Feasibility of quarantine procedures for bison (*Bison bison*) calves from Yellowstone National Park for conservation of brucellosis-free bison

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Objective—To determine the feasibility of qualifying individuals or groups of Yellowstone National Park bison as free from brucellosis.

Design—Cohort study.

Sample—Serum, blood, and various samples from live bison and tissues taken at necropsy from 214 bison over 7 years.

Procedures—Blood was collected from bison every 30 to 45 days for serologic tests and microbiological culture of blood for *Brucella abortus*. Seropositive bison were euthanized until all remaining bison had 2 consecutive negative test results. Half the seronegative bison were randomly euthanized, and tissues were collected for bacteriologic culture. The remaining seronegative bison were bred, and blood was tested at least twice per year. Cow-calf pairs were sampled immediately after calving and 6 months after calving for evidence of *B abortus*.

Results—Post-enrollment serial testing for *B abortus* antibodies revealed no bison that seroconverted after 205 days (first cohort) and 180 days (second cohort). During initial serial testing, 85% of bison seroconverted within 120 days after removal from the infected population. *Brucella abortus* was not cultured from any euthanized seronegative bison (0/88). After parturition, no cows or calves had a positive test result for *B abortus* antibodies, nor was *B abortus* cultured from any samples.

Conclusions and Clinical Relevance—Results suggested it is feasible to qualify brucellosis-free bison from an infected herd following quarantine procedures as published in the USDA APHIS brucellosis eradication uniform methods and rules. Latent infection was not detected in this sample of bison when applying the USDA APHIS quarantine protocol. (*J Am Vet Med Assoc* 2014;244:588–591)

RUMINANTS

Recognition of the potential for brucellosis transmission from YNP bison to cattle and the substantial associated economic effects have brought numerous federal and state agencies together to address the issue. The US Department of Interior, the National Park Service, USDA APHIS Veterinary Services, the US Forest Service, the Montana Department of Livestock, and Montana Fish, Wildlife, and Parks have authority for the management of bison that migrate from YNP into Montana, the management of brucellosis in bison, or the management of lands used by bison. None of the agencies, acting alone, has sufficient authority to manage YNP bison across all jurisdictional boundaries. The agencies recognize the shared responsibility and the need for cooperation in bison management; therefore, these agencies approved respective federal and state records of decision to implement the IBMP in December 2000. Management under the IBMP includes actions to protect private property, to reduce the risk of transmission of brucellosis from bison to cattle, and to maintain a viable, free-ranging population of bison in YNP. The records of decision were supported with a draft environmental impact statement that was jointly prepared by all agencies, a final environmental impact statement[1] that was prepared by the federal agencies, and a final environmental impact statement that was prepared by the Montana state agencies.

In the negotiations and hearings held in the development of the IBMP, the agencies were instructed to examine the feasibility of bison quarantine. The USDA APHIS brucellosis eradication uniform methods and rules[2] contain a protocol for the quarantine of bison from YNP and Grand Teton National Park to qualify the bison as brucellosis free (**Appendix**). Concurrent with the discussion about quarantine in the Greater Yel-

Abbreviations

FPA	Fluorescent polarization assay
IBMP	Interagency Bison Management Plan
MDOLL	Montana Department of Livestock Diagnostic Laboratory
NVSL	National Veterinary Services Laboratory
YNP	Yellowstone National Park

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lowstone Area, there have also been frequent discussions and meetings regarding bison conservation strategies in North America and the potential for restoring the species to grassland ecosystems. The agencies agree that capture and relocation of bison to other suitable habitats would be an appropriate alternative to lethal removal of bison that exceed population objectives for YNP, as defined by the IBMP.

The purpose of the study reported here was to determine whether it was feasible, following the protocol described in the USDA APHIS brucellosis eradication uniform methods and rules[2] for handling affected or restricted herds, to qualify individuals or groups of YNP bison as free from brucellosis, including latent infections. Results would provide insight and data regarding seroconversion of exposed bison, serologic testing of bison for anti–*Brucella abortus* antibodies, and tissues relevant to *B abortus* culture in bison.

Materials and Methods

The study was organized in 3 phases. Phase 1 involved enrollment and testing for seroconversion and ended with random selection for euthanasia. Phase 2 involved breeding and calving of bison that completed phase 1. Phase 3 involved translocation and assurance testing of bison that completed phase 2.

