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Reproductive Fate of Brucellosis-Seropositive Elk (*Cervus canadensis*): Implications for Disease Transmission Risk

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ABSTRACT: Brucellosis is a disease caused by the bacterium *Brucella abortus* that infects elk (*Cervus canadensis*) and cattle (*Bos taurus*). There is the potential for transmission from wildlife to livestock through contact with infected material shed during abortions or live births. To understand the impact of exposure on pregnancy rates we captured 30–100 elk per year from 2011 through 2020, testing their blood for serologic exposure to *B. abortus*. Predicted pregnancy rates for seropositive animals were 9.6% lower in prime-age (2.5–15.5 yr; 85%, 95% confidence interval [CI]: 74–91%) and 37.7% lower in old (>15.5 yr; 43%, 95% CI: 19–71%) elk as compared with seronegative animals. To understand the risk of seropositive elk shedding *B. abortus* bacteria and the effects of exposure on elk reproductive performance, we conducted a 5-yr longitudinal study monitoring 30 seropositive elk. We estimated the annual probability of a seropositive elk having an abortion as 0.06 (95% CI: 0.02–0.15). We detected *B. abortus* at three abortions and two live births, using a combination of culture and PCR testing. The predicted probability of a pregnant seropositive elk shedding *B. abortus* during an abortion or live birth was 0.08 (95% CI: 0.04–0.19). To understand what proportion of seropositive elk harbored live *B. abortus* bacteria in their tissues, we euthanized seropositive elk at the end of 5 yr of monitoring and sampled tissues for *B. abortus*. Assuming perfect detection, the predicted probability of a seropositive elk having *B. abortus* in at least one tissue was 0.18 (95% CI: 0.06–0.43). The transmission risk seropositive elk pose is mitigated by decreased pregnancy rates, low probability of abortion events, low probability of shedding at live birth events, and reasonably low probability of *B. abortus* in tissues.

Key words: Abortion, birth site, brucellosis, *Cervus canadensis*, culture, livestock, PCR, pregnancy.

INTRODUCTION

Brucellosis is a zoonotic disease caused by the bacterium *Brucella abortus*, which is endemic in elk (*Cervus canadensis*) in the Greater Yellowstone Ecosystem (GYE) in the US. The disease is a significant management concern because of the potential for transmission between elk and domestic livestock. Brucellosis is characterized by abortions, usually early in the third trimester, and primarily during the first pregnancy after infection (Cheville et al. 1998; Cross et al. 2015). The presence of brucellosis in elk populations may reduce landowner tolerance for elk. A primary concern for livestock producers operating in areas where elk populations host brucellosis is elk-to-livestock disease transmission, and the financial losses and restrictions imposed to reduce this risk. In Montana, livestock that test seropositive for brucellosis are removed and the remaining herd is quarantined until passing two rounds of testing with no detections. Aside from removal and quarantine, livestock producers operating within the Montana Department of Livestock brucellosis Designated Surveillance Area are required to vaccinate livestock and annual testing for early detection is recommended. Management actions that target elk include surveillance through capture and testing, special management hunts, and separation from livestock (e.g., fencing, hazing).

The primary transmission route for *B. abortus* is through contact with contaminated fluids or tissues shed during abortions or live births (Thorne et al. 1997; Cheville et al. 1998). Previous work in the GYE using cross-sectional data has shown that approximately 16% of seropositive pregnant elk have abortions (Etter and Drew 2006; Cross et al. 2015). Elk abortions peak in March–May; live births typically occur mid-May to mid-June (Cross et al. 2015). It has generally been assumed that seropositive elk that have live births have controlled the infection and

are not shedding many, if any, bacteria. In addition, live births are generally considered less likely to lead to transmission because female elk tend to isolate themselves to give birth (Barbknecht et al. 2011), and because environmental conditions in late May and June are not conducive to bacterial survival (Aune et al. 2012; Brennan et al. 2017). *Brucella abortus* can survive on reproductive tissues, vegetation, and soil for 21–81 d under moist and shaded conditions (e.g., under snow), but sunlight and desiccation can kill the bacteria in a few days (Aune et al. 2012). Recent livestock outbreaks in the GYE were traced to transmission from elk (Bienen and Tabor 2006; Kamath et al. 2016) and surveillance of elk herds has shown an increasing seroprevalence across the GYE (Cross et al. 2010; Brennan et al. 2017). Spatiotemporal predictions of potential elk-to-livestock transmission risk have been developed using resource selection functions, seroprevalence, elk population counts, and transmission timing (Merkle et al. 2018; Rayl et al. 2019). Serologic exposure data are typically collected from single capture events of individuals or from hunter harvest samples, offering a glimpse of an individual's *B. abortus* exposure status. Data are still lacking on the transmission risk seropositive individuals pose over time through frequency of abortions, shedding of *B. abortus* at live birth sites, and active infections as evidenced by live bacteria in tissues (but see Thorne et al. 1978).

