

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

---

USDA National Wildlife Research Center - Staff  
Publications

U.S. Department of Agriculture: Animal and Plant  
Health Inspection Service

---

2009

# Pathogenesis And Epidemiology Of Brucellosis In Yellowstone Bison: Serologic And Culture Results From Adult Females And Their Progeny

Jack C. Rhyan

*US Department of Agriculture, jack.c.rhyan@aphis.usda.gov*

Keith Aune

*Wildlife Conservation Society*

Thomas Roffe

*US Fish and Wildlife Service*

Darla Ewalt

*US Department of Agriculture*

Steve Hennager

*US Department of Agriculture*

*See next page for additional authors*

Follow this and additional works at: [http://digitalcommons.unl.edu/icwdm\\_usdanwrc](http://digitalcommons.unl.edu/icwdm_usdanwrc)



Part of the [Environmental Sciences Commons](#)

---

Rhyan, Jack C.; Aune, Keith; Roffe, Thomas; Ewalt, Darla; Hennager, Steve; Gidlewski, Tom; Olsen, Steve; and Clarke, Ryan, "Pathogenesis And Epidemiology Of Brucellosis In Yellowstone Bison: Serologic And Culture Results From Adult Females And Their Progeny" (2009). *USDA National Wildlife Research Center - Staff Publications*. 1060.  
[http://digitalcommons.unl.edu/icwdm\\_usdanwrc/1060](http://digitalcommons.unl.edu/icwdm_usdanwrc/1060)

This Article is brought to you for free and open access by the U.S. Department of Agriculture: Animal and Plant Health Inspection Service at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in USDA National Wildlife Research Center - Staff Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Jack C. Rhyan, Keith Aune, Thomas Roffe, Darla Ewalt, Steve Hennager, Tom Gidlewski, Steve Olsen, and Ryan Clarke

# PATHOGENESIS AND EPIDEMIOLOGY OF BRUCELLOSIS IN YELLOWSTONE BISON: SEROLOGIC AND CULTURE RESULTS FROM ADULT FEMALES AND THEIR PROGENY

Jack C. Rhyan,<sup>1,9</sup> Keith Aune,<sup>2,7</sup> Thomas Roffe,<sup>3,8</sup> Darla Ewalt,<sup>4</sup> Steve Hennager,<sup>4</sup> Tom Gidlewski,<sup>1</sup> Steve Olsen,<sup>5</sup> and Ryan Clarke<sup>6</sup>

<sup>1</sup> National Wildlife Research Center, Animal and Plant Health Inspection Service, US Department of Agriculture, Fort Collins, Colorado 80521, USA

<sup>2</sup> Montana Department of Fish, Wildlife and Parks, Bozeman, Montana 59717, USA

<sup>3</sup> Biological Resource Division, US Geological Survey, US Department of Interior, Bozeman, Montana, 59717, USA

<sup>4</sup> National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, US Department of Agriculture, Ames, Iowa 50010, USA

<sup>5</sup> National Animal Disease Center, Agricultural Research Service, US Department of Agriculture, Ames, Iowa 50010, USA

<sup>6</sup> Western Region, Animal and Plant Health Inspection Service, US Department of Agriculture, Belgrade, Montana 59714, USA

<sup>7</sup> Current address: Wildlife Conservation Society, Bozeman, Montana 59715, USA

<sup>8</sup> Current address: US Fish and Wildlife Service, Bozeman, Montana 59717, USA

<sup>9</sup> Corresponding author (email: jack.c.rhyan@aphis.usda.gov)

**ABSTRACT:** Our objective in this prospective study was to determine the natural course of *Brucella abortus* infection in cohorts of seropositive and seronegative, female bison (*Bison bison*) and their offspring in Yellowstone National Park (YNP) for 5 yr. We collected specimens from 53 adult females and 25 calves at least once and from 45 adults and 22 calves more than once. Annual seroconversion rates (negative to positive) were relatively high (23% for calves and juvenile bison, 6% in the total sample of adult female bison in our study, and 11% in the adult females that began the study as seronegatives). Antibody was not protective against infection, even for calves that passively received antibody from an infected mother's colostrum. Antibody levels stayed remarkably constant, with only a slow decline over time. We found only two seroconversions from a weak positive status to negative. Infected bison aborted and shed viable bacteria. Risk of shedding infective *Brucella* was highest for bison in the 2 yr following seroconversion from negative to positive. In one bison, we detected shedding for 3 yr following seroconversion. Regardless of serostatus of dams and neonates, most calves were seronegative by 5 mo of age. There was no relationship between the antibody status of the dam and the tendency of a calf to seroconvert to positive during the duration of the study.

**Key words:** *Bison bison*, Bovidae, *Brucella abortus*, brucellosis, epidemiology, serology, Yellowstone National Park.