Bison quarantine candidate selection—One hundred two bison (2005 and 2006) were selected for the first cohort and 112 (2008) for the second cohort. Bison were selected during management activities performed under the IBMP. Bison were processed through handling facilities at the western and northern boundary of YNP. Blood samples (approx 7 mL) were collected from each eligible bison, which included both male and female calves (10 months of age), and official backtags were applied to each bison. All blood was collected in standard evacuated serum tubes. At the boundary handling facilities, serum was separated in a portable centrifuge for 10 minutes at 352 × *g*, and transferred to microcentrifuge tubes. A card test[3] and field FPA[4] were performed to determine the preliminary serologic status of each bison. Bison calves that had negative serologic results on both tests were identified with official identification and were transported to the bison quarantine facility at Corwin Springs, Mont. Serum samples from bison selected for quarantine were then sent to the MDOLL for confirmatory serologic testing. The MDOLL performed 7 serologic tests for *B abortus* to confirm serologic status of the bison, including FPA,[4] complement fixation,[5] card,[3] standard tube,[6] standard plate,[7] buffered acidified plate antigen,[8] and ethacridine lactate tests.[9] Standard protocols as issued by the NVSL were followed for serologic testing. Results were analyzed by the designated brucellosis epidemiologist, and bison were designated as reactors (seropositive), suspect, or nonpositive (seronegative) for *B abortus*. For this study, bison were classified as reactors for *B abortus* if the results of an official serologic test indicated they had been exposed to or infected with *B abortus*. Bison were classified as suspect if the result of an official serologic test suggested exposure but was inconclusive.[2] The isolation of *B abortus* from culture samples from an individual bison would also have resulted in categorization of that bison as a reactor. Those bison with nonpositive results of all confirmatory tests were enrolled in the study. All bison that completed phase 1 testing were then vaccinated with RB51[a] at approximately 18 months of age. All bison that completed an initial parturition event also received an RB51[a] booster 6 months after calving.

Bison were housed in a facility designed in accord with recommendations in the brucellosis eradication uniform methods and rules[2] with 2 fences at least 10 feet apart to prevent contact with any animals outside of the facility. Multiple pastures were used, each no smaller than 7 acres and up to 25 acres. Bison cohorts were allocated into smaller subsets during calving to more efficiently capture calves after birth but were handled and considered as 1 cohort throughout the duration of the quarantine period.

Bison handling and phlebotomy—After appropriate candidates were selected, bison in quarantine were recaptured and tested every 30 to 45 days until all bison tested negative for *B abortus* 2 consecutive times. Bison were restrained by use of a commercially available chute designed for the handling of bison,[b] and blood was collected via the jugular vein (21 mL) and transferred to standard serum and heparinized tubes. Serum was collected for serologic testing at MDOLL for the 7-test panel and was also sent to NVSL for FPA testing. Blood collected in heparinized vacuum tubes was shipped for *B abortus* culture by NVSL. Any bison testing seropositive or as a persistent suspect for *B abortus* antibodies was euthanized and tissues were collected for *B abortus* culture. Euthanasia was performed with a captive bolt gun followed by exsanguination or by IV administration of pentobarbital after sedation. All bison were tested 2 more times 30 days apart after the last bison seroconverted. The study protocol, which encompassed all animal handling and testing procedures, was reviewed and approved by the Bison Quarantine Feasibility Study Animal Care and Use Committee.

Brucellosis culture—All bison that tested positive for brucellosis via serologic testing were euthanized and tissues were submitted for bacteriologic culture for *B abortus*. At the end of phase 1, 88 seronegative bison (43 females and 45 males) chosen randomly from both cohorts were also euthanized and tissues were collected for bacteriologic culture. Tissues collected included swab specimens from the vagina, rectum, uterus, and lymph nodes (including the mammary, scrotal, popliteal, subiliac, superficial cervical, internal iliac, accessory hepatic, jejunal, cranial tracheobronchial, pterygoid, superficial parotid, and medial retropharyngeal lymph nodes) as well as mammary tissue, ileum, kidney, liver, spleen, ovaries, uterus, testicles, epididymides, and seminal vesicles. Procedures for the culture of *Brucella* bacteria from diagnostic samples as well as subsequent biochemical identification were performed by traditional methods.[10] In some instances, identification was confirmed by *B abortus*, *B melitensis*, *B ovis*, and *B suis* PCR assay (ie, AMOS) or *B abortus* species-specific PCR assay.[11,12]