Although abortion is considered the hallmark clinical sign of recent brucellosis infection (Cheville et al. 1998), the longer-term reproductive effects and risk of shedding infectious materials are unknown. Studies that try to document active infection through culture of *B. abortus* from seropositive elk tissues are limited and the correlation between positive serostatus and infectiousness is generally weak (Cotterill et al. 2020).

A better understanding of how serologic exposure to *B. abortus* relates to infection status, long-term pregnancy rates, pregnancy outcomes, and the probability of bacterial shedding at both abortion and live birth sites would improve our knowledge of transmission risk. This may allow agencies to tailor management actions and responses and improve allocation of resources in time and space to reduce transmission risk. Our study aimed to evaluate the impact of exposure to brucellosis on pregnancy rates, pregnancy outcome, shedding of *B. abortus* at birth sites, and the existence of live *B. abortus* in tissues of seropositive elk through repeated sampling and birth event monitoring of individual seropositive elk across multiple years.

MATERIALS AND METHODS

Study areas

Our study area included the annual range of 15 elk herds in southwestern Montana (44°42′–45°42′N, 112°48′–110°6′W), including Bangtails, Blacktail, Clarks Fork, Deer Creeks, Greeley, Madison, Mill Creek, North Madison, Pioneer Mountains, Ruby Mountains, Sage Creek, Silver Run, Sixmile, Tendoy Mountains, and Tobacco Root Mountains (Fig. 1). We selected herds for sampling within and proximate to the Montana Department of Livestock Designated Surveillance Area for brucellosis, which is a periodically updated boundary meant to encompass the known geographic distribution of *B. abortus*-infected elk. Herd selection was further influenced by landowner cooperation and capture access and potential for transmission risk to nearby livestock operations.

Serology, pregnancy status, and pregnancy outcome

Capture operations occurred from 2011 through 2020, sampling one herd each year. Within each herd, we used helicopter net gunning to capture 30–100 female elk during January to early March. Exact sample size depended on herd size and availability of elk; target sample sizes of 100 elk were designed to detect at least one seropositive elk, with 95% confidence, if herd seroprevalence was ≥3%. We targeted adult (>2 yr old) female elk. We blindfolded and hobbled elk for handling and sampling. If elk body temperature exceeded 40 C we administered the nonsteroidal anti-inflammatory drug flunixin meglumine and processed the animal as quickly as possible. During recaptures of seropositive elk via dart gun we administered butorphanol, azaperone, and medetomidine (Zoopharm LLC, Laramie, Wyoming, USA) for anesthesia and naltrexone and atipamezole as a reversal. For individuals recaptured via net gun we administered xylazine (Vet One, Boise, Idaho, USA) for sedation and

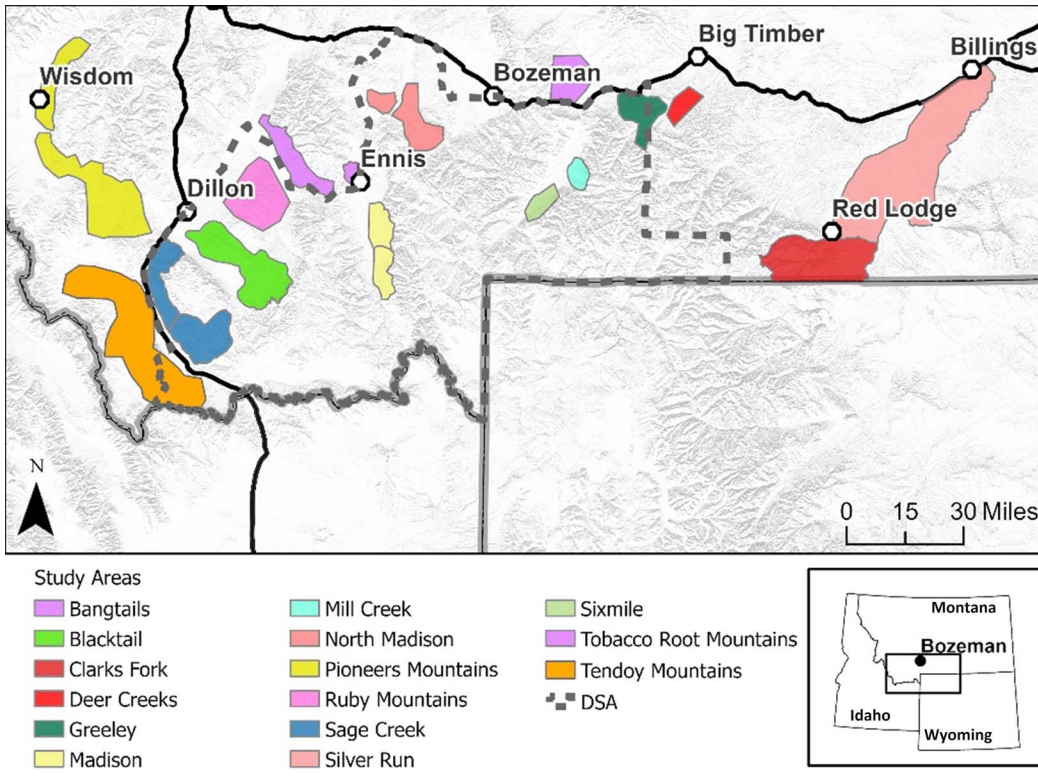

FIGURE 1. Map showing the study areas for pregnancy outcome in *Brucella abortus*-seropositive elk (*Cervus canadensis*) in southwestern Montana, USA, 2011–2020. The Montana Department of Livestock's brucellosis Designated Surveillance Area (DSA) is shown as a dashed gray line.

