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Management and Conservation

Environmental Persistence of *Brucella abortus* in the Greater Yellowstone Area

KEITH AUNE,[1] *The Wildlife Conservation Society, 301 N. Wilson, Bozeman, MT 59715, USA*
JACK C. RHYAN, *National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA*
ROBIN RUSSELL, *Montana Department of Fish, Wildlife and Parks, 1400 S. 19th, Bozeman, MT 59718, USA*
THOMAS J. ROFFE, *USFWS, DOI, 1400 S. 19th Avenue, Bozeman, MT 59718, USA*
BARBARA CORSO, *USDA/APHIS/VS/CEAH, Fort Collins, CO 80521, USA*

ABSTRACT Bison (*Bison bison*) and elk (*Cervus elaphus*) of the Greater Yellowstone Area (GYA) are the last remaining reservoirs of bovine brucellosis (*Brucella abortus*) in the United States. An important factor in evaluating the risk of transmission to cattle is the persistence of bacteria and infectious birth materials shed on pastures where cattle graze. We selected 2 study areas near the northern and western boundaries of Yellowstone National Park (YNP) to determine the persistence of bacteria on fetal tissue, soil, and vegetation, and scavenging on infectious materials from birth and abortion sites. We performed 3 independent field experiments to determine: 1) persistence of *Brucella abortus* (RB51) purposely applied to fetal tissues, 2) scavenging of fetuses by native scavengers, and 3) natural contamination of birth or abortion sites in the GYA. Results from these field experiments established that *Brucella* bacteria can persist on fetal tissues and soil or vegetation for 21–81 days depending on month, temperature, and exposure to sunlight. Bacteria purposely applied to fetal tissues persisted longer in February than May and did not survive on tissues beyond 10 June regardless of when they were set out. *Brucella abortus* field strain persisted up to 43 days on soil and vegetation at naturally contaminated bison birth or abortion sites. Fetuses were scavenged by a variety of birds and mammals in areas near YNP and more rapidly inside YNP than outside the Park boundary. Models derived from our data determined a 0.05% chance of bacterial survival beyond 26 days (95% Credible Interval of 18–30 days) for a contamination event in May. May 15 is the final date for hazing all bison into Yellowstone National Park under the current interagency bison management plan. With these data managers can predict when it is safe to graze cattle onto pastures previously occupied by bison.

KEY WORDS abortion, bacterial persistence, birth site, bison, *Bison bison*, *Brucella abortus*, brucellosis, fetal disappearance, Greater Yellowstone Area, Yellowstone National Park.

Bison (*Bison bison*) and elk (*Cervus elaphus*) of the Greater Yellowstone Area (GYA) are the last known remaining reservoirs of bovine brucellosis (*Brucella abortus*) in the United States. Paired serology and culture tests show about 50% of all seropositive bison, and almost 70% of high titer bison cows, have detectable infection (Roffe et al. 1999, Rhyan et al. 2001). Abortion and fetal losses are more common in brucellosis-infected bison, especially recently infected, primiparous cows. Fluids and tissue from brucellosis abortions contain billions of *Brucella* organisms/gram of matter and have previously been shown to contaminate the natural environment of the GYA (Rhyan et al. 1994).

Since the 1980s, free-ranging bison that reside most of the year inside Yellowstone National Park (YNP) frequently migrate outside of YNP onto public and private lands during winter. When potentially infected bison migrate to public grazing allotments or private agricultural lands during winter and spring, there is concern that bison aborting due to brucellosis or shedding infective fluids and membranes might transmit the bacteria to cattle that graze these pastures during spring and summer. Environmental contamination caused by an aborted fetus, birth tissues, feces, or vaginal fluids can persist on soil and vegetation leaving recoverable quantities of living *B. abortus* (Cook 1999). Managers lack information about how prevalent these contaminated sites are in the GYA and how long these sites remain contaminated with infected materials and viable *B. abortus*.

The ability of *B. abortus* to survive in agriculturally managed environments is an important factor determining the risk for brucellosis transmission to other hosts (Kuzdas and Morse 1954, Wray 1975). Survival of bacteria in bovine fetuses is reported to be 135 days in winter when covered with leaves (Cotton 1919), >2 months in a cool environment (Merck Manual 1998), and 6 months in a shaded fetus (Wray 1975). Most of the early research on persistence of *B. abortus* in different media (soil, urine, etc.) has been limited to

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[1]*E-mail: kaune@wcs.org*

laboratory environments or intensely managed agricultural lands not representative of the natural landscapes of the GYA. Cook et al. (1999) used *B. abortus* strain RB51 as a surrogate for field strain and found that the organism survived on the bottom surface of a bovine fetus an average of 60.5 days in February but only 4.7 days in June. Additionally, Cook et al. (2004) examined the length of time that a fetus remained in the environment in northwestern Wyoming before it was scavenged, and found that on the National Elk Refuge and Grand Teton National Park most fetuses disappeared within 69.5 hours. To our knowledge, these 2 studies provide the only geographically relevant information available to GYA managers tasked with making decisions about when cattle should be allowed to graze pastures following use by free-ranging bison.