Postcalving testing—Female bison were chemically immobilized via IM injection with a dart[c] that contained

thiafentanil[d] (0.01 to 0.015 mg/kg [0.005 to 0.007 mg/lb]) and xylazine hydrochloride[e] (0.05 to 0.07 mg/kg [0.023 to 0.032 mg/lb]) within 5 days after giving birth. Bison calves were manually captured and restrained. Blood samples as well as milk from each of the 4 mammary glands, vaginal swab specimens, and vaginal discharge, if present, were collected from each adult. Cows were treated with oxytocin[f] (20 units, IM) if milk was not readily available to encourage the milk ejection reflex. Blood was collected from each calf into 1 serum tube and 1 heparinized blood tube as well as a conjunctival swab specimen. Calves were identified to dam, and sex was recorded. Swabs were immediately transferred to World Health Organization media[g] for transport and bacterial culture. After sample collection, anesthesia was reversed with naltrexone[d] (0.3 to 0.4 mg/kg [0.136 to 0.182 mg/lb], IM and SC [50:50]) and tolazoline hydrochloride[h] (0.4 to 0.7 mg/kg [0.182 to 0.318 mg/lb], IV and IM [50:50]) and the pair of bison was released.

Results

Between January 16 and April 20 of 2005, 2006, and 2008, 17, 85, and 112 bison calves, respectively, were transported to the quarantine study facilities. Eight calves (1 in the first cohort and 7 in the second cohort) determined to be reactors (seropositive) by preliminary confirmatory testing at MDOLL were removed from the study. Twenty-six bison (6 in the first cohort and 20 in the second cohort) were classified as reactors and 2 (first cohort) as suspect during serial serologic testing from May through December of each respective year. All 36 bison (34 seropositive and 2 suspect) were euthanized and tissues were collected for *B abortus* culture. *Brucella abortus* biovar 1 was cultured from all but 3 of the seropositive bison (91%). *Brucella* organisms were not cultured from the 2 suspect bison. No bison seroconverted after 205 days in quarantine, 20 of 26 (77%) seroconverted within 90 days, and 22 (85%) seroconverted within 120 days. After enrollment, 8 of 101 (8%) seroconverted, including both reactors and suspects in the first cohort, and 20 of 105 (19%) reactors in the second cohort.

Of the 88 seronegative bison euthanized after phase 1 serologic testing, none had positive results of culture for any tissues. The minimum duration of residency at the quarantine facility for any individual bison in phase 1 was 151 days for the first cohort and 188 for the second cohort. The maximum duration of residency for any individual bison in phase 1 was 449 days for the first cohort and 215 for the second cohort. Forty five bison in the first cohort (8 males and 37 females) and 39 (5 males and 34 females) in the second cohort were moved to phase 2 of the study for breeding and calving. In phase 2, no bison cows tested positive by either serologic tests or by bacteriologic culture of vaginal swab specimens, milk, or reproductive fluids collected before, during, or after parturition. All calves (n = 67) born to females in quarantine were solitary births (ie, no twins were born).[13,14] All 67 calves had negative results of bacteriologic culture of conjunctival swab specimens and were seronegative at birth and remained seronegative and had negative results of bacteriologic culture of blood throughout testing. Bison translocated from quarantine for phase 3 remained seronegative for *B abortus* after release (for the first cohort, this was 40 months into phase 3 as of April 2013).

Discussion

Results of this study indicated that it is feasible to take sub-adult seronegative bison from an infected population and, following the rigorous quarantine protocol for approved bison quarantine facilities published in the brucellosis eradication uniform methods and rules,[2] qualify them as brucellosis free in < 3 years. The females in this study were estimated to be in the range of 6 to 12 months old when enrolled, and the youngest female completed phase 2 at approximately 3.5 years of age, having been kept < 3 years in residence at the quarantine facility. Because the primary mode of *B abortus* transmission in the YNP herd is via abortion and birthing events,[15] enrolling bison < 1 year of age minimized the field exposure of each individual to *B abortus* because the primary period of exposure would have been confined to their own calving season. We believe this limited calfhood exposure was an important factor in keeping seroconversion to the levels detected after enrollment. This conclusion was reinforced by the occurrence of higher post-enrollment seroconversion for the bison of the second cohort. This observation may be attributed to exposure of the calves to abortions in the capture facility pens beginning several weeks prior to their transportation to the quarantine study facilities.