reversed with tolazoline (Zoopharm). All elk received unique ear tags and animal identifications (IDs) for individual ID. We captured all elk in accordance with approved animal welfare (Montana Fish, Wildlife & Parks 2018). The age of all elk was estimated on the basis of tooth eruption and wear patterns. A tooth was extracted from all collared elk captured since 2016 using lidocaine (Vet One) as a local analgesic and from known individuals postmortem (e.g., euthanized or harvested elk) for cementum annuli analysis to determine their exact age.

Blood was collected via jugular venipuncture from all elk and tested for serologic exposure to brucellosis. From 2011 through 2015 we tested elk for serologic evidence of exposure in the field using the Card (National Veterinary Services Laboratory, Ames, Iowa, USA) and fluorescence polarization assay (FPA) plate tests (Ellie Lab, Germantown, Wisconsin, USA). Field test results were only used for decisions regarding deployment of GPS collars and inclusion in the pregnancy outcome study. Final serostatus was determined by an epidemiologist at the Montana Veterinary Diagnostic Laboratory (Diagnostic Lab). Over the course of the study the Diagnostic Lab testing protocol changed, adapting to the latest science and recommendations from the US Department of Agriculture. Tests may have included any combination of the following: FPA tube, FPA plate, rapid automated presumptive, buffered acidified plate antigen, complement fixation, rivanol precipitation, Card, or standard plate test. Elk that field-tested seropositive received a GPS collar (Lotek Wireless Inc, Ontario, Canada; Vectronic Aerospace, Berlin, Germany) that remained on the animal permanently. If the Diagnostic Lab confirmed these elk as seropositive for exposure to *B. abortus*, we recaptured and monitored them annually for up to 5 yr during January–March to assess pregnancy status and pregnancy outcome. Elk enrolled in the pregnancy-outcome and seropositive-elk study came from Blacktail, Greeley, Mill Creek, North Madison, and Sage Creek. Elk captured during 2016–2020 in the Bangtails, Clarks Fork, Deer Creeks, Madison, Ruby Mountains, Silver Run, Sixmile,

and Tendoy Mountains herds were tested for serostatus only at the Diagnostic Lab and are only included in the pregnancy rate analysis. We did not recapture seronegative elk.

Annual pregnancy status of seropositive elk was determined on the basis of levels of pregnancy-specific protein "b" (PSPB) in the blood serum (BioTracking, Idaho, USA). During initial captures from 2011 through 2015, any elk that field-tested positive was also assessed for pregnancy in the field by rectal palpation, and if pregnant outfitted with a vaginal implant transmitter (VIT; Advanced Telemetry Systems, New Mexico, USA; Lotek Wireless) to monitor and determine pregnancy outcome. All elk confirmed as seropositive from 2011 through 2015 were recaptured annually for up to 5 yr and outfitted with a VIT if rectal palpation indicated they were pregnant. Final pregnancy determinations after each capture were based on PSPB results. We monitored pregnancy outcomes by tracking VITs from time of capture until the VITs were expelled. Expulsion was indicated with a rapid telemetry signal.

We investigated events as soon as possible with the goal of sampling the site within 24 h. We categorized events as abortion, live birth, or unknown on the basis of inspection of the site, female elk behavior, and time of year. Live birth sites often have smooth depressions in the ground next to the VIT where the female cleans the newborn calf and the ground of any birthing material. Female elk typically localize near a birth site for several days and will bark at anyone who comes near (Vore and Schmidt 2001). The normal calving window is 15 May–30 June (Barbknecht et al. 2009; Cross et al. 2015). We defined an event as a live birth when a live calf was observed, or when a cleaned depression was found at the site, or if the female elk barked at researchers and localized around the site for several days. We defined an event as an abortion when a fetus was observed at the site, or when no live calf was observed at the VIT site or with the female and the site lacked a cleaned depression, or the female elk quickly left the site and did not return. Time of year was used in conjunction with other indicators to determine event type. We defined events as unknown when determination of an abortion or birth was not possible.