While attempting to prevent exposure of cattle to infected bison or parturition products, such as fluids, reproductive tissues, or an aborted fetus, animal health and wildlife management authorities in the GYA need specific data to inform management decisions including 1) time intervals that infected parturition products remain infectious, 2) time intervals that fetuses remain in the environment before being scavenged, and 3) time intervals that contaminated soil or vegetation remains infectious. To address the need for this information in the GYA, state and federal agencies commissioned this study under the Interagency Bison Management Plan (IBMP) and Environmental Impact Statement (EIS). Thus, our study was intended to provide site-specific guidance to decisions about temporal and spatial separation between cattle and bison as outlined in the IBMP (Plumb and Aune 2002). Our primary study objective was to determine the extent and duration at which *Brucella* infected tissues from abortion or birth events contaminate environments in the GYA.

STUDY AREA

Grazing lands at the west (near West Yellowstone, MT) and north (near Corwin Springs, MT) entrances of the YNP were the primary areas where bison frequently migrated to in winter and where cattle graze on public and private pastures. These 2 areas were environmentally distinct and climate (particularly snow conditions) was more severe in the western region than the northern area (Gates et al. 2005). Our emphasis was on the private lands adjacent to YNP managed for livestock production and the U.S. Forest Service grazing allotments. These grazing landscapes were typically occupied by cattle from mid-June through October.

We selected 2 bacterial persistence study sites within areas where biosecurity measures could be implemented by regulation as necessary (Fig. 1). One site was north of Yellowstone National Park (YNP) within a privately owned retired game ranch surrounded by game proof fence near the town of Corwin Springs. Our second study site was west of YNP in a fenced property used for community garbage disposal and recycling. Public access to the area was restricted to specific daylight times and it was monitored Monday through Saturday by city sanitation employees.

METHODS

Persistence of *Brucella* Organisms on Fetal Tissues

We collected 96 bovine and 280 bison fetuses in utero or removed them from the uterus at slaughter plants in Colorado and South Dakota and froze specimens for transportation to Montana. We thawed fetuses, placed them in plastic bags, and soaked them in RB51 solution 2 days prior to field deployment. We prepared the soaking solution from concentrated inoculums of *B. abortus* strain RB51 prepared at the National Veterinary Services Laboratory (NVSL; Ames, IA) mixed with peptone and water. To emulate a naturally infected fetus we poured 750 ml of RB51 solution containing $\geq$1 billion colony forming units (cfu)/ml, over the fetus in plastic bags. Additionally, we injected 50 ml of the solution into the abdominal cavities of the fetuses and then rolled the specimens thoroughly to contaminate the entire fetus.

We classified fetuses by weight, crown rump length, hair color, and sex. We sorted fetuses to ensure that each study site received fetuses of approximately equal size, color, and sex ratio. We placed 16 bovine or bison fetuses in open wire dog cages on each of the 2 study sites in February, March, April, and May. We sutured plastic fistulas (modified 60 cc syringes) into the abdominal wall to allow us to routinely swab the abdominal cavity. We covered 8 wire dog kennels at each site with shade cloth that intercepted 75% of Ultraviolet (UV) radiation whereas 8 other kennels were left uncovered to expose fetuses to sunlight. In addition, we placed 2 fetuses in shaded and unshaded cages with temperature probes attached to the top surface, bottom surface, and in the abdomen of the fetuses and 15 cm above the ground in the open air. We connected temperature probes to CR10X Campbell data-loggers (Campbell Scientific Inc., Logan, UT) powered with 12-volt marine batteries.

To determine variation in bacterial survival in various micro-environments we collected skin biopsy samples from the top and bottom surfaces of each fetus and swabbed the abdomen twice weekly. Biopsy samples and swabs were placed in vials of 0.5–1.0 ml of World Health Organization (WHO) media, frozen on dry ice, and shipped to the National Animal Disease Center (NADC) in Ames, Iowa. At NADC the samples were cultured for *B. abortus* RB51 followed by polymerase chain reaction (PCR) confirmation of cultures. In 2001, we considered a fetus culture negative for *B. abortus* strain RB51 only after 2 negative results from all 3 sample sites but discovered that samples occasionally were culture positive for *B. abortus* strain RB51 beyond the first 2 negative samples. We modified our criteria in 2002 and 2003 to judge a fetus negative after 4 consecutive negative culture results at which time we collected and incinerated the fetus.

