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

P. Ryan Clarke, DVM, MPH; Rebecca K. Frey, MS; Jack C. Rhy an, DVM, MS; Matt P. McCollum, MS; Pauline Nol, DVM, PhD; Keith Aune, MS

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)

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 to 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 statement1 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 rules2 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-

From USDA APHIS Veterinary Services, 2150 Centre Ave, Fort Collins, CO 80526; USDA APHIS Veterinary Services, 4101 Laporte Ave, Fort Collins, CO 80523; and Montana Fish, Wildlife, and Parks, 1400 S 19th Ave, Bozeman, MT 59715. Mr. Aune’s present address is Wildlife Conservation Society, 301 N Wilson Ave, Bozeman, MT 59715. Funding provided by USDA APHIS; Montana Fish, Wildlife, and Parks; and the Montana Department of Livestock. The authors thank Chris Quance, Jean Block, Doug Knopp, Jeremy Zimmer, Antonio Feuntes-Sanchez, Neil Anderson, Dennis Tilton, Gerald Wiscomb, and Drs. Brent Thompson, Mark Atkinson, Jennifer Ramsey, Dan Tyres, Tom Linfield, and Marty Zaluski for technical assistance.

Address correspondence to Dr. Clarke (patrick.r.clarke@aphis.usda.gov).

ABBREVIATIONS

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

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1. There is no citation for the final environmental impact statement.
2. There is no citation for the USDA APHIS quarantine protocol.
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 rules² 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 bison, including 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 test¹ and field FPA⁴ were performed to confirm serologic status of the bison, including FPA, complement fixation,⁵ card,³ standard tube,⁶ standard plate,⁷ buffered acidified plate antigen,⁶ 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 RB51³ at approximately 18 months of age. All bison that completed an initial parturition event also received an RB51³ booster 6 months after calving.

Bison were housed in a facility designed in accord with recommendations in the brucellosis eradication uniform methods and rules⁶ 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,⁴ 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 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 that contained...
Ruminants were treated with oxytocin (20 units, IM) if milk was not charge, if present, were collected from each adult. Cows mary glands, vaginal swab specimens, and vaginal dis-

Bison calves were manually captured and restrained. 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 for transport and bacterial culture. After sample collection, anesthesia was reversed after 205 days in quarantine, 20 of 26 (77%) seroconverted from the 2 suspect bison. No bison seroconverted organisms were not cul-

biovar 1 was cultured from all but 3 of the sero-

abortus culture. After sample collection, anesthesia was reversed after 205 days in quarantine, 20 of 26 (77%) seroconver-
ted from the 2 suspect bison. No bison seroconverted.

May through December of each respective year. All 36 cohort) as suspect during serial serologic testing from the second cohort) were classified as reactors and 2 (first 

confirmed 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 sero-

Bison translocated for transport and bacterial culture for any tissues. The minimum duration of resi-
dency 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 213 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 for the study of breeding and calving. In phase 2, no bison cows tested positive by either serologic tests or by bacteriologic culture of vagi-


calving. In phase 2, no bison cows tested positive by either serologic tests or by bacteriologic culture of vagi-

nal swab specimens, milk, or reproductive fluids col-

lected before, during, or after parturition. All calves (n = 67) born to females in quarantine were solitary births (ie, no twins were born). All 67 calves 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, 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 quaran-
tine facility. Because the primary mode of B abortus transmission in the YNP herd is via abortion and birthing events, 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 calf-

hood 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 sec-

ond 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. 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. 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. 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 in-
fec tions are pregnancy and parturition in females and puberty in both sexes. 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 anticipate-
ed 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 parturi-
tion 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.
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.

References

Appendix
Brucellosis testing protocols for bison by age and sex.

<table>
<thead>
<tr>
<th>Group</th>
<th>Minimum No. of tests required to release</th>
<th>Minimum test interval</th>
<th>Minimum quarantine period (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexually mature males (≥ 3 years old)</td>
<td>3</td>
<td>First: start of quarantine period Second: at least 180 days after first test Last: at least 12 months after first test</td>
<td>1</td>
</tr>
<tr>
<td>Pregnant females (≥ 3 years old)</td>
<td>5</td>
<td>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</td>
<td>1.5</td>
</tr>
<tr>
<td>Nonpregnant sexually mature females (≥ 3 years old)</td>
<td>3</td>
<td>First: before breeding Second: between 30 and 90 days after each bison has calved Last: 6 months after last bison has calved</td>
<td>1.5</td>
</tr>
<tr>
<td>Immature males (&lt; 3 years old)</td>
<td>3</td>
<td>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</td>
<td>1</td>
</tr>
<tr>
<td>Immature females (&lt; 3 years old)</td>
<td>3</td>
<td>First: before breeding Second: between 30 and 90 days after each bison has calved Last: 6 months after last bison has calved</td>
<td>2.5</td>
</tr>
<tr>
<td>Calves*</td>
<td>1</td>
<td>One test at 6 months of age</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*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.)