(Received of these data have been previously presented in other publications [13–16, 19].)

**MATERIALS AND METHODS**

For this evaluation, data from naive bison and cattle from 8 and 6 efficacy studies, respectively, were compared.

**Breeding and experimental challenge.** Bovine heifers were pasture bred at 14 to 16 months of age. Bovine heifers were pasture bred at 2.5 years of age. The pregnancy status and stage of gestation in bison and cattle were determined by rectal palpation between 40 and 90 days of gestation by a board-certified theriogenologist. Pregnant bison and cattle were transferred to a biosafety level 3 containment facility 2 to 3 weeks prior to challenge, where they were housed for the duration of the study. The bison and cattle were intraconjunctivally challenged between 170 and 180 days of gestation with 1 × 10⁶ CFU of *B. abortus* strain 2308™ (2308®; 50 μl of inoculum per eye). The concentration of viable bacteria within each challenge was determined by serial dilution in saline and standard plate counts. Prechallenge blood samples were obtained via jugular venipuncture. For experimental challenge of bison, animals were fasted for 24 h prior to being anesthetized with carfentanil (Wildnul; 0.007 to 0.008 mg/kg of body weight; Wildlife Pharmaceuticals, Fort Collins, CO) and xylazine (0.10 to 0.13 mg/kg; Mobay Corp, Shawnee, KS) administered intramuscularly via pneumatic dart (Pneudart, Williamsburg, PA). Following the intraconjunctival challenge of bison, the anesthetic was reversed with an intravenous injection of naltrexone (Trexonil; 0.88 to 0.97 mg/kg; Wildlife Pharmaceuticals, Fort Collins, CO).

Cattle were euthanized within 1 week of the anticipated parturition date or within 48 h of abortion or parturition by intravenous injection of sodium pentobarbital (Sleepaway; Fort Dodge Laboratories, Fort Dodge, IA). Bisons were euthanized within 72 h of abortion or parturition by intravenous injections of sodium pentobarbital. Fetal viability was assessed in both cattle and bison, and fetal sex and crown-to-rump length were determined. Maternal samples obtained at necropsy for bacteriologic evaluation included lymphatic tissues (bronchial, hepatic, internal iliac, mandibular, mesenteric, parotid, prescapular, retropharyngeal, and supramammary lymph nodes); samples of milk and mammary tissue from all four quarters; maternal placentome, spleen, liver, and lung; and vaginal and conjunctival swabs. Samples obtained from fetuses/calves included rectal swabs, lung, liver, spleen, bronchial lymph node, and abomasal contents. Fetal and maternal blood was also obtained at necropsy for bacteriologic and serologic evaluation.

Abortion was defined as the premature birth of a *Brucella*-infected, nonviable fetus at any time after experimental challenge with *B. abortus* strain 2308®. Mam-

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TABLE 1. Standard tube agglutination titers of bison and cattle before challenge and at necropsy after conjunctival challenge
with 10^7 CFU of *B. abortus* strain 2308 at the time of abortion or after full-term delivery

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Prechallenge</th>
<th>Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bison</td>
<td>38</td>
<td>0.81 ± 0.12A</td>
<td>2.38 ± 0.12B</td>
</tr>
<tr>
<td>Cattle</td>
<td>22</td>
<td>0.26 ± 0.12A</td>
<td>2.47 ± 0.19B</td>
</tr>
<tr>
<td>Full term</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bison</td>
<td>7</td>
<td>1.24 ± 0.21</td>
<td>1.69 ± 0.09</td>
</tr>
<tr>
<td>Cattle</td>
<td>24</td>
<td>0.11 ± 0.08A</td>
<td>0.70 ± 0.19B</td>
</tr>
</tbody>
</table>

a Data are presented as mean (log_{10}) titer ± SEM. Prechallenge and necropsy means followed by different letters differ (P < 0.05) for that species within the abortion or full-term groups.

b Cattle prechallenge or necropsy means are less (P < 0.05) than paired bison prechallenge or necropsy mean titer.

mery or fetal/uterine infection was defined as the recovery of the 2308 challenge strain from milk, mammary gland, supramammary lymph node, placentome, vaginal swab, or any fetal sample. Maternal infection was defined as the recovery of the 2308 challenge strain from any maternal sample.

**Bacterial culture.** For bacterial culture, tissues were triturated in 0.85% NaCl (saline) using a tissue grinder and placed on tryptose agar plates containing 5% bovine serum as previously described (4, 13). The swabs were directly plated on tryptose agar plates containing 5% bovine serum. The initial plates received 200 μl of the 2-ml total volume of the tissue homogenate, with dilutions extending to 10^-7. The concentration in the tissue was determined by performing standard plate counts.

