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Effects of Pregnancy Prevention on *Brucella abortus* Shedding in American bison (*Bison bison*)

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ABSTRACT: Products of parturition are the predominant source of *Brucella abortus* for transmission in bison (*Bison bison*). Our objective was to assess whether preventing pregnancy in *Brucella*-seropositive bison reduced *B. abortus* shedding. *Brucella*-seropositive and -seronegative bison from Yellowstone National Park, Wyoming, USA were used in a replicated experiment. Each of two replicates (*rep1*, *rep2*) included a group of seropositive females treated with a single dose of gonadotropin-releasing hormone-based immunocontraceptive (*Treatment rep1*, $n=15$; *Treatment rep2*, $n=20$) and an untreated group (*Control rep1*, $n=14$; *Control rep2*, $n=16$) housed separately. Seronegative sentinel females were placed in each group to monitor horizontal transmission. Seronegative males were co-mingled for breeding each year. Pregnant females were removed from treatment groups in the first year, but not thereafter. Each January–June we monitored for *B. abortus* shedding events—any parturition associated with culture-positive fluids or tissues. We analyzed probability of shedding events using a negative binomial generalized linear mixed model fit by maximum likelihood using Laplace approximation. Over 5 yr, we observed zero shedding events in *Treatment rep1* vs. 12 in *Control rep1*. All five *Control rep1* sentinels but zero (0/5) *Treatment rep1* sentinels seroconverted. In the second replicate, *Treatment rep2* had two shedding events over 3 yr and *Control rep2* had five events over 2 yr. Sentinels in both *Control rep2* (3/6) and *Treatment rep2* (5/6) seroconverted by trial endpoint. *Treatment rep1* showed a reduced shedding probability relative to *Control rep1*, *Treatment rep2*, and *Control rep2* (log odds value −25.36 vs. −1.71, −1.39, and −0.23, respectively). Fixed effect predictor covariates, year and age, had no explanatory value. These data suggest that successful contraception of brucellosis-seropositive female bison prevents shedding of *B. abortus* by individual animals. However, contraceptive treatment may or may not sufficiently reduce disease transmission to reduce brucellosis prevalence in an affected herd.

Key words: Bison, *Brucella abortus*, immunocontraception, wildlife disease management.

INTRODUCTION

Bovine brucellosis, caused by *Brucella abortus,* primarily affects bovids, although species from other families may be affected as well, including humans. *Brucella abortus* infection causes reproductive failures such as abortion, stillbirths, and weak neonates (Rhyan et al. 2001; Olsen and Tatum 2010). The predominant mode of transmission is via infectious products of parturition, as aborted or nonviable fetuses, neonates, and associated tissues and fluids may be heavily colonized by bacteria. Contact with these materials by herd members facilitates a mucosal route of introduction of *B. abortus* into susceptible animals (Rhyan and Nol 2019). Other, less frequent, modes of transmission of *B. abortus* include ingestion of infected milk, or contact with infected genitalia or feces (Cheville et al. 1998).

Brucellosis, probably introduced by cattle (*Bos taurus*), was first detected in bison of the

Greater Yellowstone Area (GYA) of the US in the early twentieth century, and had emerged in the GYA elk (*Cervus canadensis*) population by the 1930s (US Department of the Interior 2000; Rhyan and Nol 2019). Since the 1950s, a cooperative state–federal brucellosis eradication program has largely eliminated *B. abortus* from US domestic cattle and bison herds. Nevertheless, disease reservoirs in GYA free-ranging bison and elk populations still persist, and spillovers to domestic livestock occur (Ragan 2002; Olsen 2010). Annually, managers expend great efforts to maintain temporal and spatial separation between wild bison herds and domestic livestock, although data indicate that infected elk are the primary source of *B. abortus* cases in domestic cattle and bison in the GYA (Rhyan et al. 2013b; Kamath et al. 2016; National Academies of Sciences, Engineering, and Medicine 2020).

Test-and-remove is the most common management tool applied to brucellosis-affected livestock herds in the US (Ragan 2002). However, traditional lethal approaches to disease management of livestock are not necessarily compatible with what is considered feasible or socially acceptable when it comes to managing and conserving wildlife (Bienen and Tabor 2006). Research suggests that immunocontraception could be used as a nonlethal method to decrease *B. abortus* transmission in GYA bison herds (Miller et al. 2004; Rhyan et al. 2013a). We describe here an experiment designed to assess whether preventing pregnancy, using a gonadotropin-releasing hormone (GnRH) –based immunocontraceptive, in *B. abortus*–seropositive female bison would reduce probability of *B. abortus* shedding within a treated herd.