Fetal Disappearance Rates in the Greater Yellowstone Ecosystem

We conducted an independent study to determine the length of time a bovine fetus might persist within or adjacent to YNP before decomposition or being scavenged. In 2001, we obtained permission to begin the study in YNP and on 2 ranches adjacent to the Park, 1 in the west study area and

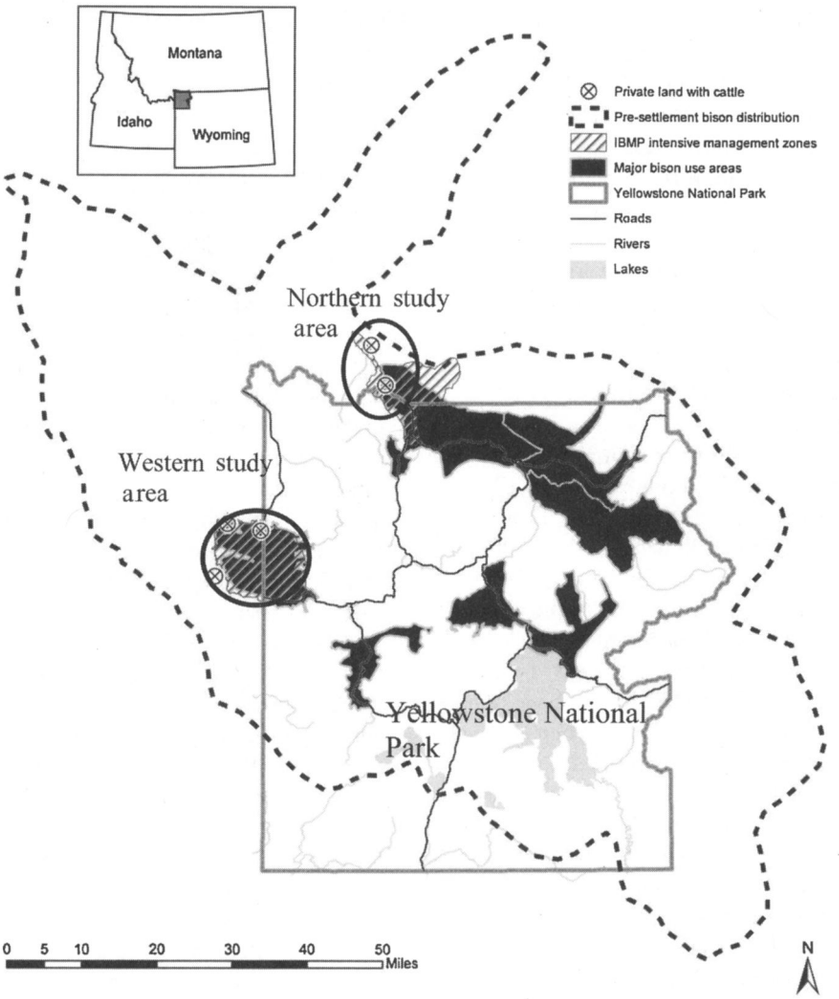

Figure 1. Map depicting the study areas, bison use areas, and Interagency Bison Management Plan (IBMP) intensive management zones in and near Yellowstone National Park, USA, 2001–2003.

another in the north study area. In 2002 and 2003, we expanded our study area to include portions of the Gallatin National Forest following the completion of an environmental assessment that evaluated any effects associated with placing bison fetuses in grizzly bear (*Ursus arctos*) and gray wolf (*Canis lupus*) habitat.

We defined suitable winter-spring (Feb–May) habitat from radio telemetry data for 53 bison monitored from 1996 to 2000 (Rhyan et al. 2009). In 2001, we placed fetuses on a 1 km grid within suitable winter-spring habitats to avoid concentrating scavengers on the landscape. We systematically placed 16 fetuses on this grid at the beginning of March, April, and May for a total of 48 fetuses. We modified the deployment schedule in 2002 and 2003 to randomly place 4 fetuses each week to better emulate the expected occurrence of abortions during late winter-early spring (Cheville et al. 1998).

We attached a trap transmitter (Advanced Telemetry Systems, Isanti, MN) to the rear leg of each fetus. A magnetic switch on the transmitter was deactivated with a magnet when the fetus was placed in the field. We attached a wire from the magnet to a steel spike positioned beneath the carcass so that the magnet would detach from and activate the transmitter when a carcass was disturbed. If a scavenger transported a fetus away from the station we relocated the fetus (within 1–3 days after disturbance) to determine how far it was moved and how much tissue was consumed. We continued monitoring all fetuses until they were fully consumed or decomposed.

We placed motion-activated cameras on 50% of the stations in 2001 to identify scavengers and determine time of scavenging events. We mounted cameras above ground on a portable jack stand or anchored to a tree 10–15 m from the carcass. We discontinued using cameras to monitor fetus disappearance sites after 2001 because of apparent effects of camera flash on scavenging activity. Consequently, we censored data from 2001 for stations monitored by cameras.

When placing and monitoring fetuses, we minimized human scent using latex gloves to handle fetuses. In 2002 and 2003, we transported each bison fetus in utero to study sites and extracted fetuses on-site to further reduce human scent and to better emulate an abortion event including

fetal fluid, membranes, and placental tissue. Field teams checked each station ≥3 times each week and opportunistically between regular checks as schedules allowed. Field checks were made from a safe distance (>50 m) using binoculars and telemetry to determine if fetuses were disturbed by scavengers. To minimize human disturbance at study sites, we did not approach fetuses unless visual evidence or telemetry indicated scavenging activity. In our statistical analysis we included year as a covariate to determine if adjustments in our methods influenced model outcomes.