Brucella abortus shedding during birth and abortion events

To determine if *B. abortus* was shed during the event, we collected environmental samples from the VIT expulsion site and sampled the VIT itself. To sample the VIT, we used a sterile polyester-tipped applicator (Puritan Medical Products Company LLC, Guilford, Maine, USA) to swab all surfaces (main housing, both wings) and placed the swab in a tube with World Health Organization (WHO) growth medium, a sucrose-based liquid medium designed to enrich bacterial survival and growth for culture (Joint Food and Agriculture Organization/WHO Expert Committee on Brucellosis 1986). The VIT was also placed in a Whirl-Pak and covered with WHO medium. Environmental samples collected at the site included soil, vegetation, tissue, secretions, and swabs of any fluids (e.g., blood). We stored all samples in WHO medium and immediately placed them inside a cooler with ice packs. We moved samples to a refrigerator and submitted them for testing as soon as possible. Samples collected more than 48 h after the event were considered unreliable, given the potential for contamination and degradation of *B. abortus*; data on bacterial detection from these events were excluded from analysis. We submitted all samples to the Diagnostic Lab to culture and identify *B. abortus* if present. Samples with bacteria suspected to be *B. abortus* were forwarded to the National Veterinary Services Laboratory (NVSL) for final identification. Starting with samples collected in 2015, we also began submitting a second VIT swab placed in a phosphate-buffered solution to the Wyoming Game and Fish Department Wildlife Health Laboratory for a PCR test that detected *B. abortus* DNA using the protocol developed by Ewalt and Bricker (2003). This method enables detection of bacteria that are no longer viable and thus would not be detected by culture. On the basis of the NVSL culture and PCR results, we categorized each site as *B. abortus* "detected" or "not detected." Detection of *B. abortus* from any sample, via culture or PCR, led to the classification of *B. abortus* detected for that event.

Presence of *B. abortus* in seropositive elk

We monitored seropositive elk captured from 2011 through 2015 for up to 5 yr and then recaptured them in January to early March via helicopter net gunning. We then euthanized them in the field by intravenous administration of 50 mL of a solution containing pentobarbital sodium (390 mg/mL; Vet One) and phenytoin sodium (50 mg/mL; Vet One). Whole carcasses were brought to the Diagnostic Lab for necropsy and samples were collected

to determine if *B. abortus* was present. We selected 5 yr to ensure an adequate sample size of pregnancies. Landowner capture concerns resulted in the euthanasia of Mill Creek elk after 4 yr of monitoring. Seropositive elk that died from other causes (e.g., natural mortality, vehicle collision) were also sampled when a complete, intact carcass was available within 24 h of mortality. We necropsied elk and collected tissue samples including lymph nodes (supramammary, popliteal, prefemoral, prescapular, iliac, hepatic, mesenteric, parotid, mandibular, bronchial, retropharyngeal), organs (kidney, liver, spleen, tonsil), and reproductive tract (mammary gland, uterus, ovaries, cervix, placentome, placenta, fetus, amniotic fluid, abomasal fluid). We also collected swabs (vaginal, rectal, uterine, tonsil crypts), plasma, and feces. Tissue samples, swabs, and feces were submitted for culture to the NVSL to determine presence of *B. abortus* and to the University of Wyoming for PCR testing (limited to 2019 necropsies only). Plasma was tested for antibodies at the Diagnostic Lab and submitted for PCR testing at the University of Wyoming. The middle incisor (I1) was extracted and submitted for cementum annuli analysis to determine exact age.

Data analysis

We used a generalized linear model (GLM) fit with a logit link to estimate the effect of serostatus on pregnancy rate. We only included the initial capture of each elk to avoid bias in repeated sampling of only seropositive elk. Because of age-specific variation in elk pregnancy rates, we censored yearling and included age class (prime, old) as a covariate in pregnancy models (Paterson et al. 2022). We classified prime age as 2.5–15.5 yr and old age as $\geq$16 yr (Paterson et al. 2022). We assumed that pregnancy rates remained relatively stable across herds and years. The GLMs were fit using the lme4 package in R v4.1.2 (R Core Team 2022).

We used a generalized linear mixed model, also in R v4.1.2, to estimate the probability that the pregnancy outcome for a seropositive elk would be an abortion and evaluate the effect of serostatus on pregnancy outcome. For this analysis we used repeated captures of seropositive individuals that were pregnant on the basis of PSPB testing. Animal ID was included as a random effect in the model to account for potential variation in active infection among individuals. Additionally, we estimated the probability that *B. abortus* would be detected at the event site of a seropositive elk to evaluate the effect of serostatus on shedding of *B. abortus* at pregnancy outcome events. Finally, we used a GLM fit with a logit link to estimate the probability that a seropositive elk has *B. abortus* present in any of its tissues.

RESULTS

From 2011 through 2020, we captured and sampled 1,062 adult ($\geq$2 yr old) female elk. Twenty-one elk died because of capture operations.

Pregnancy model results

Pregnancy data were available for 67 seropositive (prime = 63, old = 4) and 497 seronegative (prime = 485, old = 12) individuals (Table 1). Seropositive status and age category both significantly influenced pregnancy rate. The estimated coefficient for serology and age category were $\beta_{\text{serology}} = -1.08$ (95% confidence interval [CI]: −1.46 to −0.70), indicating that pregnancy declined with seropositive status, and $\beta_{\text{age category}} = 1.98$ (95% CI: 1.42–2.54), indicating that pregnancy was more likely for prime-age than old elk. The predicted probability of pregnancy for a prime-age elk was 94% (95% CI: 92–96%) if the elk was seronegative and 85% (95% CI: 74–91%) if it was seropositive; an estimated 9.6% decline in pregnancy rate associated with *B. abortus* exposure. The predicted probability of pregnancy for an old elk was 69% (95% CI: 44–87%) if the elk was seronegative and 43% (95% CI: 19–71%) if it was seropositive.