## INTRODUCTION

The significance of brucellosis infection (*Brucella abortus*) in bison (*Bison bison*) of the Greater Yellowstone Area (GYA) and the risk bison pose for transmission of brucellosis to livestock is controversial and debated. The first evidence of brucellosis in bison of the GYA was found in 1917 in the introduced herd kept at the Buffalo Ranch in the Lamar Valley in Yellowstone National Park (YNP; Mohler, 1917). Abortions were noted in two bison cows and agglutination tests showed the aborting animals had antibody to *B. abortus*. Between that observation and 1992 (Cheville, 1998), no brucellosis-confirmed

abortions were reported from YNP bison. This hiatus in confirmation of brucellosis-related abortion led some to believe that the infection no longer produced abortions in YNP bison, and therefore, bison posed little, if any, risk of transmission to cattle herds on land adjacent to YNP (Meyer and Meagher, 1995). Numerous studies of animals going to slaughter, bison being translocated to other properties, animals killed as population management actions, and bison used in brucellosis vaccination trials conducted by the Department of Interior in the 1940s confirmed the presence of brucellosis antibodies in the herd (Meagher, 1973; Cheville et al., 1998). Results of these

studies were somewhat varied, but today, the herd is generally considered to have an antibody prevalence of approximately 50%.

There is continued evidence indicating that brucellosis still induces abortion in this bison population and could pose a risk of transmission to susceptible animals. Rush (1932) observed abortions in the YNP herd and suspected brucellosis as the etiology based on serologic findings of *Brucella* antibodies in 58 (53%) of 110 bison sampled. Culture studies in 1985 and 1991–92 resulted in isolation of *B. abortus* from 6 (7%) of 88 and 26 (12%) of 218 YNP bison, respectively (Cheville et al., 1998). In a more rigorous study, conducted between 1995 and 1999, *B. abortus* was isolated from tissues from 12 (46%) of 26 seropositive bison from YNP (Roffe et al. 1999). Between 1992 and 1999, before and concurrent with the work reported here, six cases of abortion or neonatal death from *B. abortus* biovar 1 infection were confirmed in YNP bison (Rhyan et al., 1994, 2001).

The purpose of this study was to determine the natural course of *B. abortus* infection in a cohort of seronegative and seropositive, free-ranging, adult, female bison and their offspring for 5 yr. Survival and reproductive rates related to antibody status and age from this study have already been reported (Fuller et al. 2006). Here, we report the natural course of *B. abortus* infection in YNP bison, based on *Brucella* serology and culture, and the reproductive outcomes of seroconverting female bison.

## MATERIALS AND METHODS

### Animal selection and sampling

We conducted 1 yr of pilot work to perfect methodologies, commencing October 1995 and concluding field operations in October 2001. All field work was conducted in YNP (44°8'N to 45°7'N, 110°0'W to 111°4'W). In the fall (generally October), adult, female bison in good condition were randomly selected for capture by chemical immobilization using carfentanil (range 3.3–6.0 mg) and xylazine (range 30–70 mg) delivered by 2-ml

dart (Pneu-Dart, Williamsport, Pennsylvania, USA) and antagonized with naltrexone (range 400–850 mg) and tolazoline (range 100–1,000 mg) or yohimbine (range 45–60 mg) by hand injection. We determined age of animals by incisor eruption and wear (Fuller, 1959; Dimmick and Pelton, 1996) and collected samples of heparinized and whole blood, milk (if present), feces, and cervical and oral swabs. Heparinized blood was immediately centrifuged, and plasma was tested for antibodies to *B. abortus* by the standard card test (Anonymous, 1965b), while the animal was immobilized. Pregnancy status was determined by rectal palpation and ultrasonography (Model SSD-500V; Aloca, Tokyo, Japan). We also later submitted serum for laboratory assay of pregnancy-specific protein B (PSPB; Haigh et al., 1991). For the study, we wanted only pregnant bison with about an equal number of seropositive/suspect and seronegative bison, in similar age distributions. While immobilized, the bison was accepted or rejected for the study based on antibody status (as determined by the card test) and pregnancy status (as determined by rectal palpation and ultrasonography). Each accepted animal was tagged with a Very High Frequency (VHF) or a Global Positioning Satellite (GPS)-containing VHF radiocollar (Aune et al. 1998) and a uniquely numbered, small, metal ear tag. We later confirmed serologic status in the laboratory using multiple serologic tests (see “Laboratory Procedures” below) and “Uniform Methods and Rules” criteria (USDA 2003). Pregnancy status was confirmed using combined palpation, PSPB level, and ultrasonography. In the rare instance of a discrepancy in pregnancy test results, an ultrasonographic image of viable fetus was regarded as positive. In this manner, we classified 26 bison as pregnant/seropositive and 27 as pregnant/seronegative.

We recaptured radiocollared bison in winter (February and March) and the subsequent fall (usually October) of each year, either through chemical immobilization or by net gun fired from a helicopter, with subsequent hobbling, and collected the same suite of samples. For study bison that produced offspring, we captured those offspring on the same schedule as the original adult females.

For winter captures, we determined pregnancy status in the field by rectal palpation, later confirmed with a PSPB assay. We implanted pregnant animals with vaginal transmitters (Advanced Telemetry Systems, Inc., Isanti, Minnesota, USA), each emitting a continuous radio signal of a unique radio frequency or rhythm (double pulse) at a rate of

approximately 60 signals/min. The vaginal transmitters were programmed to decrease the rate of signal transmission when motionless for more than 4 hr. Alternatively, some transmitters were programmed to decrease the rate of signal transmission when the device's temperature dropped below 30 C. A decreased signal rate created by an expelled vaginal transmitter was, therefore, a presumptive indication of parturition or abortion. Following expulsion, the vaginal transmitters were located, aseptically collected, and later, cultured for *Brucellae*. Additionally, we examined the site for evidence of parturition or abortion (tissues or fluids) and collected any observed material (including apparently contaminated soil or vegetation) for culture and, if tissue was collected, histopathology.