Brucella abortus Contamination at Naturally Occurring Bison Birth or Abortion Sites

We located naturally occurring birth or abortion sites during a brucellosis epidemiology study conducted from 1996 to 2002 in and adjacent to YNP (Rhyan et al. 2009). From 1996 to 2002, we radio-marked bison following field immobilization according to techniques identified in Aune et al. (1998) and Rhyan et al. (2009). We palpated each bison to determine pregnancy and implanted pregnant animals with vaginal implant transmitters (VIT; Advanced Telemetry Systems; Bowman and Jacobson 1998). In 2002, we opportunistically radio-marked additional bison when captured during annual field operations prescribed under the IBMP. We handled these bison in a standard cattle squeeze and fitted them with radiocollars and VITs after an initial screening test was negative for brucellosis. We used motion or temperature sensitive VITs that emitted an increased pulse rate when expelled from the vaginal cavity.

We monitored movements and reproductive status of radio-marked bison intensively and observed them 3–6 times per week throughout late winter and early spring. We conducted ground and aerial telemetry searches to locate individual animals, VITs, and sites of potential birthing activity (Carstensen et al. 2003). We initiated field expeditions as soon as we determined VITs were expelled (usually 1–7 days) to directly observe marked animals and determine if female bison were accompanied by a calf (Seward et al. 2005, Barbknecht et al. 2009). We also included chance observations of calving or abortion in unmarked bison and occasionally encountered aborted fetuses or placental tissue expelled by unmarked bison. We recorded Global Positioning System location, habitat conditions, number and type of animals observed in or near the site, date, time, and presence of scavengers for each event. We stratified sites as either 1) birth or abortion events based on visible fetus, fluids, or tissue, or 2) premature VIT ejection events which lacked evidence of these birth products.

At each birth or abortion site, we mapped the specific location of tissue, fluids, or fetus and microsite features were labeled with spike nails. When we located fetus or birth tissues they were collected, bagged, labeled, and frozen. We swabbed soil and vegetation and placed swabs in WHO media. We sampled soil and vegetation at VIT ejection sites only in the area directly beneath implants. We placed ejected VITs in a Whirl-Pak bag (Nasco, Modesto, CA) and injected approximately 10 ml of WHO media to gently washed the implant. The WHO media was extracted using syringes, placed into sterile vials, labeled, and subsequently frozen for submission to the laboratory. We revisited all sites once a week and collected samples from each microsite feature until culture results were negative ≥2 times. We submitted samples to NADC for standard culture and PCR confirmation.

Statistical Analysis

We analyzed our data using a Cox Proportional Hazards model (Cox 1972) implemented in WINBUGS (Speigelhalter et al. 2007). We modeled the number of days fetuses remained infected with *Brucella* and the number of days until fetuses were completely scavenged as a function of covariates. Covariates we considered in models of fetus disappearance included study site (northern or western), year, month the fetus was placed on the landscape, distance to the road, and distance to YNP. Covariates we considered for inclusion in models of *Brucella* persistence in the fetus were study site, year, month the fetus was placed on the landscape, sample (top, bottom, or swab), fetus exposure to sunlight (sun or shade), and fetus size. We subdivided observations for each fetus into the event triplet (e,r,s) where e = time of entry into the study, r = last time a fetus tested positive for *B. abortus* RB51(for brucellosis persistence) or the last time fetus remnants were detected on the study site (for time to scavenging), and s = day of the first negative test for *Brucella* to declare fetuses *Brucella* negative or day a fetus was completely scavenged (Andersen and Gill 1982, Liebezeit et al. 2009). We calculated joint probabilities of fetuses testing positive and remaining unscavenged directly in WINBUGS using the results from the 2 separate analyses. We could not test whether these probabilities would be serially dependent as our results were generated independently in 2 separate study procedures. WINBUGS implements Bayesian analysis techniques by employing the Metropolis-Hastings algorithm to conduct Markov Chain Monte Carlo (MCMC) simulations (Speigelhalter et al. 2007). We ran models for 10,000 iterations but discarded the first 4,000 until we achieved stationarity to minimize effects of initial values on the posterior inference (Lunn et al. 2000). We evaluated models using the Bayesian deviance information criteria (DIC; Speigelhalter et al. 2002). We conducted a graphical assessment of the proportional hazards assumption and did not find any evidence of violations of the proportional hazards assumption (Kalbfleisch and Prentice 1980).

RESULTS

Persistence of *Brucella* Organisms on Fetal Tissues

Brucella abortus strain RB51 survived a maximum of 81 days for a fetus placed out in February. In contrast, RB51 survived a minimum of 21 days for a fetus placed out during mid-May (Table 1). The raw bacterial decay curves demonstrated an observable difference in survival probability across the late winter and spring (Fig. 2); 10 June was the latest date

Table 1. The maximum time (days) for the observed survival of *Brucella abortus* (RB51) on a shaded fetus in the Greater Yellowstone Area compared to the model predicted median time (day there is a <50% chance of survival) and 95% credible intervals, 2001–2003.