**Serology.** Prechallenge and necropsy blood samples were serologically evaluated for *Brucella* antibodies using the card and standard tube agglutination tests (1). Serum was diluted 1:2 for the standard tube agglutination test, with the initial dilution being 1:25.

**Statistical analysis.** Bacteriologic data are presented as the mean ± standard error of the mean (SEM). Serologic and colonization (CFU/g) data were converted to the logarithm of the titer for analysis. Any values of zero were converted to 1 prior to logarithmic conversion. Serologic responses of bison and cattle after challenge were compared by a generalized linear model (SAS Institute, Cary, NC) and presented as the mean titer SEM. Fisher’s exact test was used to compare incidences of abortion and infection between naive bison and cattle following experimental challenge.

**RESULTS**

In cattle (n = 24) and bison (n = 7) that did not abort, the average times between challenge and parturition did not differ (P > 0.05) and were 93.1 ± 1.9 and 88.0 ± 3.2 days, respectively. However, in animals that did abort, the time between experimental challenge and abortion was shorter (P < 0.05) for bison (45.0 ± 2.0 days; n = 38) than for cattle (59.0 ± 3.1 days; n = 22).

Seroslogic responses to infection, as measured by the standard tube agglutination test, indicated that mean titers prior to challenge were greater (P < 0.05) in bison than in cattle (Table 1). With the exception of bison that delivered full-term calves, the mean standard tube titers at necropsy of cattle and bison were greater (P < 0.05) than prechallenge titers. The mean titers after abortion did not differ (P > 0.05) between cattle and bison.

The incidence of abortion, fetal infection, uterine or mammary infection, or infection in maternal tissues after experimental challenge was greater (P < 0.05) in bison than in cattle (Table 2). Data characterizing the localization of *B. abortus* in four target tissues after abortion or full-term parturition suggest that tissue colonization (CFU/g) rates are similar (P > 0.05) in cattle and bison (Table 3). Limited data suggest that *B. abortus* colonization of target tissues is more likely in bison after full-term parturition than in cattle that do not abort after experimental challenge.

**DISCUSSION**

After analysis of data from a number of experimental challenge studies in cattle and bison, it is apparent that the pathophysiology of brucellosis has similarities and differences when bison and cattle are compared. In general, bison abort in a shorter interval after challenge and at a high percentage compared to cattle. Infection rates in uterine, mammary, and maternal tissues are also higher in bison after experimental challenge than in cattle.

However, cattle and bison are similar in *Brucella* colonization of four target tissues after abortion. The four tissues were chosen for evaluation based on the fact that they either provide lymphatic drainage for (i) the site of experimental challenge (the parotid lymph node), (ii) the uterus (placentome), or (iii) the mammary gland (supramammary lymph node) or repre-

TABLE 2. Incidence of abortion or infection in bison or cattle after experimental challenge with 10^7 CFU of *B. abortus* strain 2308 in midgestation

<table>
<thead>
<tr>
<th>Animal</th>
<th>Abortion [no./total (%)]</th>
<th>Infection [no./total (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fetal</td>
<td>Uterine/mammary</td>
</tr>
<tr>
<td>Bison</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>22/46 (48)a</td>
<td>23/46 (50)a</td>
</tr>
</tbody>
</table>

a Cattle have a significantly lower (P < 0.001) incidence of abortion or infection after *B. abortus* 2308 challenge than bison.

TABLE 3. Colonization in target tissues of bison and cattle at abortion or full-term parturition after experimental challenge with 10^7 CFU of *B. abortus* strain 2308 in midgestation

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Parotid</th>
<th>Prescapular</th>
<th>Supramammary</th>
<th>Placentome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bison</td>
<td>34</td>
<td>2.68 ± 0.12</td>
<td>1.98 ± 0.17</td>
<td>2.71 ± 0.29</td>
<td>7.38 ± 0.27</td>
</tr>
<tr>
<td>Cattle</td>
<td>5</td>
<td>2.44 ± 0.21</td>
<td>1.39 ± 0.61</td>
<td>1.21 ± 0.71</td>
<td>6.27 ± 1.63</td>
</tr>
<tr>
<td>Full term</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bison</td>
<td>7</td>
<td>1.70 ± 0.37A</td>
<td>1.03 ± 0.42</td>
<td>0.90 ± 0.90</td>
<td>2.54 ± 1.19A</td>
</tr>
<tr>
<td>Cattle</td>
<td>3</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

a Data are presented as mean numbers of CFU/g (log_{10}) ± SEM. The means within a tissue followed by different letters are statistically different (P < 0.05) between cattle and bison.
sent a lymph node not associated with a preferred localization site of *Brucella* (the prescapular lymph node). As lateral transmission of brucellosis is predominantly believed to occur through colonization and expulsion of infected placental or fetal tissues, higher numbers of bacteria in the uterine environment probably demonstrate greater potential for the spread of infection to other animals.