When we detected a decreased rate of signal from a vaginal transmitter, we made every effort to find the transmitter and visually observe the cow and any accompanying calf. During the period from March through June each year, project personnel monitored animal movements and vaginal transmitter signals daily. Within 5 days of parturition, we immobilized the cow and collected samples as in fall and winter. We attempted to capture any accompanying calf and collected specimens (feces, oral, and ocular swabs) for culture and blood for culture and serologic testing. We placed only a metal ear tag on newborn calves until the following fall or winter when, if captured, they were fitted with a VHF radiocollar. During the spring and summer, field personnel observed radioinstrumented cows for the presence of a suckling calf at their side. When bison were captured in the fall, we determined pregnancy and antibody status as above but did not place vaginal transmitters in putatively pregnant bison.

### Laboratory procedures

We froze samples of heparinized blood, milk, and swabs placed in 1-ml WHO media (National Veterinary Services Laboratories [NVSL], Ames, Iowa, USA) on dry ice each evening and kept the specimens on dry ice or in a  $-70$  freezer until shipment to the NVSL for culture, using the methods of Alton et al. (1988). We centrifuged whole-blood specimens and collected, aliquoted, and shipped sera to NVSL for a panel of nine serologic tests: standard card, standard plate (SPT), standard tube (STT; Anonymous, 1965a), rivanol, buffered acidified plate antigen (BAPA; Anonymous, 1965b), complement fixation (CF; Anonymous, 1993), particle concentrate fluorescence immunoassay

(PCFIA; IDEXX Laboratories, Westbrook, Maine, USA), rapid automated presumptive test (RAP), and a competitive enzyme-linked immunosorbent assay (D-Tec, Synbiotics Corporation, San Diego, California, USA).

### Data and statistical analyses

Animal-years for each group of animals was calculated by totaling the number of months each animal was in the study (first capture to last capture) and dividing by 12. The annual seroconversion rate for a group was calculated by dividing the total number of seroconversions for the group by the total number of animal years for the group (i.e., *number of positive seroconversions in adults/total number animal-years for adults in study*). Methods likely underestimated the seroconversion rate because individual animals converted between captures, but we used the total time between tests in the denominator of animal years, resulting in the denominator being biased high (upper limit).

A reproductive failure was defined as a female bison of reproductive age ( $\geq 3$  yr) that failed to bear a live calf or bore a weak calf that died as a neonate. Birth of a live calf was confirmed by observation of the calf or evidence of suckling at the first capture following parturition (usually May, June, July, or October). By this definition, abortion, neonatal death (pregnant in the fall or winter sampling and no visual evidence of a calf or evidence of suckling at capture following expulsion of the vaginal transmitter), or not becoming pregnant would all be classified as reproductive failures.

At each capture, we assigned the serostatus and noted changes in that status from previous captures. We assigned *seroconverter* status to those bison who changed antibody status between captures (negative to positive=*positive seroconverter*; positive or suspect to negative=*negative seroconverter*) and *nonconverter* if the antibody status remained the same between two captures. To examine the relationship between gender and positive seroconversion in offspring, we used a Pearson chi-square test in a  $2 \times 2$  contingency table, including the number of positive seroconverting calves and juveniles by gender and the number of negative nonseroconverting offspring by gender. We also used a Pearson chi-square test to assess the influence of the mother's antibody status on the tendency of offspring to be seropositive at any time in the study through a  $2 \times 2$  contingency table (positive calves in this analysis included calves that remained seropositive or seroconverted to

positive). Interval censoring (only the window when seroconversion occurred was known) and right censoring (death, collar failure, study ends before seroconversion) of the data were approximately equal across gender and sero-status of the dam, thus not biasing the conclusions.

## RESULTS

During the course of the study, we immobilized 53 adult female bison (27 [51%] seronegative and 26 [49%] seropositive or suspect upon initial capture) and collected specimens from them at least once. We captured and collected specimens more than once from 45 (85%) of the 53 bison. Of these 45 repeat-capture bison, 28 (56%) had 45 calves across the years that we were able to capture and from which we collected samples at least once during the study.

Seventeen (38%) of the 45 repeat-captured adults remained seronegative for their entire study (total 42.5 animal-years in the study, mean 2.5 yr/animal); 18 (40%) remained seropositive or suspect (total 58.6 animal-yr, mean 3.3 yr/animal); eight (18%) converted from seronegative to seropositive (total 32.9 animal-yr, mean 4.1 yr/animal); and two (4%) converted from weak positive or suspect to seronegative (total 5.7 animal-yr; mean 2.8 yr/animal). We documented 5.7 positive seroconversions/100 animal-yr and 1.4 negative seroconversions/100 animal-yr for the 45 adult bison. Among the 25 cows (56%) that began the study as seronegatives and were monitored more than once, the annual conversion rate to seropositive was 11%. Cows converted to seropositive at all ages (Table 1).

We collected specimens from 45 calves born to the original cows during the study; once from 23 of these calves (51%), twice from 12 calves (27%), and up to 11 times from the remaining 10 (22%). We also collected one-time samples from two calves born to female offspring of the original radiocollared cows. The total time in the study for the 22 calves captured

more than once was 39.6 yr (mean 1.8 yr/animal). The first capture and sampling of the 47 calves in our study occurred as newborns ( $n=12$ , 26%), 5 to 6 mo old ( $n=34$ , 72%), and yearlings ( $n=1$ , 2%).