		Top		Bottom		Swab	
Month	No.	Observed max. days	Predicted median (95% CI)	Observed max. days	Predicted median (95% CI)	Observed max. days	Predicted median (95% CI)
Feb	96	67	12 (10–15)	77	24 (29–34)	81	22 (19–26)
Mar	96	49	15 (13–17)	77	33 (38–39)	63	26 (21–30)
Apr	96	42	11 (10–13)	69	26 (21–30)	44	20 (17–24)
May	88	21	6 (5–7)	24	13 (11–15)	25	10 (8–12)

that RB51 was cultured from any tissue (fetus deployed on 15 May).

We ran 27 candidate models of brucellosis persistence, of which the month + light exposure + sample location model was the optimal model (Table 2). Year and study site effects were not contained in any of the top 3 models indicating that these factors had limited influence on the response variable. The hazard ratio indicated that the risk of RB51 bacteria dying was 2.37 times higher in May than in February given that all other covariates were held constant (Table 3). Shading from the sun and location also affected the likelihood that RB51 would persist (Tables 2 and 3). Hazards ratios indicated that the odds of bacterial survival were approximately 1.82 times greater (inverse of hazards ratio) inside the fetus (swab site) and 2.4 times greater for samples from the bottom of the fetus compared to samples from the top surface given that all other variables were held constant. Shading the carcass from the full intensity of sunlight with shade cloth also increased the probability that bacteria would survive (Table 3). The proportional hazards model predicted <0.05% likelihood that RB51 would survive longer than 125 days in February on the bottom of a fetus shaded from sun (95% credible interval 108–145 days; Fig. 3). Predicted probabilities of persistence indicate that a sample from the bottom of a shaded fetus contaminated during May would have <0.05% probability of persistence after 53 days (95% credible interval 45–63 days; Fig. 3).

Fetal Disappearance Rates in the Greater Yellowstone Ecosystem

We photographed or identified by track evidence 13 scavenger species including gray wolf, coyote (*Canis latrans*), black bear (*Ursus americanus*), grizzly bear, mountain lion (*Felis concolor*), red fox (*Vulpes vulpes*), striped skunk (*Mephitus mephitis*), pine marten (*Martes martes*), bald eagle (*Haliaeetus leucocephalus*), turkey vulture (*Cathartes aura*), raven (*Corvus corax*), magpie (*Pica pica*), and an unidentified hawk (*Buteo* spp.). Most mammals scavenged during evening or at night whereas birds scavenged during daylight hours. Six species (elk [*Cervus elaphus*], American bison [*Bison bison*], white-tailed jack-rabbit [*Lepus townsendi*], mule deer [*Odocoileus hemionus*], pronghorn [*Antilocapra americana*], and Canada goose [*Branta canadensis*]) investigated fetuses, but did not scavenge. White-tailed jack rabbits, mule deer, pronghorns, and Canada geese approached fetuses but did not make physical contact, whereas several elk and bison mildly or aggressively nudged, sniffed, or licked fetuses.

Fetuses placed in the field were frequently transported away from the original site to be consumed by scavengers and occasionally were cached for later consumption. We found portions of carcasses in trees (moved by martens and birds), buried in soil (by coyotes and fox), or in dens (inhabited by wolves). Many (106 of 204; 51.9%) fetuses were moved ≥30.5 m (100 ft.) from the original deployment

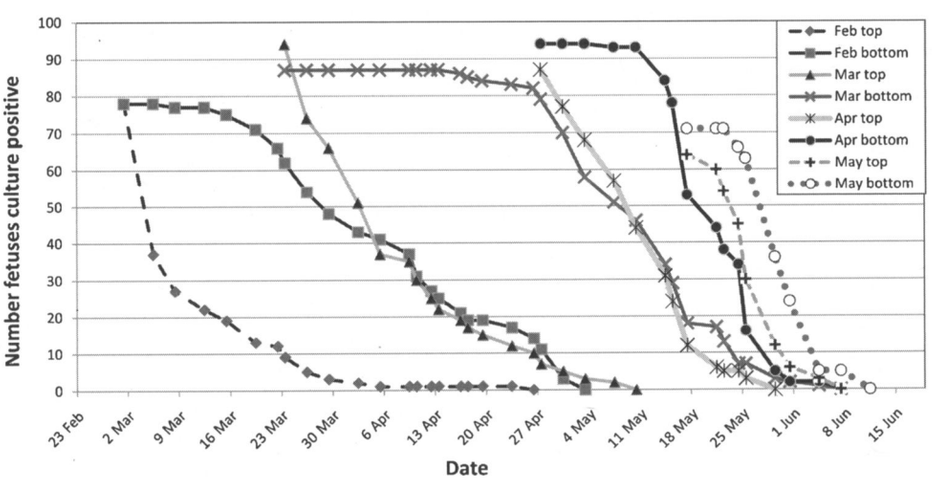

Figure 2. Raw survival curves for *Brucella abortus* (RB51) bacteria on bison fetuses placed out in various months for 3 years combined showing number of fetuses culture positive on the top and bottom surfaces over time, 2001–2003.