MATERIALS AND METHODS

Animals

Animals were captured at the National Park Service's Stephens Creek, Montana, US (45°02′56.8″N, 110°45′04.8″W) bison facility, as they migrated out of Yellowstone National Park (YNP) in late winter and early spring of 2011 and 2014. Eighty-three nonpregnant female bison, approximately 1–2 yr old, brucellosis seropositive and seronegative, were captured and used for the study. Between 2012 and 2014, ten 2–3-yr-old seronegative males were also captured. Additionally, four seronegative 2-yr-old males were purchased from a commercial source in 2012. Bison were collected in a series of traps and corrals, approximately 9.7 ha, using passive feeding or horses. Animals were sorted and manually restrained in a chute for examination and selection by age, sex, and pregnancy status. We determined age of animals by incisor eruption and wear (Dimmick and Pelton 1996; Fuller et al. 2007). We used rectal palpation to select nonpregnant females. Blood was collected from the jugular vein of eligible animals, using a 30-mL syringe with a 16-gauge needle, for chute-side brucellosis serological testing using a fluorescence polarization assay (FPA) and a standard card test (US Department of Agriculture, Animal and Plant Health Inspection Service [USDA-APHIS] 2003, 2006a). Blood was also transferred to serum separator tubes (Vacutainer, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA), allowed to clot at approximately 4 C, and centrifuged at 2,000 × G for 10 min. Serum was aliquoted into 2–3-mL cryovials and submitted to the Montana Veterinary Diagnostic Laboratory (MVDL), Bozeman, Montana, US, for additional brucellosis serology. Animals received unique identifications in the form of numbered ear tags (Y-Tex, Cody, Wyoming, USA) in both ears and a radio-frequency identification button tag (AllFlex USA, Dallas Fort Worth Airport, Texas, USA) inserted at the base of the left ear. We transported the bison 11 km in a stock trailer to the USDA-APHIS Veterinary Services (VS) Bison Facilities in Corwin Springs, Montana, US. The facilities comprised seven pastures totaling approximately 36 hectares, including holding corrals and bison working areas.

Experimental design

We established two study replicates, in 2012 and 2014–2015. The first replicate (*Replicate 1*) was made up of 29 seropositive females and 10 seronegative females. The second replicate (*Replicate 2*) was assembled over 2 yr and included animals captured in 2011 and 2014, and offspring of *Replicate 1* (36 seropositive females, 12 seronegative females).

In April 2012, *Replicate 1* was sorted into two 10-ha pastures. One pasture (*Control replicate [rep]1*) contained seropositive females (n=14) and

seronegative sentinel females ($Sentinel_{Control\ rep1}$; n=5) to detect transmission through seroconversion. A second pasture (*Treatment rep1*) contained seropositive females (n=15) and seronegative sentinel females ($Sentinel_{Treatment\ rep1}$; n=5). Males were housed separately from females until breeding. We allowed comingling of males with females during July to October of each year, two males per pasture. For first breeding of *Replicate 1*, we used commercially sourced males; in subsequent years we used YNP males.

In April 2014, we assigned 20 seropositive females to *Treatment rep2* (n=20), accompanied by six seronegative females ($Sentinel_{Treatment\ rep2}$). In 2015, we assembled *Control rep2* (n=16), along with six seronegative females ($Sentinel_{Control\ rep2}$). Both groups were housed in 12-ha pastures. Males comingled with females (two per pasture) from July to October each year.

Calves remained with their dams until approximately 9 mo old, at which time they were transferred to a separate pen or to another facility to enter into a brucellosis quarantine protocol (Clarke et al. 2014), retained to supplement *Replicate 2*, or were shipped to slaughter.

Immunocontraceptive treatment

Gonadotropin-releasing hormone-based immunocontraceptive (GonaCon; USDA-APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA) was prepared as described by Miller et al. (2008) and Frey et al. (2024). Seropositive females assigned to treatment groups were manually restrained and injected intramuscularly (IM) 2.5 cm ventral from the hip, bilaterally, with 3,000 µg GnRH in 2 mL adjuvant, divided into 1 mL (1,500 µg GnRH) in each hip, 3 mo before exposure to males. Treatment group animals were given one dose only on initiation of each replicate. *Treatment rep1* received immunocontraceptive in May 2012 and *Treatment rep2* in May 2014. None of the seropositive bison in control groups, nor any sentinels, received immunocontraceptive treatment. The first year of each replicate occurred on January 1 following immunocontraception treatment–control group assembly. Figure 1 summarizes the design and timeline of this two-replicate study.

Monitoring and sample collection

In January each year, under manual restraint, we collected blood as described above and vaginal swabs (polyester-tipped, polyester stick applicator swabs; Fisher Scientific, Hampton, New Hampshire, USA), placed in World Health Organization media (supplied by MVDL) from females for brucellosis serology and *Brucella* culture. We checked pregnancy status through rectal palpation, and placed vaginal transmitters in pregnant animals (Advanced Telemetry Systems Inc., Isanti, Minnesota, USA) as described (Rhyan et al. 2009). In order to begin each replicate trial with maximum treatment efficacy, immunocontraceptive-treated animals identified as pregnant in the January handling before the first calving season (year 1) were removed from the study. In subsequent years, however, treated animals that became pregnant (i.e., those with "breakthrough pregnancies") remained in the treatment group paddocks to allow for waning efficacy of the treatment over time.