All of the calves born to seronegative dams and caught as newborns were seronegative at birth. Most of the newborn-caught calves born to seropositive cows had antibody titers to *B. abortus* detected on one or more tests (Table 2). At 5 mo, however, the majority of calves were seronegative regardless of their dam's antibody status.

Two calves born to seropositive dams had two or more positive serologic tests when first captured, either as a newborn (calf 898) or at 5 mo of age (calf 812). At recapture, 5 and 7 mo later, respectively, these calves were negative on all tests. Calf 880, born to cow 853, which seroconverted during or immediately after that calving season, was seronegative the following October and February despite suckling milk that was culture-positive at both captures. In contrast, calf 818, born to a strongly seropositive cow (830), had high antibody titers to *B. abortus* on all tests when first sampled at 5 mo of age. Four months later, the calf remained strongly seropositive, and whole blood was culture-positive for *B. abortus*. This animal remained seropositive for the entire 4 yr it was in the study.

Conversion from seronegative to seropositive occurred in seven calves (15%) born to the radiocollared cows during the study. An additional two calves (869 and 877; 4%) were positive on one serologic test only when first caught at 5 mo of age and were later seropositive on multiple tests. Positive seroconversion occurred in calves born to both seronegative and seropositive dams (Table 1). The annual positive seroconversion rate for the 22 calves captured more than once was 23% (nine seroconversions/39.6 animal-yr). For animals born while in the study, first detection of positive seroconversion occurred from 5 mo to 33 mo of age but most often occurred

TABLE 1. Age and culture results of bison from Yellowstone National Park seroconverting to positive for brucellosis.<sup>a</sup>

Bison No.	Dam No. and serostatus at calf's birth	Sex	No. captures	Date first captured	Time in study (first to last capture)	Bison age at SC first detected	Date SC first detected	Date and specimen positive <i>B. abortus</i> culture
805 <sup>b</sup>		F	16	October 1995	6 yr	9 yr	October 2000	February 2001: blood April 2001: milk, vagina, feces
820	805 neg	M	5	October 1996	2 yr	1 yr, 9 mo	February 1998	
819	805 neg	F	11	October 1997	4 yr	1 yr, 5 mo	October 1998	May 2000: milk
884	805 neg	M	4	May 1998	2 yr, 5 mo	5 mo	October 1998	
806 <sup>b</sup>		F	12	October 1995	6 yr	6 yr	October 1996	October 1996: blood
895	806 pos	M	2	October 1999	1 yr, 4 mo	1 yr, 9 mo	February 01	February 2001: blood
833 <sup>b</sup>		F	12	October 1996	4 yr, 8 mo	8 yr	October 1999	
877	833 neg	F	2	October 1998	1 yr	1 yr, 5 mo	October 1999	
844 <sup>b</sup>		F	8	October 1997	3 yr, 7 mo	3 yr	May 1998	October 2000: blood May 2001: milk
848 <sup>b</sup>		F	9	October 1997	3 yr, 5 mo	6 yr	3/01	March 2001 vagina
875	848 neg	M	3	October 1998	2 yr	11 mo	April 1999	
853 <sup>b</sup>		F	7	October 1997	2 yr, 4 mo	4 yr	October 1999	October 1999: milk February 2001: milk
6691 <sup>b</sup>		F	7	February 1998	3 yr, 8 mo	3 yr	October 1998	October 1999: vagina March 2000: vagina
869	6691 pos	M	2	October 1999	1 yr, 8 mo	2 yr, 1 mo	June 2001	
6820 <sup>b</sup>		F	6	February 1998	3 yr, 3 mo	10 yr	February 1999	
887	3038 pos	M	3	October 1998	2 yr, 4 mo	2 yr, 9 mo	February 2001	
893	6752 neg	F	6	October 1998	3 yr	2 yr, 5 mo	October 2000	

<sup>a</sup> SC = seroconversion from negative to positive; neg = negative; pos = positive; M = male; F = female.

<sup>b</sup> Original cows in the study.

TABLE 2. Serologic results of calves born to seropositive or suspect and seronegative dams and captured and sampled during the study.

Calves and dams	No. seropositive or suspect/ No. calves sampled (%)		No. of calves that seroconverted negative to positive while in study
	Newborn	5–6 mo of age	
Calves born to seronegative dams (n=20)	0/5 (0)	2 <sup>a</sup> /17 (12)	6 <sup>b</sup>
Calves born to seropositive/suspect dams (n=27)	5 <sup>c</sup> /7 (71)	3 <sup>d</sup> /20 (15)	3 <sup>e</sup>

<sup>a</sup> One calf (No. 877) was positive on only the standard plate test; one calf (No. 884) was strongly seropositive after having been seronegative as a newborn.

<sup>b</sup> No. includes calf No. 877 that was seropositive on the standard plate test at 5 mo. of age and was positive on all serologic tests 1 yr later.

<sup>c</sup> The two seronegative newborns are calf No. 899, whose dam was only a serologic suspect at the calf's birth, and a calf that had not suckled at capture because of multiple congenital anomalies.