Table 2. Deviance information criteria (DIC) results of modeling *Brucella abortus* (RB51) persistence on a bison fetus exposed to sun or shaded with samples taken from the top, bottom, or inside (swab) of a fetus using Cox proportional hazards for the months of February–May, 2001–2003. ΔDIC for a model is the difference between the minimum DIC for a set of models, and the DIC for the model. DIC wt = −0.5 × ΔDIC score for that model divided by the sum of these values across all models.

Brucella model	DIC	ΔDIC	DIC wt
Month + sun or shade + sample	8,488.39	0.00	1.00
Month + sample	8,501.58	13.19	0.00
Study site + sample + sun or shade	8,585.69	97.30	0.00

site; maximum distance a fetus was moved was 2 miles by a red fox. We detected movement of fetuses between private and public lands in 7 instances.

Motion sensitive cameras monitored 50% of bovine fetuses deployed in 2001. Fetuses disappeared more rapidly at sites without cameras (10.7 days) than those with cameras (17.1 days; $F_{96} = 8.93$; $P = 0.004$). The mean number of days between fetus deployment and complete consumption was 18.2 days and ranged from 1 to 78 days ($n = 204$, SD = 20.1). All fetuses were consumed by scavengers except 2 in the north study site that gradually decomposed.

We ran 23 models of fetal disappearance, of which the study site + YNP + interaction (Study site × YNP) model was the optimal model (Table 4). Year and month effects were not contained in either of the top 2 models, indicating that these factors had little or no association with the response variable. A fetus was 1.78 times more likely to be scavenged in YNP than in multiple-use lands adjacent to the Park (Table 5). Although our top model included study site as an important predictor of fetal disappearance, differences between the 2 study sites was small (Fig. 4). For example, at day 10 outside YNP the median predicted probability of persistence at the northern site was 0.596 with a 95% credible interval (0.541, 0.657) and the median predicted probability of persistence at the western study site outside YNP was 0.571 (95% credible interval: 0.429, 0.765).

Combined Model

Using the best predictive models for *Brucella* persistence and time to scavenging we calculated the joint probability of a *Brucella* contaminated fetus remaining in the GYA. The curves of joint probability for survival became steeper as the winter-spring season progressed from February to May (Fig. 5). The combined model predicted a <0.5% probability that a fetus deposited on 15 May in the northern study area would remain unscavenged and that *Brucella* would survive on the bottom surface beyond 10 June (mean persistence for May is 26 days, 95% credible interval = 18–30 days). Converting this credible interval into dates around 10 June yielded a range from 2 June to 14 June.

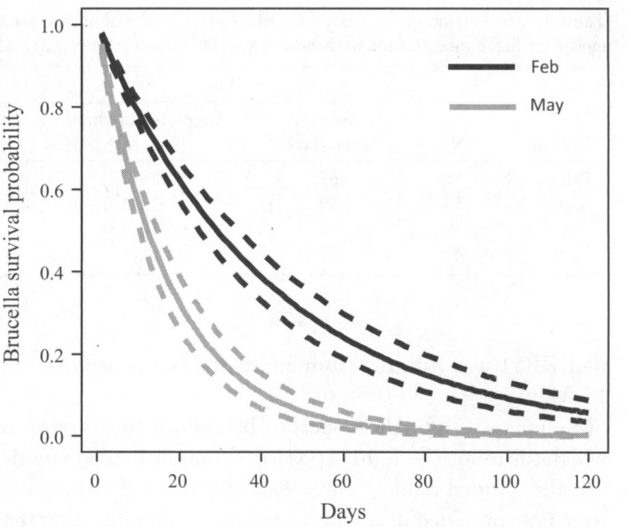

Figure 3. The predicted probabilities of *Brucella abortus* (RB51) survival on the bottom surface of a bison fetus that is shaded from the sun as derived from a Cox proportional hazards model and comparing the months of February and May, 2001–2003. Dotted lines indicate the 95% credible intervals.

Brucella abortus Contamination at Naturally Occurring Bison Birth or Abortion Sites

We conducted field investigations at 152 potential birth or abortion sites from marked or unmarked bison. Approximately 49% of these birth or abortion sites were located with the aid of VITs whereas 51% were located during chance encounters while conducting field activities. The greater proportion of birth or abortion sites presented expelled tissue, fluids, or an aborted fetus (63.2%) whereas at others we found an ejected implant without visible evidence of tissue or fluids (37.8%). Birth or abortion sites were located and investigated from February through July with the majority (91.4%) found in April–June (Table 6).

Fourteen of 152 (9.2%) sites investigated and sampled were positive for *B. abortus* biovar-1, the most common field strain

Table 3. Parameter estimates and hazards ratios from Cox proportional hazards models predicting persistence of *Brucella abortus* (RB51) on bison fetal tissues in the Greater Yellowstone Area, 2001–2003. We present the median and 95% confidence intervals (0.025–0.975) for the top model as selected by deviance information criteria.

