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BRUCELLA ABORTUS IN BISON. II. EVALUATION OF STRAIN 19 VACCINATION OF PREGNANT COWS

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ABSTRACT: Protection against *Brucella abortus* induced abortion and infection provided by strain 19 (S19) vaccination was evaluated in American bison (*Bison bison*). Forty-eight pregnant bison were manually inoculated (MI) with S19 vaccine, 44 were ballistically inoculated (BI) with an absorbable hollow pellet containing lyophilized S19, and 46 were manually injected with buffered saline as non-vaccinated controls (NVC). All bison were *Brucella* spp. seronegative prior to the experiment, in the second trimester of pregnancy, and were randomly assigned to experimental groups. Approximately 60 days post-vaccination, abortions were observed in the vaccinated bison. *Brucella abortus* strain 19 was recovered from a bison that had recently aborted, her fetus, and from 11 of 12 other aborted fetuses. Fifty-eight percent (53 of 92) of vaccinated bison aborted, and no abortions were observed in the NVC bison. One cow aborted during her second post-vaccinal pregnancy and S19 was identified from the dam and fetus indicating that chronic S19 infections can occur in bison. Positive antibody titers were present 10 mo post-vaccination in 73% (66 of 91) of the bison. Thirteen mo post-vaccination, 30 MI vaccinates, 27 BI vaccinates, and 30 NVC bison were challenged during the second trimester of pregnancy with 1×10^7 CFU of *B. abortus* strain 2308 via bilateral conjunctival inoculation. Protection against abortion was 67% ($P \leq 0.0001$) for vaccinated bison compared to 4% in NVC. Protection against *B. abortus* infection was determined to be 39% ($P \geq 0.001$) for vaccinates and 0% (zero of 30) for NVC. Persistent antibody titers, vaccine induced abortions, and chronic S19 infections indicate that the S19 vaccine doses used in this study are not suitable for pregnant bison.

Key words: *Brucella abortus*, bison, *Bison bison*, vaccination, strain 19 vaccine, vaccinal abortions, retained vaccinal titers, experimental infection, brucellosis.

INTRODUCTION

Brucellosis has been documented as a problem in free-ranging bison (*Bison bison*) in the United States since 1930 (Creech, 1930; Rush, 1932; Tunnicliff and Marsh, 1935; Thorpe et al. 1965) and in Canada since 1947 (Moore, 1947; Corner and Connell, 1958; Choquette et al., 1978; Tessaro, 1986, 1989). During the first few months of 1989, more than 500 bison that moved north out of Yellowstone National Park (YNP) into Montana were shot and samples collected. Serologic tests (card, rivanol, standard tube agglutination and complement fixation) indicated that >50% of the YNP bison sera were positive for *Brucella* spp. antibodies (Ferlicka, 1989). Isolates of *B. abortus* collected from bison from YNP were pathogenic in cattle (Colgrove et al., 1985). Davis et al. (1990) experimentally infected bison with 1×10^7 colony forming units (CFU) of *B. abortus* strain 2308 and documented that (1) the pathogenesis and abortifacient properties of *B. abortus* in bison does not differ from that observed in cattle, (2) serologic tests used in cattle must be utilized with caution in bison due to the delayed antibody response in bison, and (3) infected bison are as capable as cattle of transmitting *B. abortus* to susceptible cattle. Brucellosis in bison which is primarily limited to *B. abortus* biovar 1 continues to be a problem in North America in private herds (Flagg, 1983) as well as in some free-ranging herds on public lands (Tessaro, 1986, 1989; Davis, 1990).

Strain 19 (S19) vaccine is currently the only vaccine licensed by the United States

Department of Agriculture (USDA) for use in the United States in the prevention of brucellosis in animals. The efficacy of S19 vaccination in adult bison had not been evaluated prior to this study. The principal objectives were to determine the protection against *B. abortus* induced abortions and infections in adult bison vaccinated with manually injected S19, and S19 delivered ballistically.