Pregnancy outcomes

During 2011–2015 when we enrolled seropositive elk in the pregnancy outcome study, 45 elk tested laboratory seropositive for exposure to *B. abortus*. Six of these 45 elk tested seronegative in the field and were released without a GPS collar. One elk died during capture, leaving 38 seropositive elk in the pregnancy outcome study. The sample size of collared, seropositive elk diminished over the years because of collar malfunction, mortality, and lack of pregnancy.

TABLE 1. Number of pregnant and open (nonpregnant) adult (≥2 yr old) elk (*Cervus canadensis*) by serostatus, age category (prime: 2.5–15.5 yr; old: >15.5 yr), and year from 2011 through 2020, Montana, USA. Pregnancy data from recaptures of seropositive elk are not included.

	Seronegative				Seropositive			
	Prime		Old		Prime		Old	
Year	Open	Pregnant	Open	Pregnant	Open	Pregnant	Open	Pregnant
2011	3	43	0	0	3	9	0	0
2012	0	62	0	0	1	4	0	0
2013	6	51	0	0	0	0	0	0
2014	5	52	0	0	2	7	0	0
2015	1	36	1	1	3	13	0	2
2016	3	28	0	1	1	4	0	0
2017	1	42	1	2	1	8	1	1
2018	1	57	2	2	0	6	0	0
2019	3	36	0	1	0	0	0	0
2020	4	51	1	0	0	1	0	0
Total	27	458	5	7	11	52	1	3

Eight seropositive elk never provided birth event data because they died or were lost because of collar malfunction (n=3), were never pregnant during the 5 yr of the study (n=2), or did not provide any birth event data because either they did not receive a VIT when they were pregnant (i.e., fetus not felt during palpation at capture; n=2) or when they did receive a VIT the birth event was unknown (n=1). This resulted in a total of 30 seropositive individuals providing 108 elk-years of sampling, with 82 elk-years of pregnancies monitored with a VIT (Table 2, Fig. 2).

From the 82 elk-years of monitoring we documented 65 pregnancies with known outcomes. We documented four abortions: three confirmed with fetuses at the site and one suspected on the basis of timing. We documented 61 live birth events: 35 (57%) confirmed by observation of a calf at the birth site and 26 (43%) suspected on the basis of timing, elk behavior, and birth site characteristics. We classified 17 events as unknown, including three events where the VIT was retained, two events where the VIT fell out prematurely,

TABLE 2. Number of seropositive elk (*Cervus canadensis*) sampled, number of pregnancies monitored with a vaginal implant transmitter, and pregnancy outcome (live birth, abortion, or unknown) by year from 2011 through 2018 for elk enrolled in the pregnancy outcome study, Montana, USA. Seropositive elk were first recruited into the pregnancy outcome study during 2011–2015 and monitored for up to 5 yr from 2011 through 2018.

			Pregnancy outcome		
Year	Seropositive elk	Pregnancies monitored	Live birth	Abortion	Unknown
2011	5	4	4	0	0
2012	10	9	6	2	1
2013	8	7	5	0	2
2014	15	12	11	1	0
2015	27	22	19	0	3
2016	21	11	8	0	3
2017	13	9	5	0	4
2018	9	8	3	1	4

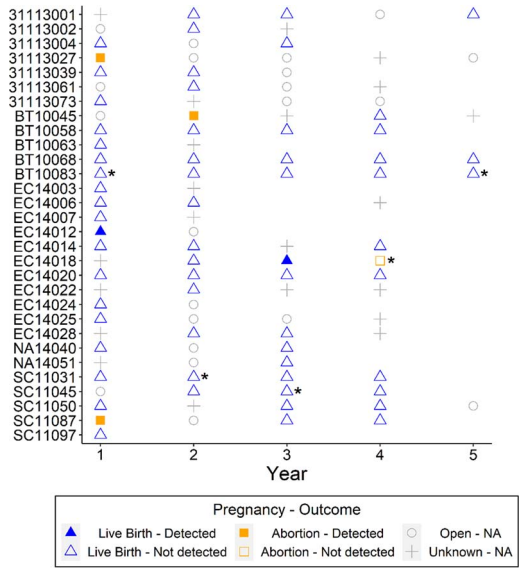

FIGURE 2. *Brucella abortus*-seropositive elk (*Cervus canadensis*) enrolled in the pregnancy outcome study from 2011 through 2018, Montana, USA, which had at least one known pregnancy outcome event (live birth, abortion), by year of monitoring. Pregnancy outcomes include open (circle), live birth (triangle), abortion (square), and unknown (cross). Events where *B. abortus* was detected are solid red, events where *B. abortus* was not detected are hollow and blue, and events without testing (e.g., open, unknown) are gray. Blanks indicate elk that were not sampled that year (mortality, not captured). * indicates that sampling occurred more than 48 h after the event, rendering the detection outcome unreliable because of environmental degradation.

seven events where the VIT beacon failed and could not be tracked, one event where the VIT was found with no sign of a birth site, and four elk that died in the spring before any event occurred.