Brucella model	Median	0.025	0.975	Hazard ratio
Brucella intercept	−2.62	−2.80	−2.45	
Mar vs. Feb	−0.13	−0.30	0.05	0.88
Apr vs. Feb	0.12	−0.06	0.29	1.12
May vs. Feb	0.86	0.68	1.04	2.37
Swab vs. top	−0.59	−0.74	−0.43	0.55
Bottom vs. top	−0.85	−1.01	−0.70	0.42
Shade vs. sun	−0.26	−0.37	−0.13	0.77

Table 4. Deviance information criteria (DIC) results of modeling bison fetus persistence at 2 study sites, northern and western and inside or outside Yellowstone National Park (YNP) using Cox proportional hazards, 2001–2003. ΔDIC for a model is the difference between the minimum DIC for a set of models, and the DIC for the model. DIC wt = −0.5 × ΔDIC score for that model divided by the sum of these values across all models.

Fetus models	DIC	Δ DIC	DIC wt
Study site + YNP + study site × YNP	1,938.08	0.00	0.40
Study site + YNP	1,938.68	0.60	0.30
Study site + YNP + month	1,941.04	2.96	0.09

Table 5. Parameter estimates and hazards ratios from Cox proportional hazards models predicting persistence of bison fetuses in the Greater Yellowstone Area, 2001–2003. We present the median and 95% confidence intervals (0.025–0.975) for the top model as selected by deviance information criteria.

Fetus scavenging model	Median	0.025	0.975	Hazard ratio
Fetus intercept	−2.94	−3.15	−2.77	
In Yellowstone vs. out	0.58	0.19	0.94	1.78
West vs. north	0.38	0.17	0.65	1.48
Study site × YNP	−0.34	−0.90	0.25	

found in the GYA. Two of 56 VIT ejection sites (3.6%) and 12 of 96 birth or abortion sites (12.5%) were culture positive. An aborted fetus was located on 6 of 12 positive birth or abortion sites. The persistence of bacteria was determined through multiple sample efforts at 9 of the 14 positive sites investigated. The remaining 5 sites were not available to be resampled before they were destroyed by environmental factors including heavy snow, flooding, or trampling by large bison herds. We discovered culture positive sites during the months of April and May. *Brucella abortus* persisted on the April sites ($n = 6$) from 10 to 43 days but remained viable for only 7–26 days on May sites ($n = 3$).

DISCUSSION

Persistence of *Brucella* Organisms on Fetal Tissues

Using RB51 as a surrogate for field strain, we found that *Brucella* persisted for days (or even months) on undisturbed fetal tissue exposed to the natural environment in the GYA. Persistence of RB51 on fetal tissues highlights the importance of temporally separating grazing cattle and bison to avoid indirect exposure and potential transmission of *B. abortus*. The most prominent risk period for indirect transmission was from February through early June.

Our results fall within the range of *Brucella* persistence reported from other studies across a wide range of climates and from throughout the world (Cameron 1932, Spink 1956). Previous research suggests that viability of *B. abortus* outside the host is influenced by the prevailing environmental conditions (Nielson and Duncan 1990). Bacterial survival outside a host is dependent on environmental factors including exposure to UV light, humidity, and temperature effects

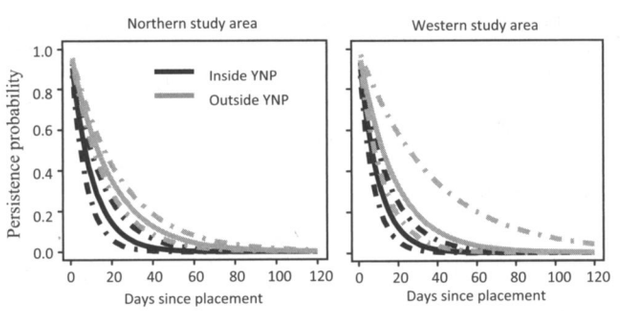

Figure 4. The predicted probabilities of persistence for a bison fetus placed in the northern and western study areas inside and outside of Yellowstone National Park (YNP) derived from a Cox proportional hazards model, 2001–2003. Dotted lines indicate the 95% credible intervals.

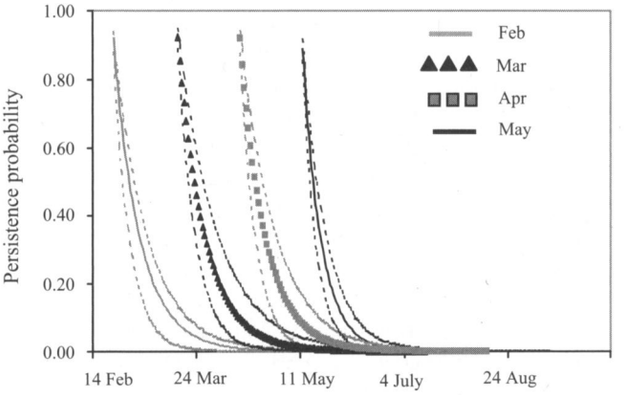

Figure 5. The predicted probabilities for the combined likelihood that a fetus remained both contaminated with *Brucella* bacteria and available on the landscape in the Greater Yellowstone Area derived from a Cox proportional hazards model for each month, February–May, 2001–2003. Dotted lines indicate the 95% credible intervals.