<sup>d</sup> Seropositive calves include No. 818 that had high titers on multiple tests and was culture-positive 4 mo. later, No. 812 that was positive on particle concentrate fluorescence immunoassay (PCFIA) only and was negative 7 mo later, and No. 869 that was positive on complement fixation and suspect on PCFIA only but seroconverted to positive on multiple tests at 2 yr.

<sup>e</sup> No. includes calf No. 869, described in footnote d.

during the second year of life. Six (46%) of the 13 bull calves/juveniles that were captured at least twice (21.7 animal-yr, mean 1.7 yr/animal) seroconverted, and three (33%) of the nine females captured at least twice (17.9 animal-yr, mean 2 yr/animal) seroconverted.

We isolated *B. abortus* biovar 1 from one or more specimens at one or more captures from eight bison that seroconverted to positive during the study (Table 1). The time delay between first detection of seroconversion and the positive culture varied from immediate to 2.5 yr (cow 844). Specimens from three additional seropositive animals were also culture-positive for *B. abortus* biovar 1, including the blood of cow 813 and the milk of cow 827, both once-caught bison, and the blood of calf 818 at 9 mo of age.

The duration of infection detected by culture of collected specimens varied. We isolated *Brucella* only once from some bison and up to 3 yr after seroconversion in bison 844 (from milk). Our 17 isolates of *Brucella* were almost evenly divided among milk ( $n=6$ ), blood ( $n=6$ ), and vaginal swabs ( $n=4$ ). Bison 805 was culture-positive in feces during late-stage pregnancy. We

found culture-positive vaginal swabs or exudates following abortion (cow 848), just before calving (805), and during fall and winter when not pregnant (6691).

After positive seroconversion, reproductive results for the eight original cows and two of the calves born in the study were varied (Table 3). Of the 24 postseroconversion reproductive seasons monitored for the 10 bison cows, we confirmed 11 live calves (confirmation by observation of calf or evidence of nursing calf at capture), 11 reproductive failures, and two undetermined outcomes. Of the reproductive failures, four were considered abortions based on a positive pregnant status in fall or winter and a negative pregnant test in spring. One had lost its pregnancy status by February, two by March, and one by June. Four other reproductive failures were not pregnant on one or more occasions from October through May, and three did not have adequate testing during normal gestation, but no calf was observed. We found *Brucella* culture-positive vaginal exudate and an involuting uterus indicative of a recent abortion event in one of the March-aborting cows. Three of the abortions (cows 806, 844, and 848) occurred in the gestation concurrent



TABLE 3. Reproductive results of seroconverting female bison of reproductive age.<sup>a</sup>

Animal No.	LC/GBSC <sup>b</sup>	Age SC <sup>c</sup>	LC/GASC <sup>d</sup>	Reproductive outcomes of gestations concurrent with and subsequent to SC				
				First	Second	Third	Fourth	Fifth
805	5/5	9 yr	1/1	LC <sup>e</sup>				
806	1/1	6 yr	3/5	RF (Ab)	Und	LC	LC	LC
833	2/3	8 yr	1/2	LC	Und			
844	0/0	3 yr	0/4	RF (Ab)	RF	RF (Ab)	RF	
848	2/3	6 yr	0/1	RF (Ab)				
853	1/2	4 yr	0/1	RF (open)				
6691	0/1	3 yr	1/4	RF <sup>f</sup>	LC <sup>g</sup>	RF (open)	RF	
6820	1/1	10 yr	2/3	RF (open)	LC	LC		
819 (805's calf)	0/0	1 yr, 5 mo	2/2	LC <sup>h</sup>	LC			
893 (6752's calf)	0/0	2 yr, 5 mo	1/1	LC				
Totals (%)	12/16 (75)		11/24 (46)	4/10	3/6	2/4	1/3	1/1

<sup>a</sup> SC = seroconversion; LC = live calf; GBSC = gestations before SC; GASC = gestations after (concurrent with or subsequent to) SC; RF = reproductive failure; Ab = abortion; Und = undetermined; open = not pregnant

<sup>b</sup> No. of confirmed LC born per No. of monitored GBSC (*live calves/gestations before SC*).

<sup>c</sup> Age at which SC was first detected.

<sup>d</sup> No of confirmed LC born per No. of monitored GASC (*live calves/gestation after seroconversion*).

<sup>e</sup> Calf not observed, but there was evidence of nursing calf in October.

<sup>f</sup> No. 6691 was pregnant and seronegative in February 1998, was not recaptured May 1998, was seropositive with no evidence of calf in October 1998.

<sup>g</sup> No. 6691 calved late summer and had culture positive vaginal swab in October and February after calving.

<sup>h</sup> Calf No. 819 had multiple congenital anomalies; calf was euthanized and necropsied, and results were culture negative, whereas dam's milk was culture positive.

with, or immediately after, seroconversion and, in one case (cow 844), again 2 yr later. Three other recently seroconverted cows had live calves following seroconversion. Another cow (805), captured in late pregnancy (April), was *B. abortus* culture-positive in vaginal exudate, milk, and feces. The cow was next captured in October, when it had evidence of a suckling calf, so was considered to have given birth to a live calf.

Chi-square statistics indicated no significant relationship between gender and positive seroconversion ( $P=0.54$ ). Our analysis also showed no relationship between antibody status of bison cows and the tendency of a calf to convert to seropositive or remain seropositive during the duration of the study ( $P=0.19$ ). There was a significant difference ( $P=0.03$ ) in the proportion of newborn seropositive calves born to seronegative dams and seropositive dams.