Colonization data from maternal lymph nodes taken at necropsy after abortion suggest that live bacteria in lymphatic tissues are lower in number than in placental tissues, but bacterial numbers are relatively uniform across lymph nodes. Recognizing that colonization in the suprammary lymph node is unlikely to accurately reflect the concentrations (CFU/ml) of *Brucella* bacteria in milk at parturition, it was interesting that this lymph node did not contain more bacteria than the prescapular lymph node, a systemic lymph node not associated with the site of experimental infection or providing lymphatic drainage for a preferential site of *Brucella* localization. Although vertical transmission appears to be of less epidemiologic importance, brucellosis can be transmitted to calves through infected milk. Only a small percentage of animals in which *Brucella* was isolated from uterine or fetal tissues did not have localization within mammary tissues. In general, our colonization data suggest that *Brucella* broadly and uniformly localizes across lymphatic tissues of bison and cattle during acute infections.

The persistence of *B. abortus* in lymphatic and uterine tissues of nonaborting bison was not surprising, as previous studies have documented that bison (25) take up to 22 weeks after inoculation to clear the attenuated RB51 vaccine strain compared to 12 weeks in cattle (13). Clearance of a virulent field strain of *B. abortus* would be anticipated to take longer than an attenuated vaccine strain.

Dose-response data in cattle after conjunctival exposure to virulent *B. abortus* suggest that, depending upon the challenge strain, 10⁵ CFU is insufficient to induce clinical effects (2) but 10⁷ CFU induces infection in 78% of naive cattle (10). In large ruminants, current experimental challenge protocols typically require 10⁷ CFU for conjunctival exposure of large ruminants for repeatable and consistent clinical infections (5, 8, 11). It was estimated that 7 × 10⁵ CFU of S2308 most closely mimics the epidemiologic features of infected herds (11). Assuming a minimal infectious dose of 10⁵ CFU for *B. abortus*, it is estimated that 1 g of placental tissue from aborting bison and cattle would contain on average 360 and 20 infectious doses, respectively. Due to the large amount of fluids and materials expelled at the time of parturition, any abortion event in cattle or bison would be capable of infecting large numbers of animals that come in contact with the aborted materials. From a biological perspective, the difference between cattle and bison in tissue colonization after abortion is probably insignificant in regard to the ability to transmit brucellosis.

The appropriate experimental challenge dose for evaluating vaccine efficacy in wildlife reservoirs of brucellosis remains controversial. The conjunctival challenge dose for strain 2308 was set at 700,000 bacteria, as infection rates in naive cattle after experimental exposure were felt to most closely parallel natural exposure (11). In comparison, an experimental challenge dose of 1.5 × 10⁷ CFU was the minimal dose for inducing 100% infection rates in naive cattle (11). Others have reported that higher challenge doses may overwhelm protective immunity induced by vaccination (6). In the current study, the overall rate of abortion was slightly less than in a previous bison study, in which 96% of naive bison aborted following challenge with 10⁷ CFU of *B. abortus* strain 2308 (7). Although it has been postulated that experimental challenge doses should not induce 100% infection rates in naive animals, it should be recognized that vaccine efficacy under field conditions may be influenced by a number of factors, including, but not limited to, environmental or nutritional stress, coexisting diseases, stage of pregnancy, exposure dosage, and previous immunologic exposure to the disease. Although the experimental challenge does appear to be more pathogenic in bison than in cattle, data indicate the model is capable of demonstrating differences in protection between vaccinated and naive bison (19). Recognizing that the experimental challenge model may be a more strenuous measure of protection in bison than in cattle, it may be of greater value than evaluation of efficacy using a reduced dose of 2308. As a vaccination program for free-ranging wildlife will be of long duration, costly, and unlikely to be combined with an adequate test and removal program, a strenuous test of vaccine efficacy will be more useful for selecting a vaccine that could be implemented in the most effective disease control program.

The results of our study suggest that naive bison and cattle have similarities and differences after experimental exposure to a virulent *B. abortus* strain. Bison have higher abortion and infection rates and abort earlier than naive cattle. This could be important for understanding the epidemiology and transmission of brucellosis in free-ranging bison under field conditions. In comparison, serology and tissue colonization after experimental challenge were similar between the two species. Although our data suggest that bison may be more susceptible to infection with *Brucella*, some pathogenic characteristics of brucellosis were similar between bison and cattle.

REFERENCES