During January–May each year, we monitored pregnant animals daily via observation and VHF radio telemetry to document transmitter expulsion events. If no live calf was present after the expulsion signal occurred, to assess pregnancy status we chemically immobilized the dam approximately 24 h after onset of the expulsion signal. We immobilized animals using etorphine (0.01 mg/kg) or thiafentanil (0.015–0.02 mg/kg; Wildlife Pharmaceuticals, Windsor, Colorado, USA) plus xylazine hydrochloride (0.05–0.07 mg/kg; AnaSed, Lloyd Inc., Shenandoah, Iowa, USA), or using premixed butorphanol–azaperone–medetomidine (0.06–0.09 mg medetomidine/kg; BAM, Wildlife Pharmaceuticals). All immobilizing drugs were delivered IM via dart (Pneudart, Williamsport, Pennsylvania, USA). Immobilizing drugs were antagonized with naltrexone (Wildlife Pharmaceuticals; 50 mg IM per mg thiafentanil or 25 mg IM per mg etorphine) and 300 mg tolazoline IM (Tolazine, Akorn Animal Health, Lake Forest, Illinois, USA). In cases where BAM was used, animals were reversed with atipamazole (Wildlife Pharmaceuticals) at 5 mg per 1 mg medetomidine, naltrexone (0.05–0.125 mg/kg IM), and tolazoline (1 mg/kg IM). In animals deemed still pregnant, we obtained a blood sample and replaced the vaginal transmitter. On evidence of abortion or stillbirth, we collected blood, vaginal swabs, feces, and milk, if present, from the dam, and we collected the placenta and fetus when possible. We collected milk from each quarter of the udder into 50-mL polyethylene centrifuge tubes (Fisher Scientific). Feces were placed

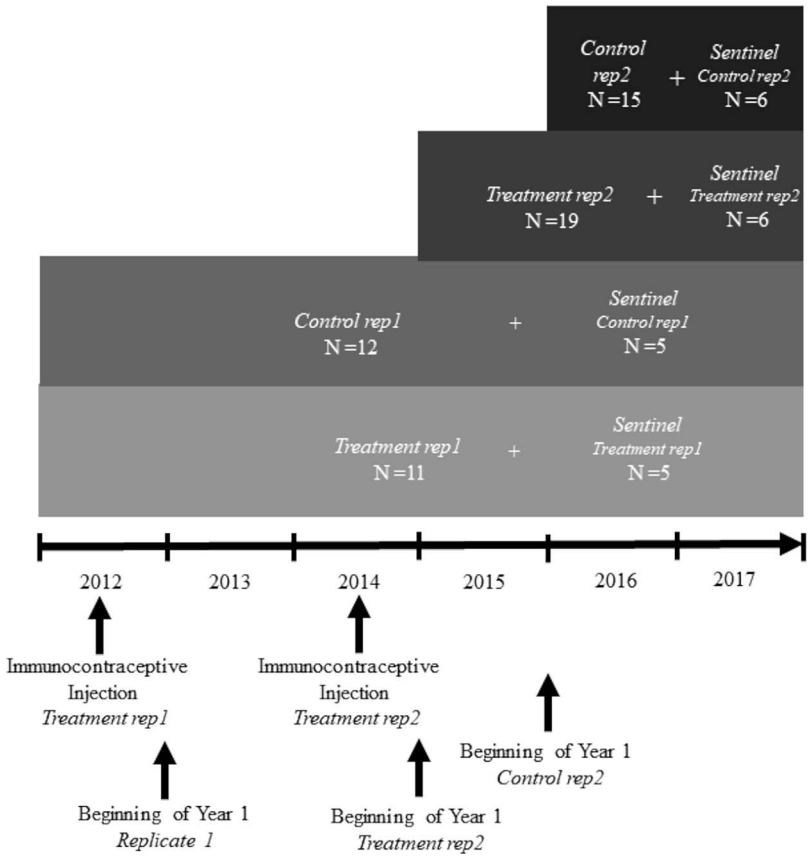

FIGURE 1. Timeline of an experimental evaluation of immunocontraception as a brucellosis management tool. The experiment included two replicates (*rep1*, *rep2*). Each replicate included a group of immunocontraceptive-treated seropositive females (*Treatment*) and a group of untreated seropositive females (*Control*) housed in separate paddocks. *Treatment* groups received a one-time intramuscular administration of 3,000 μg gonadotropin-releasing hormone-based immunocontraceptive in May of the year before onset of the replicate. Smaller numbers of seronegative female sentinels (denoted as $Sentinel_{group}$) were housed with each of the four primary experimental groups (*Treatment rep1*, *Treatment rep2*, *Control rep1*, *Control rep2*) to monitor for transmission. Study years were defined as January–December. The first replicate trial ran for 5 yr (2013–2017); the second replicate trial had a staggered start and ended after 2 or 3 yr of observing *Control rep2* (2016–2017) or *Treatment rep2* (2015–2017), respectively.