(Neilson and Duncan 1990, Wray 1975, Sinclair et al. 2008). Cook (1999) reported that month influenced survival of *B. abortus* strain RB51 and survival was greatest in February and on the underside of the fetus. He also found a strong correlation between UV and temperature on survival of bacteria. Our findings confirm that month and subsequent exposure to direct sunlight influenced survival of *Brucella* organisms. We observed that sun-protected areas of the fetus (ventral surface) and shading extended the survival of RB51 on fetal tissues.

Fetal Disappearance Rates in the Greater Yellowstone Ecosystem

Scavenging rates appeared to be different in various geographic areas and management jurisdictions across the GYA. Although scavenging resulted in the rapid removal of most fetuses, they disappeared more rapidly inside YNP than outside the Park boundaries. Scavenging bison fetuses outside YNP (this study) also took longer than was reported by Cook et al. (2004) for the National Elk Refuge, Wyoming. Scavenging of fetuses required greater time in multiple use landscapes than protected areas in YNP. We speculate that increased scavenger activity, abundance, and density inside protected areas hastened the rate of scavenging thereby reducing the potential for exposure from an infected fetus, tissue, or fluids expelled during a bison abortion or birth event. Synergistic effects of human disturbance, harvest programs, and reduced scavenging activity may increase risk of

Table 6. Year and month that we discovered bison birth or abortion sites in the Greater Yellowstone Area, 1996–2002.

Year	1996	1997	1998	1999	2000	2001	2002	Total
Feb	0	0	0	1	0	0	0	1
Mar	0	1	0	3	1	2	0	7
Apr	1	2	5	11	7	19	5	50
May	5	8	11	13	10	12	12	71
Jun	0	5	2	1	2	6	2	18
Jul	0	0	4	1	0	0	0	5
Total	6	16	22	30	20	39	19	152

exposure from *Brucella* induced abortion and expelled tissues because they remain longer on multiple use lands.

Camera monitoring of fetuses detected an important curiosity behavior by ungulates, including bison, elk, and mule deer. Although we could not quantify the rates and degree of contact between these fetuses and susceptible host species for *B. abortus*, we found that interactions with fetuses occurred. Maichak et al. (2009) reported and quantified behavioral interactions with aborted fetuses on and adjacent to feedgrounds and Cook et al. (2004) reported consumption of fetuses by elk and bison on the National Elk Refuge. Our results suggested that contact with these fetal tissues could be a significant risk factor for inter- and intra-species transmission of brucellosis among native ungulates throughout the GYA.

The Combined Model

Risk of indirect *Brucella* transmission from bison to cattle was associated with persistence of bacteria on fetal tissues and the rate at which infected tissues were naturally removed by scavengers. To our knowledge, the probability of bacterial survival and risk for indirect transmission of brucellosis from bison to other susceptible hosts had not been evaluated prior to our study. Our combined model predicts that *Brucella* organisms are unlikely to survive after 11 June provided bison have been removed from grazing pastures by 15 May. Our combined predictive model illustrated that bacterial decay and scavenging interacted to rapidly eliminate infectious material from the natural environment. Additionally, our model enables managers to adaptively prescribe an appropriate temporal separation period bounded by specific levels of confidence to safely allow cattle grazing on pastures previously visited by bison.

Brucella abortus Infection at Naturally Occurring Bison Birth or Abortion Sites

Although the sample size from natural abortion sites was small ($n = 9$), we demonstrated that bacterial survival of field strain *B. abortus* mimicked survival of RB51 in our experimental study. Furthermore, our field observations were within the range observed in experiments with dry soil (Cameron 1932) but lower than survival observed in moist soil (Daminova 1968). Environmental persistence of *B. abortus* was not only affected by scavenging, climate, and sunlight but also by physical and mechanical impacts associated with flooding and animal trampling.

Prevalence of infected sites at abortion or birth sites (12.5%) was higher than anticipated. We provided the first field-based estimate of the frequency of *B. abortus* shedding in free ranging bison (12.5%) and the only direct evidence for environmental persistence of *Brucella* bacteria in the GYA. The culture of field strain *B. abortus* from tissues, soil, and vegetation at birth or abortion sites and evidence of bacterial persistence at these sites confirmed risk associated with indirect transmission around those events. We were unable to fully explore that risk because we did not measure the rate of susceptible host contact with sites or determine the concentration of bacteria that would represent an infectious contact.

MANAGEMENT IMPLICATIONS

Our findings confirmed the need to temporally separate cattle and brucellosis-exposed bison when managing the risk of transmission associated with sharing grazing habitats adjacent to Yellowstone National Park. Our predictive model enables managers to quantify the uncertainty associated with various temporal separations strategies. The combined model data could be used to reduce persistent management tensions in the GYA associated with allowing bison and cattle to share pastures at different time schedules. Furthermore, our results demonstrate that preserving a complete component of natural scavengers in this environment will benefit disease management by rapidly removing *B. abortus* infected materials from the landscape. Wildlife managers should seek to maintain a complete assemblage of scavengers on these landscapes to enhance the rapid removal of potentially infectious tissue associated with bison abortions or stillborns.

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