Results of this study indicated that a seronegative 6- to 12-month-old bull bison from an infected population can be considered brucellosis free after 3 years in quarantine. This finding reinforces the quarantine protocol for handling affected or restricted herds as first proposed and published in the brucellosis eradication uniform methods and rules.[2]

A positive serological result is an accurate indicator of infection and supports the approved testing protocols for older bison as outlined in the brucellosis eradication uniform methods and rules.[2] Older bison (≥ 3 years) in an infected herd, by nature of their length of residency, have a higher probability of exposure to *B abortus* and therefore a higher probability of a positive serologic test result at initial screening.[16] Although a serologic titer is not an absolute indicator of active *B abortus* infection, in a quarantine situation, it is the only practical gauge on which to base enrollment.

The crucial events that seem to reveal low-level infections are pregnancy and parturition in females and puberty in both sexes.[1] Therefore, capture and collection of tissues and swab specimens immediately after birth were deemed essential to determine with more certainty that these bison were not shedding *B abortus*. The result of a successful quarantine feasibility study was anticipated to be live bison eligible for translocation to public and tribal herds. Study bison were vaccinated with RB51 in anticipation of the regulatory requirements likely to be imposed by these receiving entities and not as a required element for conducting the study.

Older bison that have survived at least 1 parturition prior to enrollment without seroconverting would seem to be eligible for a shorter duration of residency in a quarantine test group as outlined by the USDA APHIS brucellosis eradication uniform methods and rules.[2]

Regardless of age, the key event for all females would still be the completion of a term pregnancy free of any indicators of brucellosis.

This study provided data that reinforce the testing protocol framework in the USDA APHIS brucellosis eradication uniform methods and rules. The ability to obtain brucellosis-free bison from exposed populations now gives herd managers, whose previous population control options were primarily limited to slaughter, an outlet to remove live bison from their herds. In the future, any entity, whether private, tribal, academic, or governmental with the intention of operating a facility to obtain brucellosis-free bison can use the methodology used in this study as a foundation to create a testing regimen applicable to their source population.

a. Colorado Serum Co, Denver, Colo.
b. Pearson Livestock Equipment, Thedford, Nev.
c. Pneu-Dart Inc, Williamsport, Pa.
d. Wildlife Pharmaceuticals Inc, Windsor, Colo.
e. Anased, Lloyd Inc, Shenandoah, Iowa.
f. National Veterinary Services Laboratory, Ames, Iowa.
g. Osborn, Bimeda Inc, Oakbrook Terrace, Ill.
h. Tolazine, Lloyd Inc, Shenandoah, Iowa.
i. Olsen S, USDA Agricultural Research Service National Animal Disease Center, Ames, Iowa: Personal communication, 2010.

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Appendix

Brucellosis testing protocols for bison by age and sex.

Group	Minimum No. of tests required to release	Minimum test interval	Minimum quarantine period (y)
Sexually mature males (≥ 3 years old)	3	First: start of quarantine period Second: at least 180 days after first test Last: at least 12 months after first test	1
Pregnant females (≥ 3 years old)	5	First: before calving Second: between 30 and 90 days after each bison has calved during first and second calvings Last: 6 months after last bison has calved during first and second calvings	1.5
Nonpregnant sexually mature females (≥ 3 years old)	3	First: before breeding Second: between 30 and 90 days after each bison has calved Last: 6 months after last bison has calved	1.5
Immature males (< 3 years old)	3	First: start of quarantine period Second: at least 180 days after first test Third: at least 12 months after the first test, and at least 3 years of age	1
Immature females (< 3 years old)	3	First: before breeding Second: between 30 and 90 days after each bison has calved Last: 6 months after last bison has calved	2.5
Calves*	1	One test at 6 months of age	0.5

*Calves born to females that were pregnant upon entry into the quarantine and calves born in an individual test group in which reactors have been detected should not be released as calves.

(Adapted from USDA APHIS. *Brucellosis eradication: uniform methods and rules*. Veterinary services publication 91–45–013. Fort Collins, Colo: USDA APHIS, 2003.)