in Whirl-Pak bags (Nasco, Fort Atkinson, Wisconsin, USA). Placentas were collected into 1-gallon resealable plastic storage bags. Swabs, milk, feces, and placenta were stored at –80 C until shipment to USDA-APHIS VS, National Veterinary Services Laboratories (NVSL) for *Brucella* culture (culture methods described under Bacteriology). Fetuses/dead calves were collected in doubled large plastic bags and transported to MVDL for necropsy and culture. Personnel handling any potentially infectious materials took all necessary precautions, including using appropriate personal protective equipment.

On observation of an apparently normal full-term calf, the dam was immobilized within 5 d of parturition and the samples listed above were collected. We also collected conjunctival swabs (as described above for vaginal swabs), blood, and feces from the calf. In June or July of each year, we manually restrained nonpregnant animals for blood and vaginal swab collection, and handled (either manually or through chemical immobilization) the males to collect blood for brucellosis testing to verify seronegativity before breeding.

Aborted fetuses, stillborn calves, and adult animals that died or were humanely euthanized before

the end of the trial were transported to the MVDL for necropsy and tissue collection for *Brucella* culture. At conclusion of the study, we humanely euthanized the remaining *Treatment* animals onsite and collected blood and tissues. Nontreated *Control* and *Sentinel* animals were shipped to slaughter, where blood and tissues were collected at the slaughter establishment. Postmortem tissues collected from mortalities, aborted fetuses, stillbirths, and animals euthanized at study termination included mandibular, parotid, medial retropharyngeal, prescapular, internal iliac, tracheobronchial, hepatic, mesenteric, prefemoral, mammary and popliteal lymph nodes, mammary gland, uterus, ovary, placenta, ileum, lung, liver, spleen, kidney. We used appropriate personal protective equipment and safety procedures when working with all carcasses and tissues. We sent samples collected at the end of the study to NVSL for serology and culture.

Serological testing

We tested for antibodies against *Brucella*. On initial collection of the animals, standard card and FPA tube tests were carried out chute-side. Throughout the remainder of the study, standard card, FPA tube, buffered acidified plate antigen (BAPA), complement fixation (CF), and serological tests, as described in the 2001 USDA Brucellosis Uniform Methods and Rules and in NVSL protocols, were performed at the MVDL or NVSL using standard procedures (USDA-APHIS 2003, 2006a, 2006b, 2006c, 2011). We defined positive animals as those testing positive on either CF or FPA. Animals considered suspect produced results in the suspect range of CF or FPA, or were positive on standard card or BAPA without a positive result on either CF or FPA.

Bacteriology

Swabs, feces, milk, and tissues shipped to the NVSL for bacteriologic culture underwent standard isolation protocols (Alton et al. 1988) with a modification to use a blender to homogenize tissues. All isolation procedures were performed using appropriate biosafety level 3 facilities and procedures. Isolates and DNA were inactivated before removal for molecular procedures and viability testing was performed to ensure inactivation on all samples. Tissues obtained from necropsy at MVDL also were cultured as described in Alton et al. (1988); and *Brucella*-suspect isolates were shipped to NVSL for confirmation. At NVSL, isolates were identified using a modified AMOS PCR, which includes primers to differentiate vaccine strains (Bricker and Halling 1994a, 1994b; Bricker et al. 2003; Ewalt and Bricker 2003). Tissues with confirmed growth of *B. abortus* were considered culture positive, as was the animal from which the tissues came.

Statistical analysis

We analyzed the probability of shedding events (defined as parturition associated with culture-positive fluids or tissues; positive milk excluded) produced by any nonsentinel female using a generalized linear mixed model fit by maximum likelihood using Laplace approximation. The GLMER model used is a function within the various linear mixed models of the R programming environment packages 'lme4' and 'lmerTest' (R Core Team 2022). We used a negative binomial approach given the large number of nonshedding events (n=160; defined as any nonsentinel animal that did not produce a shedding event regardless of pregnancy status), versus shedding events (n=19). This created an overdispersed and clumped data set that seemed best suited to a negative binomial distribution for modelling purposes (Bolker 2015). We opted to keep the four groups separate because of differences in the research practices between the two replicates, which seemed large enough to warrant their separation. It would have been difficult to account for these differences in the model. These differences included variation in immunocontraceptive mixing methods (Frey et al. 2024), removal of animals, delayed start of *Control Rep2*, and early cessation of *Replicate 2*. In addition, because of the year delay in establishing *Control Rep2*, we did not include data from the first year of *Treatment Rep2* in the analysis, because it lacked a control during that time. Keeping the groups separate resulted in treatment status by replicate becoming one of the fixed effect variables. We acknowledge that this is a somewhat less standard than combining the data and testing only for the effect of treatment, but as described above, the introduced biases in the data required a different approach.