We censored one abortion and four live birth events because of sampling that occurred more than 48 h after the event, rendering the detection result unreliable. Of the 60 acceptably sampled events, *B. abortus* was detected at 3/3 abortions (100%) and 2/57 live births (4%; Fig. 2). Culture testing detected *B. abortus* at three abortions and one live birth that PCR did not detect, whereas PCR detected *B. abortus* at one live birth that was not detected by culture. PCR testing was not available at the time of the three adequately sampled abortions, preventing a comparison. Samples with *B. abortus* detected included fetal tissue, soil, and VIT swabs (Supplementary Material Fig. S1).

Model results predicting type of outcome event showed that animal ID (an individual elk) was not significant. We used the fixed effects to estimate the predicted probability of a seropositive elk having an abortion as 0.06 (95% CI: 0.02–0.15). Model results for shedding of *B. abortus* at event sites showed that animal ID did account for some of the variance. We proceeded with the fixed effects to estimate the predicted probability of a seropositive elk shedding *B. abortus* at an event as 0.08 (95% CI: 0.04–0.19).

Presence of *B. abortus* in seropositive elk

Of the 45 seropositive elk captured from 2011 through 2015, 17 were available for a full necropsy after mortalities; lost elk reduced our sample size. The Diagnostic Lab performed all 17 necropsies on seropositive elk, submitting 17–28 samples per individual for culture testing, and in 2019 for PCR testing also (Fig. 3). *Brucella abortus* was detected in three seropositive individuals: by culture in the popliteal lymph node of two elk, and by PCR in the placentome and plasma of one of those elk and in the retropharyngeal lymph node of a third elk that was undetected by culture. Model results estimated the predicted probability of a seropositive elk having *B. abortus* in any tissue as 0.18 (95% CI: 0.06–0.43).

DISCUSSION

The reproductive performance of *B. abortus*-seropositive individuals and their associated disease transmission risk is best understood across multiple years because infectiousness generally peaks the year after exposure and most elk control or clear the infection. Our finding that exposure to *B. abortus* decreased elk pregnancy rates by approximately 9.6% in prime-age elk and by 37.7% in old elk was similar to that of previous studies that found a 7–31% reduction in pregnancy rates of seropositive elk (Cotterill et al. 2018b; Yang et al. 2019). It is important to note the very small sample size for old elk (n=4