MATERIALS AND METHODS

Experimental animals

Bison were obtained from a privately owned brucellosis free herd located near Gillette, Wyoming (USA; 43°40′ to 43°41′N, 105°28′ to 105°29′W). The ranch had been vaccinating all female calves at 8 to 10 mo of age for the last 5 yr. All bison cows >5-yr-old on the ranch that had not been vaccinated with S19 were serologically negative as determined by the card, rivanol precipitation (RIV) (National Animal Disease Laboratory, undated), hemolysis-in-gel (HIG) (Nielsen et al., 1983), and cold complement fixation tube (CCFT) tests (Jones et al., 1963), and a bison conjugated enzyme linked immunoassay (BisELISA) (Heck et al., 1980). The bison cows selected for the study were 6- to 8-yr-old; 90 to 120 days pregnant, as determined by rectal palpation and known breeding dates, and had not been vaccinated against brucellosis. All bison in the study were identified with ear tattoos.

Vaccination

In November 1986, 48 bison cows were manually injected (MI) subcutaneously via sterile disposable needle and syringe with 2 ml of diluent containing 5.3×10^8 CFU of S19 liquid vaccine provided by the National Veterinary Services Laboratory (NVSL; Ames, Iowa 50010, USA). This S19 dosage was within the range recommended by the United States Department of Agriculture/Animal and Plant Inspection Service/Veterinary Service (1985) for use in adult cattle. Forty-four bison cows were ballistically inoculated (BI) either subcutaneously or intramuscularly from a distance of >3 m with a 6.53 mm absorbable hollow pellet (BallistiVet® Inc., White Bear Lake, Minnesota 55127, USA) containing lyophilized S19 at a dose of $\geq 1.7 \times 10^9$ CFU (Angus, 1989). All remote vaccine delivery systems are subject to inherent variables such as injection site, route of inoculation (subcutaneous, intramuscular, intravenous, intraperitoneal, etc.), amount of bleeding from the wound at the injection site, in vivo reconstitution and contaminants at the injection site. All of these variables can effect the amount and route of vaccine actually delivered to an animal via the BallisitiVet/BioBullet® System, so a precise dosage of S19 was difficult to determine. This S19 dose and the remote delivery system was the same as that utilized in Wyoming to vaccinate elk (*Cervus elaphus nelsoni*) on winter feedgrounds (Herriges et al., 1988, 1989; Angus, 1989). An additional 46 bison cows were hand injected subcutaneously with 2 ml of physiologically buffered saline as non-vaccinated controls (NVC). After vaccination, the bison in the study were returned to the range with 837 other bred bison.

Challenge with *B. abortus* strain 2308

In November 1987, all bison cows on the ranch were bled, palpated to confirm pregnancy, and 30 each of the MI, BI, and NVC were randomly selected without regard to their history of vaccine induced abortions or serologic profiles, and shipped via truck to the Veterinary Medical Park, Texas A&M University (College Station, Texas 77843, USA). Three were injured during transit from Wyoming and died. Approximately 1 mo after arrival, blood samples were collected and 30 MI, 27 BI, and 30 NVC bison were individually challenged with 1×10^7 CFU of *B. abortus* strain 2308 via bilateral conjunctival inoculation. After challenge, three groups of 29 bison (10 MI, 9 BI, and 10 NVC randomly selected) were each placed in three spacially separated isolation paddocks of approximately 1 ha to equalize pen effect. Eight bison (two MI, three BI, and three NVC) were injured during handling procedures and were subsequently euthanatized. All bison were fed a commercial 11% protein complete bulk ration daily at approximately 3% of their body weight and round-baled pasture hay ad libitum.