We selected three fixed effect variables to inform or predict shedding. These included treatment status with four factor levels, treatment or control separated by replicate, year (scaled) and animal age (scaled). Scaling was done to avoid giving these variables undue weight as numeric values, allowing the model more numeric stability

and better convergence (Fox 2015). Each year in the study represents the calendar year following a breeding season (again, year 1 began in the January following an animal's first exposure to males in the study). We included unique animal ID as a random effect to account for the repeated measure in this longitudinal study design.

Ethics approval

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the USDA-APHIS, Wildlife Services, National Wildlife Research Center (Protocol QA-1858) and the IACUC of the USDA-APHIS, VS, Bison Quarantine Research Facility (Title: Evaluation of GonaCon, an immunocontraceptive vaccine as a means of decreasing shedding of *Brucella abortus* in bison). In addition, this study was made available for public comment via an environmental impact statement in 2012, in which "no significant impact" was the final determination (USDA-APHIS 2012a, 2012b).

RESULTS

Treatment failures, losses, and censored animals

Within year 1 of *Replicates 1* and *2*, treatment failure (i.e., breakthrough pregnancy) occurred in 3/15 *Treatment rep1* bison and 1/20 *Treatment rep2* bison. These individuals were removed from the study. In addition, 10 bison from the first replicate and three cows from the second died over the course of the study due to trauma, peritonitis, dystocia, or pneumonia. Ultimately, eight animals were censored from the statistical analysis due to immunocontraceptive failure in year 1 (four), mortality before calving in year 1 of both replicates (three), and one *Control Rep1* animal that never bred and was removed after year 3.

Shedding events and seroconversion of sentinels

Control rep1 animals produced 12 shedding events out of 47 birth events that occurred and 57 possible birth events over five reproductive seasons (Table 1 and Supplementary Material Table S1). The first *Control rep1* shedding event occurred late January, preceding detection of the first seroconversion and abortion (albeit culture-negative) in a $Sentinel_{\text{Control rep1}}$ animal 15 d later. Two more *Control rep1* animals produced shedding events and two more $Sentinel_{\text{Control rep1}}$ animals seroconverted and produced shedding events in year 1. In contrast, *Treatment rep1* animals gave birth to seven culture-negative live calves out of 50 possible births. The five $Sentinel_{Treatment\ rep1}$ animals produced 22 culture-negative parturitions during the first trial. No sentinels seroconverted in the *Treatment rep1* throughout the study.

Control rep2 animals produced five *B. abortus* shedding events out of 17 birth events and 29 possible births, and $Sentinel_{Control\ rep2}$ sustained four shedding events out of 10 birth events over 2 yr (Table 1 and Supplementary Material Table S1). Two of the three *Control rep2* shedding events in year 1 preceded the first two seroconversions or shedding events in $Sentinel_{Control\ rep2}$. In *Treatment rep2*, one of six females with breakthrough pregnancies in the second or third years produced two *B. abortus* shedding events. No shedding events were detected among the $Sentinel_{Treatment\ rep2}$ females, but one animal was detected as seropositive in January of year 3. The five remaining $Sentinel_{Treatment\ rep2}$ animals remained seronegative through the third calving season, but four of these animals were identified as seropositive in December of year 3.

Only the fixed effect variable, "treatment status," which equated to the four group assignments, provided predictive value for shedding events ($P \leq 0.001$; Table 2). *Control rep1*, *Control rep2*, and *Treatment rep2* had similar values (log odds −1.71, −0.23, and −1.39, respectively; Table 2a). *Treatment rep1* had a log odds value of −25.36 indicating a much lower shedding probability relative to the other three groups. Fixed effect predictor covariates, year (scaled) and age (scaled), had no explanatory value, and were ultimately removed from the model. The random effect of unique animal ID, represented by the interclass correlation coefficient value output, was very low and indicates no influence on the model outcome (Table 2b).