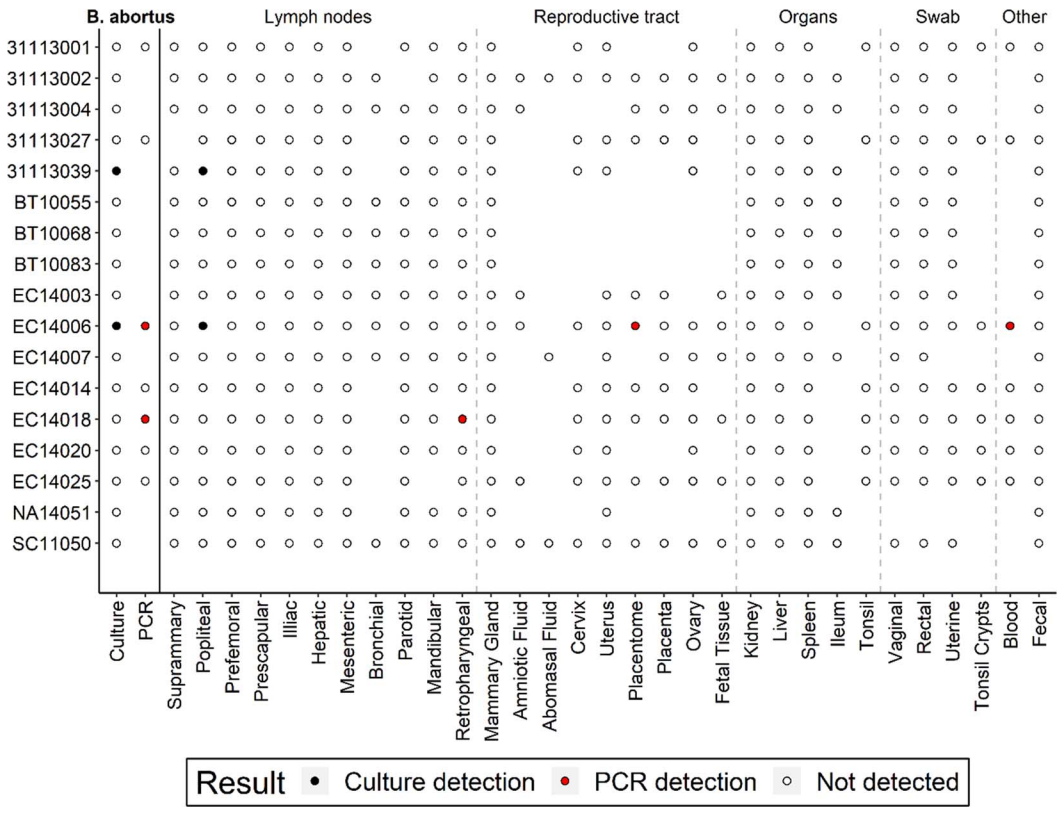

FIGURE 3. Samples from necropsied *Brucella abortus*-seropositive elk (*Cervus canadensis*) grouped by test result for *B. abortus*, and then sample type: lymph nodes, reproductive tract, organs, swabs, and other, collected from 2016 through 2019, Montana, USA. Detection of *B. abortus* is indicated by solid circles: black for culture and red for PCR. Samples where *B. abortus* was not detected are indicated by open circles. Blanks represent samples that were not collected for that individual (i.e., not pregnant).

seropositive, $n=12$ seronegative), which resulted in uncertainty around our predicted probabilities of pregnancy. We believe that the trend of decreasing pregnancy for seropositive and particularly for old seropositive elk is accurate even if the data available generate large confidence intervals. Although this information helps with the overall understanding of brucellosis impacts on elk reproductive performance, reduced pregnancy rates of this magnitude in the seropositive segment of the population are unlikely to affect overall population growth in the populations we studied (Gaillard et al. 1998; Eacker et al. 2017).

The number of abortions among seropositive pregnant elk in our study was low, with model predictions estimating 6% (95% CI: 2–15%) of pregnancies resulting in abortions. Cross et al. (2015) reported an estimated abortion rate of 16% (95% CI: 10–23%) for pregnant seropositive elk associated with feedgrounds in Wyoming. The higher abortion rate in herds that frequent feedgrounds may be due to higher exposure rates and increased likelihood of infectious abortion events in areas with high elk densities where contact with infectious material is likely for many elk (Maichak et al. 2009). The lower abortion rate in our study may also be attributed to monitoring the same seropositive elk over an extended period, with each successive year being farther from initial infection when abortion is most likely. The seropositive elk in our study also did not experience the same densities as feedground elk. Although abortions are the most likely vector of *B. abortus* transmission, live births have been acknowledged as potential transmission vectors and

our results confirm previous findings (Thorne et al. 1997; Brennan et al. 2017) that *B. abortus* is sometimes shed at live birth sites. Detection of *B. abortus* in 2/61 (3%) live births for seropositive elk suggests a real, but very low, risk. Management actions designed to reduce transmission risk, such as separation of elk and livestock, are generally concentrated during the abortion period (March through May) and not the birthing period (mid-May to mid-June). Live births can occur into late June (Cross et al. 2015) and delaying livestock grazing turn-out dates that overlap elk calving ranges until July may help reduce transmission risk.

Serologic testing is still the primary diagnostic tool for *Brucella* spp. infections worldwide and enables diagnosis of exposure in live animals, but seropositive individuals may not have an active infection and culture rates are nearly always much lower than seropositivity rates (Etter and Drew 2006; O'Leary et al. 2006; Truong et al. 2016). We found only 3/17 seropositive elk (18%) culture positive, compared with 31–42% of seropositive elk from Rainey Creek feedground in Idaho (Etter and Drew 2006). Culture-positive rates appear to be higher among feedground elk, where seroprevalence and density are generally high, possibly resulting in more infectious individuals and events (Creech et al. 2012; Cotterill et al. 2018a). Our delayed culture sampling of seropositive elk 4–5 yr after initial serologic testing may have allowed some elk to clear infections and contributed to a lower culture-positive rate compared with feedground elk with serologic and culture testing conducted at the same time. It is also possible that *B. abortus* infections were latent, colony counts were low, or we missed detecting the bacteria during sampling. Although our detection sample size was low, it supported previous work indicating that positive culture tests commonly come from samples of organs, milk, and in particular lymph nodes, especially the retropharyngeal, supramammary, internal iliac, and popliteal (O'Leary et al. 2006; Godfroid et al. 2010; O'Grady et al. 2014; Dadar et al. 2021). These tissues should be prioritized for sampling to detect *B. abortus*.

Culture testing remains the gold standard for diagnosing many bacterial infections, but its sensitivity, or ability to correctly detect individuals with the disease, is difficult to determine and may be low (Klouche and Schröder 2008; Limmathurotsakul et al. 2010; Smirnova et al. 2013; Butler et al. 2018). Bacterial culture is time consuming, susceptible to contamination and degradation from environmental exposure, and exposes laboratory technicians to risk (Al Dahouk et al. 2003; Smirnova et al. 2013). There is debate on the standard being updated and augmented with newer diagnostic techniques such as PCR (conventional, real-time, and multiplex methods) that show speed, reduced contamination, high sensitivity, and high specificity (Godfroid et al. 2010; Smirnova et al. 2013; Saytekin and Ak 2018). In our study, PCR detected *B. abortus* at one birth site and in the tissues of one elk, which traditional culture testing did not detect. Culture testing, however, did detect *B. abortus* at one birth site that PCR did not detect, although PCR testing at birth sites was limited. Even when both culture and PCR detect *B. abortus* in the same individual, it may be from different tissues, suggesting that one test may miss a detection that is picked up by the other method. Imperfect detection implies that active infections may still be missed even when both methods are used.