## DISCUSSION

Serologic, culture, and reproductive results of this study are consistent with those observed in previous experimental infections (Davis, et al., 1990; Olsen et al., 2003). Except for animals seroconverting from negative to positive, positive antibody titers to *B. abortus* were remarkably stable throughout the study, likely reflecting the long-term persistent nature of *Brucella* infection with chronic low to high levels of antigenic stimulation. Reexposure of some animals to the organism probably occurred during the study; however, spikes in positive serologic titers were not observed. Two adult bison with suspect or low titers on the first collection became seronegative during the study.

The significant relationship between *Brucella* antibody in newborn bison and the cow's antibody status, coupled with the loss of antibody by most calves within

5 mo, is an indication of passively transferred antibodies to the newborns via colostrum from seropositive dams. This is similar to the process in cattle, where most antibody titers of calves born to seropositive dams disappear within 2 to 4 mo of age with a few persisting to 6 mo (Winthrop et al., 1988). These passively transferred antibodies are unlikely to provide any significant protective benefits later in life against infection with *B. abortus* in YNP bison. Calves born to both seronegative dams and seropositive dams experienced seroconversion and infection during the study.

The annual seroconversion rate in bison calves and juveniles less than 3 yr of age was approximately 20%, and in adult females, approximately 10%. We have observed curious and precocious behavior at calving time, especially in young bulls, and have proposed that behavior as a factor resulting in increased exposure of juveniles (Rhyan, 2000). These high rates of seroconversion are significant because conversion to a positive serostatus was clearly linked with stimulation and growth of the *Brucella* organism, although the time delay for detecting *B. abortus* infection after seroconversion varied. We obtained positive culture results most often from animals that had recently seroconverted (within 2 yr of seroconversion). Following seroconversion, *B. abortus* could be isolated from blood, milk, or vaginal secretions from some animals for prolonged periods, in one of our bison up to 3 yr. These findings suggest that recently seroconverting bison pose the highest risk for transmission and that the window of opportunity for bison to shed infective *Brucella* bacteria is long (at least 3 yr). The finding that one bison (805) shed viable *Brucella* in feces during late pregnancy and unrelated to her own abortion event suggests feces could be an additional mechanism for distributing viable *Brucella*. Shedding of *B. abortus* in feces of cattle (Fitch et al., 1932) and bison (Rhyan et al., 2001) has been reported previously; however, in these reports it occurred immediately

following abortion and was attributed to the dams' ingestion of infected products of parturition. The shedding of *B. abortus* in her feces before parturition by bison 805 may have resulted from her own infection, or alternatively, she may have ingested parturition products from another infected bison.

Based on our data and that reported in the literature from natural and experimental infections in bison, we propose the natural course of brucellosis in YNP bison to be the following. The most common source of exposure to noninfected animals, excluding newborns of infected mothers, is *B. abortus*-infected products of parturition (aborted fetus, live calf, placenta, or vaginal exudate). The infected vagina of a nonpregnant cow is a possible, but less likely, alternative path for infecting noninfected animals. Newborns, born to infected mothers, may be infected at birth or through *B. abortus* in milk, but surviving calves rarely show a persistent antibody response before 5 to 6 mo of age. Most calves of seropositive cows will receive passive antibodies, which decline and are usually unmeasurable by 5 to 6 mo. These animals are still susceptible to subsequent infection and seroconversion.

Once exposed a calf or adult animal may, depending on the dose ingested, become infected. Juveniles and adults may seroconvert at any age. After infection, male bison experience seminal vesiculitis (Williams et al., 1993; Rhyan et al., 1997), epididymitis, and ampullitis (Williams et al., 1993) and, in a minority of cases, orchitis (Creech, 1930; Rhyan et al., 1997), which may affect fertility. Recently infected, female animals may bare live calves that survive; bare weak, infected, live calves that subsequently die; or may experience abortions. Infected seropositive cows likely remain seropositive and infected for a prolonged time. Antibody is not protective, and the likelihood of successful bacterial isolation from a seropositive cow is directly related to antibody levels (Roffe et al., 1999). In subsequent years, these dams may have normal

pregnancies or may experience one or more reproductive failures, including *Brucella*-related abortion, early embryonic death, or failure to get pregnant. *Brucella*-related abortions produce abundant infectious material, but live births may also produce infectious material. Before and at the time of abortion, females experience metritis and retained placentas (Williams et al., 1993; Rhyan et al., 2001). Feces from an infected dam in the periparturient period, or feces from a bison recently ingesting infective material, may contain viable *Brucella* organisms but likely does not serve as an important source of exposure to other bison.