TABLE 1. *Brucella abortus* shedding events and seroconversion observed in captive bison (*Bison bison*) during experimental evaluation of immunocontraception as a disease management tool. Shedding events were the *Brucella abortus* culture–positive births detected within each group (below) in a year. New seropositive females were the number of female bison in each sentinel group (below) that newly tested seropositive for *B. abortus* exposure in January, at calving, or in June–July of the same study year. Annual and overall totals are shown. The experiment included two replicates (*rep1, rep2*). Each replicate included a group of immunocontraceptive-treated seropositive females (*Treatment*) and a group of untreated seropositive females (*Control*) housed in separate paddocks. *Treatment* groups received a one-time intramuscular administration of 3,000 µg gonadotropin-releasing hormone-based immunocontraceptive in May of the year before onset of the replicate. Smaller numbers of seronegative female sentinels (denoted as $Sentinel_{group}$) were housed with each of the four primary experimental groups (*Treatment rep1*, *Treatment rep2*, *Control rep1*, *Control rep2*) to monitor for transmission. Study years were defined as January–December. The first replicate trial ran for 5 yr (2013–2017); the second replicate trial had a staggered start and ended after 2 or 3 yr of observing *Control rep2* (2016–2017) or *Treatment rep2* (2015–2017), respectively. See text for additional details of experimental design and study methods.

Metric	Group	n	Year					Total shedding events/total parturitions (percent)	Total shedding events/total possible parturitions (percent)
			1	2	3	4	5		
No. shedding events/parturitions (%)	*Control rep1*	12[a]	4/9 (44)	1/9 (11)	3/10 (30)	4/10 (40)	0/9 (0)	12/47 (26)	12/57 (21)
	$Sentinel_{Control\ rep1}$	5[b]	2/5 (40)	2/4 (50)	1/5 (20)	1/3 (33)	1/3 (33)	7/20 (35)	7/22 (32)
	Treatment rep1	11	0/0 (0)	0/1 (0)	0/2 (0)	0/1 (0)	0/3 (0)	0/7 (0)	0/55 (0)
	$Sentinel_{Treatment\ rep1}$	5	0/3 (0)	0/4 (0)	0/5 (0)	0/5 (0)	0/5 (0)	0/22 (0)	0/25 (0)
	Control rep2	15[c]	3/7 (43)	2/10 (20)	—	—	—	5/17 (29)	5/29 (17)
	$Sentinel_{Control\ rep2}$	6	2/6 (30)	2/4 (50)	—	—	—	4/10 (40)	4/12 (33)
	Treatment rep2	19	0/0 (0)	1/5 (20)	1/4 (25)	—	—	2/9 (22)	2/57 (4)
	$Sentinel_{Treatment\ rep2}$	6	0/4 (0)	0/6 (0)	0/5 (0)	—	—	0/15 (0)	0/18 (0)
New seropositives (number/year)	$Sentinel_{Treatment\ rep1}$	—[e]	0	0	0	0	0	—	—
	$Sentinel_{Treatment\ rep2}$[d]	—	0	0	1	—	—	—	—
	$Sentinel_{Control\ rep1}$	—	3	1	0	1	0	—	—
	$Sentinel_{Control\ rep2}$	—	2	1	—	—	—	—	—

[a] Two females died before year 4 or 5.
[b] Two previously seroconverted females died before year 4 or 5.
[c] One female died before year 2.
[d] Animals were euthanized and tested in December of year 3, by which time four more animals had become seropositive.
[e] —indicates that data is not applicable.

TABLE 2. (a) and (b). Model output for generalized linear mixed model fitted by maximum-likelihood methods using a negative binomial distribution to determine which variables predicted shedding of *Brucella abortus* in bison in a replicated experiment evaluating immunocontraception in *Brucella*-seropositive female bison as a disease management tool. The experiment included two replicates (*rep1, rep2*). Each replicate included a group of immunocontraceptive-treated seropositive females (*Treatment*) and a group of untreated seropositive females (*Control*) housed in separate paddocks. *Treatment* groups received a one-time intramuscular administration of 3,000 μg gonadotropin-releasing hormone-based immunocontraceptive in May of the year before onset of the replicate. Smaller numbers of seronegative female sentinels were housed with each of the four primary experimental groups (*Treatment rep1*, *Treatment rep2*, *Control rep1*, *Control rep2*) to monitor for transmission. Study years were defined as January–December. The first replicate trial ran for 5 yr (2013 – 2017); the second replicate trial had a staggered start and ended after 2 or 3 yr of observing *Control rep2* (2016 – 2017) or *Treatment rep2* (2015 – 2017), respectively. We selected three fixed effect variables to inform or predict shedding. These included treatment status with four factor levels: treatment or control separated by replicate, project year (scaled), and animal age (scaled). Only treatment status provided predictive value in regards to shedding. The difference in log odd values between the *Control rep1* and *Treatment rep1* is large. *Treatment rep1* had no shedding events. Though *Treatment rep2* indicates a value that could be interpreted within the realm of statistical significance, this is more likely an artifact of small sample size and a single individual being positive two consecutive years. The random effect of unique animal ID, represented by the ICC value output, was very low and indicates no influence on the model outcome.