Sample collection and examination

After S19 vaccination, the bison in the experimental groups were observed daily and any indication of abortion such as retained placentas, vaginal discharges, or direct observation of fetuses was recorded by the ranch operators. Ear-tag numbers of aborting or suspect bison were noted. Tissues from 12 aborted fetuses were collected and stored at −20 C. Lung, abomasum, abomasal contents, meconium, and mediastinal lymph node were collected at necropsy. A BI cow observed aborting was killed by gunshot and parotid, suprapharyngeal, and supramammary lymph nodes, placenta, uterus, and milk samples were collected at necropsy.

In September 1987, all breeding cows on the ranch were bled, and lactational status deter-

mined. Lactational status was used as a correlate of the rancher's records of abortions and retained placentas. Fourteen of 837 non-experimental cows in the herd became serologically reactive. Eight were slaughtered in November 1987 and six in November 1988. Tissues were collected for culture.

At the termination of pregnancy after challenge with *B. abortus* 2308, blood, placenta, uterine swabs, and milk samples from each quarter of the udder were collected from the dam. Blood and rectal swabs were collected from live born-calves and they were ear-tagged. Following abortion, the fetus was recovered within 8 hr and necropsied. Lung, abomasum, abomasal contents, meconium, and mediastinal lymph node were collected for culture. Tissues and swabs were stored at −70 C until plated on Farrell's restrictive media (Farrell, 1974) and blood agar for bacteriologic isolation and identification. Strain 19 differs from other strains of biovar 1 in requirement for CO_2, sensitivity to thionine blue, penicillin, safranin O, and erythritol (Nicoletti, 1990). Sera were stored at −70 C. Sera were evaluated for *Brucella* sp. specific antibodies by the card, RIV, CCFT, and HIG tests, and BisELISA. After termination of pregnancy, cows and calves were removed from their group.

Statistical analyses of the data

Protection against *Brucella* sp. abortion and protection against *Brucella* sp. infection provided by S19 adult vaccination were compared to NVC by Chi Squares and Fisher's Exact Test. Protection against infection was defined as the inability to isolate *B. abortus* strain 2308 from the bison dam her fetus or calf.

RESULTS

Post-vaccination abortions

Approximately 60 days post-vaccination, abortions and retained placentas were observed in both groups of vaccinated bison. *Brucella abortus* S19 was recovered from the lymph nodes and reproductive tissues of the aborting cow, her fetus, and 11 of 12 of the other recovered fetuses, primarily from abomasal contents. Serum from the cow reacted on the card, RIV, CCFT and HIG tests, and BisELISA. Abortions in the vaccinated bison continued for approximately 3 mo, but no abortions were observed in the NVC or in the other bred bison in the pasture.

By the rancher's observations, 58% (53 of 92) of vaccinates had aborted, 50% (24 of 48) of the MI and 66% (29 of 44) of the BI were observed to have aborted. No abortions were observed in the NVC or other bison. Lactational status at 10 mo post-vaccination indicated that 69% (63 of 92) of vaccinates, 60% (29 of 48) of the MI and 77% (34 of 44) of the BI were dry, compared to 13% (six of 46) in the NVC. Calving success based on lactational status from the remainder of the herd was 93% (779 of 837).

One of the bison shipped to Texas A&M University in November 1987, aborted on 31 December 1987, and S19 was isolated from the dam and fetus. She was in the BI group and had also aborted during her first pregnancy following vaccination.

Persistent vaccinal titers

Sera collected 10 mo post-vaccination indicated as many as 73% (66 of 91) of bison were serologically reactive on either the card, RIV, CCFT and HIG tests, or BisELISA. Sixty-three percent (30 of 48) and 84% (36 of 43) of bison were seroreactive to at least one test in MI and BI, respectively (Table 1). The 46 NVC bison were negative.

Exposure of non-vaccinated bison to S19

Two percent (14 of 837) of non-vaccinated cows sharing winter pastures with the vaccinated bison became seroreactive (Table 2). *Brucella* spp. were not isolated from bison tissues collected at slaughter.