Several questions remain concerning the epidemiology and pathogenesis of brucellosis in bison. The role of the male and of venereal transmission in the spread of brucellosis among bison is unknown. In one study, Robison and others (1998), reported the lack of seroconversion in bison cows bred by one infected bison bull shedding organisms in the semen. The possibility of undetected, latent infection in young bison exposed as calves also exists and could be the source of infection for some of the offspring in our study. This condition occurs infrequently in cattle (“heifer syndrome”) and usually manifests at sexual maturity; at which time, animals may abort, shed organisms, and develop antibodies to *B. abortus* (Wilesmith, 1978; Winthrop, et al., 1988). The cause of some animals’ reproductive failures in years after seroconversion needs to be determined. Fuller et al. (2007) applied multiple logistic regression to data from this study and found that brucellosis infections reduced birth rates in two age categories (3 yr olds and >3 yr old), and these effects were pronounced in bison that seroconverted the same year. Additional analyses of data from this study and additional data demonstrated significantly lower pregnancy rates across all age classes among seropositive bison as compared with seronegative bison, suggesting the disease may play a role in reducing

fecundity in chronically infected bison (Geremia et al., 2009). Reproductive failures in years following seroconversion could be due to mid-term or late-term abortions, early embryonic deaths, or chronic, low-grade endometritis preventing implantation and pregnancy.

Regardless of these unanswered questions, the preponderance of data indicate that the epidemiology and pathogenesis of brucellosis in chronically infected wild bison, such as the herd in YNP, is very similar to that in chronically infected cattle (Manthei and Carter, 1950; Enright, 1990). Differences certainly exist, such as the quantitative immune response to exposure or response to vaccines. These differences are small but may have important implications for the effectiveness of vaccines in bison. Our findings on the epidemiology of brucellosis in bison have important implications for managing the disease in free-ranging wildlife. Risk to noninfected populations of wildlife or livestock is highest from bison in their first pregnancy following seroconversion. High antibody-containing animals pose the greater risk of shedding *Brucella*. Despite decades of infection in the YNP herd, bison remain infected for the long term, and antibody is not protective. More work is needed to determine the extent of subsequent reproductive failures caused by brucellosis and the risk for transmission they pose. In addition, we need to better understand the frequency and role of live, infected calves and those that remain infected into adulthood and potentially shed *Brucella* during reproductive events.

#### ACKNOWLEDGMENTS

We thank the following people for assisting and accommodating us in the field: K. Coffin, L. Jones, S. Sweeney, and K. Altenhoffen with US Geological Survey; N. Anderson, A. Whitelaw, W. Maples, and C. O’Rourke with Montana Department of Fish, Wildlife and Parks; J. Mack, W. Brewster, B. Siebert, C. Daigle-Berg, M. Keetor, B. Phillips, G. Plumb, and M. Biel with the National Park Service; and N. Cheville, M. Gilsdorf, M. Philo, M.

McCullum, S. Coburn, and P. Nol with the US Department of Agriculture. We thank J. Stradley, D. Chapman, R. Stradley, J. Olson, P. Nolan, J. Innes, and R. Small for assistance in aerial tracking and helicopter capture. We thank P. Geer at the National Veterinary Services Laboratories for laboratory assistance. We also thank A. Gertonson, T. Linfield, and M. Bridges with the Montana Department of Livestock, and C. Coffin and D. Tyers with the US Forest Service, for administrative and logistic support, and D. Hunter for assistance in animal immobilization.

#### LITERATURE CITED

- ALTON, G. G., L. M. JONES, R. D. ANGUS, AND J. M. VERGER. 1988. Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France, 190 pp.
- ANONYMOUS, 1965a. Standard agglutination test procedures for the diagnosis of brucellosis: Diagnostic reagents manual 65D. US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Ames, Iowa, 8 pp.
- Anonymous 1965b. Supplemental test procedures for the diagnosis of brucellosis. Diagnostic reagents manual 65E. US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Ames, Iowa, 23 pp.
- . 1993. *Brucella abortus* complement fixation test micro method. US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, Diagnostic Bacteriology Laboratory, Ames, Iowa, 30 pp.
- AUNE, K. E., T. ROFFE, J. RHYAN, J. MACK, AND W. CLARK. 1998. Preliminary results on home range movements, reproduction and behavior of female bison in northern Yellowstone National Park. In International symposium on bison ecology and management in North America, L. Irby and J. Knight (eds.). Montana State University, Bozeman, Montana, pp. 61–70.
- CHEVILLE, N. F., D. R. MCCOLLOUGH, AND L. R. PAULSON. 1998. Brucellosis in the Greater Yellowstone Area. National Research Council, National Academy of Sciences, National Academy Press, Washington, D.C., 186 pp.
- CREECH, G. T. 1930. *Brucella abortus* infection in a male bison. North American Veterinarian 11: 35–36.
- DAVIS, D. S., J. W. TEMPLETON, T. A. FICHT, J. D. WILLIAMS, J. D. KOPEC, AND L. G. ADAMS. 1990. *Brucella abortus* in captive bison, I: Serology, bacteriology, pathogenesis, and transmission to cattle. Journal of Wildlife Diseases 26: 360–371.
- DIMMICK, R. W., AND M. R. PELTON. 1996. Criteria of sex and age. In Research and management techniques for wildlife and habitats, T. A. Bookhout (ed.). The Wildlife Society, Bethesda, Maryland, pp. 169–214.
- ENRIGHT, F. M. 1990. The pathogenesis and pathobiology of *Brucella* infection in domestic animals. In Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press, Ann Arbor, Michigan, pp. 301–320.
- FITCH, C. P., L. M. BISHOP, AND W. L. BOYD. 1932. A study of bovine blood, urine and feces for the presence of *B. abortus* Bang. Society for Experimental Biology and Medicine Proceedings 29: 555–558.
- FULLER, J. A., R. A. GARROTT, P. J. WHITE, K. E. AUNE, T. J. ROFFE, AND J. C. RHYAN. 2007. Reproduction and survival of Yellowstone bison. The Journal of Wildlife Management 71: 2365–2372.
- FULLER, W. A. 1959. The horns and teeth as indicators of age in bison. Journal of Wildlife Management 23: 342–344.
- GEREMIA, C., P. J. WHITE, R. A. GARROT, R. W. WALLEN, K. E. AUNE, J. TREANOR, AND J. A. FULLER. 2009. Demography of central Yellowstone bison: effects of climate, density, and disease. In The ecology of large mammals in central Yellowstone: Sixteen years of integrated field studies, R. A. Garrot, P. J. White and F. G. R. Watson (eds.). Academic Press, San Diego, California, pp. 255–279.
- HAIGH, J. C., C. GATES, A. RUDER, AND R. SASSER. 1991. Diagnosis of pregnancy in wood bison using a bovine assay for pregnancy-specific protein B. Theriogenology 36: 749–754.
- MANTHEI, C. A., AND R. W. CARTER. 1950. Persistence of *Brucella abortus* infection in cattle. American Journal of Veterinary Research 11: 173–180.
- MEAGHER, M. M. 1973. The bison of Yellowstone National Park. In National Park Service Scientific Monograph Series No. 1. National Park Service, Washington, D.C., 161 pp.
- MEYER, M. E., AND M. MEAGHER. 1995. Letter to the editor: Brucellosis in free-ranging bison (*Bison bison*) in Yellowstone, Grand Teton, and Wood Buffalo National Parks: A review. Journal of Wildlife Diseases 31: 579–598.
- MOHLER, J. R. 1917. Report of the chief of the Bureau of Animal Industry, Pathological Division: Abortion disease. In Annual reports of the Department of Agriculture (1917). US Department of Agriculture, Washington, D.C., pp 105–106.
- OLSEN, S. C., A. E. JENSEN, W. C. STOFFREGEN, AND M. V. PALMER. 2003. Efficacy of calthood vaccination with *Brucella abortus* strain RB51 in protecting bison against brucellosis. Research Veterinary Science 74: 17–22.
- RHYAN, J. C. 2000. Brucellosis in terrestrial wildlife and marine mammals. In Emerging diseases of