(a)

Fixed effect			
Treatment status	Log odds—mean	Confidence interval	*P* value
Control rep1[a]	−1.71	−2.58 to −0.85	<0.001
Treatment rep1	−25.36	−2,032.36 to 1,981.65	0.980
Control rep2	−0.23	−1.34 to 0.89	0.691
Treatment rep2	−1.39	−2.89 to 0.12	0.071

(b)

Random effect	
Unique animal ID	
Total random effect variance	3.08
Random effect variance	0.32
Interclass correlation coefficient	0.10
Number of individuals	57
Number of observations	179

[a] The model assigned group as intercept.

Final *Brucella* culture and serostatus

Supplementary Material Table S1 presents individual animal data on culture status at final disposition. Table 3 summarizes the initial serologic status and final serologic and culture status of all study animals. Four originally seropositive animals became seronegative at some time during the study and remained so until final disposition. Four animals originally seropositive were intermittently seronegative or suspect during the study but were ultimately seropositive at final disposition.

DISCUSSION

Our findings suggest that halting reproduction in *Brucella*-seropositive bison can reduce *B. abortus* shedding events, if not prevent them entirely. In both replicates, no shedding events occurred as long as pregnant animals were removed from the groups. As we allowed breakthrough pregnancies to remain in the treatment groups in subsequent years, our intent was to observe whether shedding, and thus transmission, might continue to be reduced over time, despite a gradual return to fertility. This concept

TABLE 3. Number of *Brucella*-seropositive female bison in each group out of total animals in each group at study initiation, and number and percent of seropositive and culture-positive female bison in each group out of total animals in each group at final disposition. Study is a replicated experiment evaluating immunocontraception in *Brucella*-seropositive female bison as a disease management tool. The experiment included two replicates (*rep1*, *rep2*). Each replicate included a group of immunocontraceptive-treated seropositive females (*Treatment*) and a group of untreated seropositive females (*Control*) housed in separate paddocks. *Treatment* groups received a one-time intramuscular administration of 3,000 μg gonadotropin-releasing hormone-based immunocontraceptive in May of the year before onset of the replicate. Smaller numbers of seronegative female sentinels (denoted as $Sentinel_{group}$) were housed with each of the four primary experimental groups (*Treatment rep1*, *Treatment rep2*, *Control rep1*, *Control rep2*) to monitor for transmission. Study years were defined as January–December. The first replicate trial ran for 5 yr (2013–2017); the second replicate trial had a staggered start and ended after 2 or 3 yr of observing *Control rep2* (2016–2017) or *Treatment rep2* (2015–2017), respectively. See text for additional details of experimental design and study methods.

Group	No. seropositive/total (percent) study initiation	No. seropositive/total (percent) final disposition	Culture-positive/total (percent) final disposition
Control Rep1	12/12 (100)	10/12 (83)[a]	1/12 (8)[a]
$Sentinel_{Control\ Rep1}$	0/5 (0)	5/5 (100)	0/5 (0)
Treatment Rep1	11/11 (100)	9/11 (82)	0/11 (0)
$Sentinel_{Treatment\ Rep1}$	0/5 (0)	0/5 (0)	0/5 (0)
Control Rep2	15/15 (100)	15/15 (100)[b]	3/15 (20)[b]
$Sentinel_{Control\ Rep2}$	0/6 (0)	3/6 (50)	3/6 (50)
Treatment Rep2	19/19 (100)	19/19 (100)	5/19 (26)
$Sentinel_{Treatment\ Rep2}$	0/6 (0)	5/6 (83)	4/6 (67)

[a] Two *Control Rep1* animals died before years 4 and 5.
[b] One *Control Rep2* animal died before year 2.

appeared to be strongly supported by *Replicate 1*, wherein *Treatment rep1* did not produce any shedding events in seven parturitions among four individuals with breakthrough pregnancies; this is in contrast to 12 shedding events produced by *Control rep1*. Low probability of shedding in *Treatment rep1* was also reflected in the data analysis. However, the very different outcome observed in *Replicate 2* emphasizes that the production of shedding events in subsequent years, and the impacts of those shedding events in terms of disease transmission in a herd, are highly unpredictable. This is especially apparent in that, although only 1/6 *Treatment rep2* animals with breakthrough pregnancies after year 1 produced shedding events, these two events apparently led to seroconversion in all but one of the sentinels; an outcome effectively indistinguishable from that observed in *Control rep2*. Therefore, use of tools such as immunocontraception for disease control will likely need nearly perfect efficacy in preventing pregnancy, lasting several years, to reduce disease prevalence in an affected herd successfully.