Protection against abortion provided by S19 adult vaccination

Post-challenge abortions were observed from 23 January 1988, 39 days post-exposure (PE) until March 1988. Abortions occurred in 33% (17 of 52) of vaccinates; 43% (12 of 28) of MI aborted; and 21% (five of 24) of BI aborted. Abortions occurred in 96% (26 of 27) of NVC. Protection against abortion provided by vaccination compared to NVC were: 67% (35 of 52) ($\chi^2 = 28.99$) for S19 vaccinates; 57%

TABLE 1. Serologic response of 91 bison cows 10 mo post-vaccination with *Brucella abortus* strain 19.

Experimental group	Serologic tests				
	Number reactive card (+)[a]	Number reactive RIV ≥25[b]	Number reactive CCFT ≥40[c]	Number reactive HIG ≥10[d]	Number reactive BisELISA ≥1.000[e]
Manually injected (n = 48)	24	30	26	30	27
Ballistically injected (n = 43)	34	36	36	28	20
All S19 vaccinates (n = 91)	58	66	62	58	47

[a] Card (+), card positive reaction.
[b] RIV ≥25, rivanol precipitation test reaction at a serum dilution of ≥ 1:25.
[c] CCFT ≥40, cold complement fixation test reaction at a serum dilution of = 1:40.
[d] HIG ≥10.0, hemolysis-in-gel test reaction at a zone of ≥ 10 mm.
[e] BisELISA ≥1.000, bison conjugated enzyme linked immunosorbent assay with an optical density reading of ≥1.000.

(16 of 28) (χ^2 = 18.38) for MI; and 79% (19 of 24) (χ^2 = 30.35) for BI. Abortion rates in both vaccinated groups were significantly ($P \leq 0.0001$) lower than the NVC.

Forty-eight percent (25 of 52) of bison calves from vaccinated dams survived to weaning; calf survival in the MI group was 39% (11 of 28); and in the BI group (58% (14 of 24). None (zero of 27) of the calves in the NVC survived. *Brucella abortus* strain 2308 was isolated from all fetuses and dead calves.

Protection against infection provided by adult S19 vaccination

Brucella abortus strain 2308 was not recovered from 39% (21 of 54) of vaccinated cows or their calves. Thirty percent (nine of 30) of MI and 50% (12 of 24) of BI were protected from infection. None (zero of 30) of NVC were protected. Protection from infection provided by vaccination compared to controls were: HI+BI (χ^2 = 15.56), MI (χ^2 = 10.59), and BI (χ^2 = 19.29). All of the vaccinal groups were significantly different ($P \leq 0.001$) from NVC.

TABLE 2. Serologic response of 14 non-vaccinated bison cows 10 mo after comingling with 91 strain 19 vaccinated bison cows on winter pastures.

Cow number	Serologic tests				
	Card	RIV	CCFT	HIG	BisELISA
5	(+)[a]	400[b]	160[c]	12.0[d]	1.482[e]
9	(−)	25	20	Negative	1.238
37	(+)	200	80	11.0	0.998
67	(−)	50	40	12.0	1.206
73	(+)	100	80	10.0	1.264
218	(−)	50	40	11.0	1.189
246	(+)	100	80	10.0	1.092
262	(+)	200	80	12.0	1.227
323	(−)	25	80	10.0	1.038
374	(+)	200	160	12.0	1.482
803	(−)	25	20	Negative	0.851
809	(−)	25	10	Negative	0.697
813	(−)	Negative	10	Negative	0.590
836	(−)	50	40	Negative	0.923

[a] Card (+), card positive reaction.
[b] RIV 400, rivanol precipitation test reaction at a serum dilution of 1:400.
[c] CCFT 160, cold complement fixation test reaction at a serum dilution of 1:160.
[d] HIG 12.0, hemolysis-in-gel test reaction at a zone of 12 mm.
[e] BisELISA 1.482, bison conjugated enzyme linked immunosorbent assay with an optical density reading of 1.482.