- animals, C. Brown and C. Bolin (eds.). ASM Press, Washington, D.C., pp 161–184.
- , W. J. QUINN, L. L. STACKHOUSE, J. J. HENDERSON, D. R. EWALT, J. B. PAYEUR, M. JOHNSON, AND M. MEAGHER. 1994. Abortion caused by *Brucella abortus* biovar 1 in a free-ranging bison (*Bison bison*) from Yellowstone National Park. *Journal of Wildlife Diseases* 30: 445–446.
- , S. D. HOLLAND, T. GIDLEWSKI, D. A. SAARI, A. E. JENSEN, D. R. EWALT, S. G. HENNAGER, S. C. OLSEN, AND N. F. CHEVILLE. 1997. Seminal vesiculitis and orchitis caused by *Brucella abortus* biovar 1 in young bison bulls from South Dakota. *Journal of Veterinary Diagnostic Investigation* 9: 368–374.
- , T. GIDLEWSKI, T. J. ROFFE, K. AUNE, L. M. PHILO, AND D. R. EWALT. 2001. Pathology of brucellosis in bison from Yellowstone National Park. *Journal of Wildlife Diseases* 37: 101–109.
- ROBISON, C. D., D. S. DAVIS, J. W. TEMPLETON, M. WESTHUSIN, W. B. FOXWORTH, M. J. GILSDORF, AND L. G. ADAMS. 1998. Conservation of germ plasm from bison infected with *Brucella abortus*. *Journal of Wildlife Diseases* 34: 582–589.
- ROFFE, T. J., J. C. RHYAN, K. AUNE, L. M. PHILO, D. R. EWALT, AND T. GIDLEWSKI. 1999. Brucellosis in Yellowstone National Park bison: quantitative serology and infection. *The Journal of Wildlife Management* 63: 1132–1137.
- RUSH, W. M. 1932. Bang's disease in Yellowstone National Park buffalo and elk herds. *Journal of Mammalogy* 13: 371–372.
- US DEPARTMENT OF AGRICULTURE. 2003. Brucellosis eradication. In *Uniform methods and rules*. US Department of Agriculture, Animal and Plant Health Inspection Service, Riverdale, Maryland, Publication 91-45-013, 121 pp.
- WILESMITH, J. W. 1978. The persistence of *Brucella abortus* infection in calves: A retrospective study of heavily infected herds. *The Veterinary Record* 103: 149–153.
- WILLIAMS, E. S., E. T. THORNE, S. L. ANDERSON, AND J. D. HERRIGES, JR. 1993. Brucellosis in free-ranging bison (*Bison bison*) from Teton County, Wyoming. *Journal of Wildlife Diseases* 29: 118–122.
- WINTHROP, C. R., R. R. BROWN, D. A. STRINGFELLOW, P. R. SCHNURRENBERGER, C. M. SCANLAN, AND A. I. SWANN. 1988. Bovine brucellosis: An investigation of latency in progeny of culture-positive cows. *Journal of the American Veterinary Medical Association* 192: 182–186.

*Received for publication 20 August 2008.*