Three of five $Sentinel_{Control\ rep1}$ seroconversions and two of three $Sentinel_{Control\ rep2}$ seroconversions could almost certainly be attributed to direct exposure to parturition-associated shedding events produced by their respective control groups, as these seroconversions were detected shortly after *Brucella*-positive birth events occurred in both control groups. Unfortunately, we were unable to determine a more precise time frame regarding when transmission occurred in the remainder of sentinels that seroconverted, as detections in those animals were not made until December and January. This would have been important to document more precisely, because using a contraceptive tool relies on the premise that the vast majority of horizontal *Brucella* spp. transmissions are associated with infectious parturition events. In retrospect, more frequent testing between August and November should have occurred each year to estimate timing of seroconversion in relation to known shedding events within their groups.

Although our data do suggest that overall shedding events do diminish as animals age, age was not important as an explanatory variable in our analysis. Age, in the context of targeting younger animals for reproductive removal from a brucellosis-affected bison herd, is considered an important strategy in managing brucellosis. Models have indicated that sterilization or removal of probably infectious seropositive females at or under 5 yr of age, can potentially aid in reducing disease prevalence in the GYA bison population (Ebinger et al. 2011; Treanor et al. 2011; Hobbs et al. 2015). However, in our study, 6-yr-old and 8-yr-old recently seroconverted sentinels produced shedding events. This was probably because they had only recently been infected with *B. abortus*, suggesting that timing of infection is perhaps the more influential factor, rather than age.

Brucella abortus was recovered from only a few animals in the first replicate at termination of the study. In contrast, the *Replicate 2* had many more culture-positive animals at end of the study. As discussed above, this was probably because the second replicate animals had been more recently infected than the animals in the *Replicate 1* at time of necropsy. This outcome is consistent with findings by Treanor et al. (2011), where older female bison, over 5 yr of age, had reduced probability of harboring active infection, probably because their infections were acquired at a young age. In the case of this study, however; we must also consider the possibility that occurrence of fewer culture-positive animals in *Replicate 1* were due to sample handling or laboratory issues, such as variable durations of storage time at −70 C before culturing, but we regard this as a less likely explanation.

In both replicates, none of the healthy offspring from seropositive dams that were associated with *Brucella*-negative births, were identified as seropositive when tested at 9 mo of age, or throughout the remainder of the study, unless they were purposefully exposed to infectious birth events in following years. We therefore did not see any evidence of vertical transmission in those calves, despite being born to and having nursed from seropositive females. These offspring did initially have maternal antibodies to *Brucella*, which waned by approximately 6 mo. This is consistent with the report from Rhyan et al. (2009), where only a few (3/20) calves born to seropositive bison were seropositive at 5–6 mo. Although there is a possibility that some of these calves could be latently infected, latency is considered an uncommon occurrence (Ray et al. 1998).

It is interesting to note that 1/5 *Control rep1* sentinels did not seroconvert until the fourth reproductive season, despite the occurrence of 16 shedding events in the 3 yr prior, through which the other four sentinels became positive on serology during the first two seasons. In contrast, one *Treatment rep2* animal produced two shedding events that seem to have led to the eventual seroconversion of 5/6 associated sentinels. Differences in occurrence of transmission of *B. abortus* within free-ranging herds may be attributed to a variety of animal and environmental conditions, including herd dynamics, animal-specific bacterial shedding levels, plane of nutrition, disease resistance, ambient temperatures, severity of winters, and other environmental factors (Aune et al. 2012; Treanor 2013; Treanor et al. 2015). Controlling for such variables can be challenging; therefore, strategically deploying the most effective possible reproductive intervention would be very important to reduce disease prevalence in a bison herd successfully.

None of the male bison seroconverted during the study, despite multiple exposures to *Brucella*-infected females. Venereal transmission of *B. abortus* is thought unlikely to occur in cattle and bison. Previous studies exploring male to female transmission have been unsuccessful; however, seroconversion did occur in female bison artificially inseminated with attenuated *B. abortus* Strain 19, and virulent *B. abortus* has been isolated from semen collected from male bison in the GYA (Crawford et al. 1990; Robison et al. 1998; Frey et al. 2013; Uhrig et al. 2013). More work is needed to

determine if venereal transmission does occur, as that will influence decisions regarding the need for disease or reproductive intervention in males.

Preventing reproduction in brucellosis-positive bison might be an effective, nonlethal way to exclude these animals from participating in the epizootiology of this disease within a herd (Miller et al. 2004; Ebinger et al. 2011; Rhyan et al. 2013a). However, we observed in our study that an imperfect contraceptive tool can still lead to high-consequence shedding events that have the potential to transmit *B. abortus* to many susceptible animals in the herd. Success of immunocontraception in reducing disease prevalence would probably require a higher and longer-lasting efficacy than we observed in these bison. In addition, tools such as this should be implemented in conjunction with other tools such as vaccination against brucellosis, with adaptive modifications as prevalence changes over time. Further research is needed, such as building upon existing models in which simulations include application of pregnancy prevention at different efficacy levels and durations, to assess whether reduction in brucellosis prevalence can be attained using this strategy.

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SUPPLEMENTARY MATERIAL

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