DISCUSSION

Vaccination of pregnant bison with 5×10^8 CFU S19 which is the recommended dose for adult cattle and bison (United States Department of Agricultural/Animal and Plant Inspection Service/Veterinary Service, 1985), or the higher dose of S19 used to ballistically vaccinate elk (Herriges et al., 1988, 1989) has disadvantages. Bison like many wild ungulates are seasonal in their reproductive cycles. Commercial producers of bison annually roundup and work bison in September to November to wean calves as late as possible. Bred bison females to be S19 vaccinated at this time are in the first or second trimester of pregnancy. Vaccination of adult pregnant bison is not common and the reasons for this are obvious. The rate of vaccine induced abortions (58%) observed in vaccinated pregnant bison is not acceptable to private producers of bison, due to loss of production, and should be carefully considered by health officials, due to the potential for secondary S19 infections in other animals or humans. In the higher BI delivered dose, the vaccine induced abortion rate of 66% observed would eliminate most of the annual bison calf crop in any bison herd vaccinated in this manner and would expose the environment to considerable amounts of a human pathogen. The dose of 5×10^8 CFU S19 in pregnant cattle will cause abortions, but the rate of vaccine abortions is $<1\%$ (Nicoletti, 1977).

The high percentage (73%) of seroreactive bison at 10 mo, and the occurrence of a vaccine-induced abortion at 13 mo postvaccination indicates that chronic S19 infections may occur at the dosages used in this study. The larger percentage of vaccine-induced abortions, chronic S19 infections, and persistent titers observed in the BI compared to MI bison is probably dose related. Persistent vaccinal titers could confuse regulatory testing and sero-epidemiologic studies. USDA regulations require that bison be serologically tested by the same criteria as cattle. Serologic tests currently utilized do not distinguish vaccinal titers from those resulting from field *Brucella* spp. infections.

The lack of recovery of S19 from tissues of the 14 cows which seroconverted after comingling with vaccinated and aborting bison indicates that secondary exposure to S19 probably did not result in chronic S19 infections. The potential hazard of S19 abortion products to humans should not be overlooked.

Results of S19 adult vaccination at the doses used was discouraging. The 67% protection against abortion and the 39% protection against infection observed in the S19 vaccinated bison is similar to or lower than expected in S19 vaccinated adult cattle which is 65 to 75% and 50 to 65% respectively (Manthei, 1952, 1959; Manthei et al., 1952; Deyoe et al., 1979; Crawford et al., 1988). However, many of the vaccinated bison in this study had previously aborted due to S19. For example, the BI bison had the best protection against both post-challenge abortions (79%) and infections (50%), but this group also had the largest number of S19 vaccine induced abortions (66%) and persistent titers (84%).

Brucellosis in bison continues to be a political issue in both the United States and Canada, and a potential threat to domestic livestock herds in association with infected bison (Flagg, 1983; Tessaro, 1986, 1989; Davis, 1990). Incidence of *Brucella* spp. infected bison is high in some public herds (Ferlicka, 1989). A vaccination program could be useful in reducing brucellosis in bison. The vaccine utilized should be more protective than S19 as used in this study and without the problems associated with human pathogenicity, potential virulence or infectivity in non-target species, and interference with diagnostic serology. An efficient method of vaccine delivery to wide-ranging terrestrial sylvatic animals would also be necessary.

Strain 19 vaccination alone will not eliminate brucellosis from an infected herd of cattle (Manthei, 1959). The only proven method to eliminate brucellosis from a herd

of infected animals is to test and remove the infected animals. This has been accomplished in thousands of cattle herds, several publicly owned bison herds such as the National Bison Range, Montana and Custer State Park, South Dakota, and in most privately owned bison herds in the United States (Tessaro, 1989). If vaccination of free-ranging bison is considered, the objectives, the limitations, and the cost/benefit ratio of the program should be understood. The results of this study suggest that S19 vaccination of pregnant cows will be ineffective in a free-ranging bison herd heavily infected with brucellosis such as those in Yellowstone National Park or in Canada.

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