Use of PCR does involve limitations, including false negatives, cost, and dependence on the quality of isolated DNA (Smirnova et al. 2013). Studies have been mixed when comparing detection rates of *Brucella* spp. by culture versus PCR, with some detecting no difference between PCR and culture (Leyla et al. 2003; O'Leary et al. 2006; Saytekin and Ak 2018) and others arguing PCR to be highly sensitive and that it would probably improve detection (Dadar et al. 2021). Stage of infection, through number and location of bacteria, may also influence detection rates (O'Leary et al. 2006). Future work should examine detection rates from individuals of known exposure dates.

With the difficulty of culturing *B. abortus* and imperfect performance of PCR testing, our results indicate that using culture and PCR together may offer increased detection rates and serve as a better "gold standard."

Disease management paradigms often suggest the removal of seropositive individuals to reduce disease prevalence and transmission risk. However, only 3/17 necropsied seropositive elk had tissues that tested culture or PCR positive. Additionally, we only detected *B. abortus* at three abortions and two live births of 60 promptly sampled events, adding further credence to the limited risk seropositive elk, as a group, may pose. The current focus on managing seropositive elk and their removal to curtail transmission risk may not be as effective as once thought, particularly since positive serostatus is weakly correlated with infectiousness (Cotterill et al. 2020). If the greatest risk of shedding occurs with abortion events that typically occur the year after first exposure (Thorne et al. 1978), then seropositive elk that were exposed years ago and still retain antibodies may have a degree of immunity to becoming infectious again and may be less susceptible than naïve seronegative elk to having a brucellosis-induced abortion. Targeting young seropositive animals who have the greatest likelihood to be infectious, while retaining seropositive older age classes that may have some immunity, may be more effective at reducing transmission risk (Ebinger et al. 2011). Given that pregnancy rates appear to be lower for seropositive elk and the low incidence of *B. abortus* detection at pregnancy outcome events, the probability of a seropositive elk shedding *B. abortus* on the landscape is rather low. It is important to note, however, that the presence of *B. abortus* at live birth events does increase transmission risk to some extent and should be considered when applying best management practices to reduce elk-to-livestock transmission, which has real consequences for individual producers. Further identifying, and ultimately limiting, conditions that result in transmission of *B. abortus* to livestock from these low-probability events may be a more effective way of managing brucellosis risk than removing seropositive elk.

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SUPPLEMENTARY MATERIAL

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LITERATURE CITED

Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. 2003. Laboratory-based diagnosis of brucellosis—A review of the literature. Part II: Serological tests for brucellosis. *Clin Lab* 49:577–589.

Aune K, Rhyan JC, Russell R, Roffe TJ, Corso B. 2012. Environmental persistence of *Brucella abortus* in the Greater Yellowstone Area. *J Wildl Manage* 76:253–261.

Barbknecht AE, Fairbanks WS, Rogerson JD, Maichak EJ, Meadows LL. 2009. Effectiveness of vaginal-implant transmitters for locating elk parturition sites. *J Wildl Manage* 73:144–148.

Barbknecht AE, Fairbanks WS, Rogerson JD, Maichak EJ, Scurlock BM, Meadows LL. 2011. Elk parturition site selection at local and landscape scales. *J Wildl Manage* 75:646–654.

Bienen L, Tabor G. 2006. Applying an ecosystem approach to brucellosis control: Can an old conflict between wildlife and agriculture be successfully managed? *Front Ecol Environ* 4:319–327.

Brennan A, Cross PC, Portacci K, Scurlock BM, Edwards WH. 2017. Shifting brucellosis risk in livestock

coincides with spreading seroprevalence in elk. *PLoS One* 12:e0178780.

Butler CJ, Edwards WH, Paterson JT, Proffitt KM, Jennings-Gaines JE, Killion HJ, Wood ME, Ramsey JM, Almberg ES, et al. 2018. Respiratory pathogens and their association with population performance in Montana and Wyoming bighorn sheep populations. *PLoS One* 13:e0207780.

Cheville NF, McCullough DR, Paulson LR. 1998. *Brucellosis in the Greater Yellowstone Area*. National Academy Press, Washington, DC.

Cotterill GG, Cross PC, Cole EK, Fuda RK, Rogerson JD, Scurlock BM, du Toit JT. 2018a. Winter feeding of elk in the Greater Yellowstone Ecosystem and its effects on disease dynamics. *Phil Trans Royal Soc B Biol Sci* 373:20170093.

Cotterill GG, Cross PC, Merkle JA, Rogerson JD, Scurlock BM, du Toit JT. 2020. Parsing the effects of demography, climate and management on recurrent brucellosis outbreaks in elk. *J Appl Ecol* 57:379–389.

Cotterill GG, Cross PC, Middleton AD, Rogerson JD, Scurlock BM, du Toit JT. 2018b. Hidden cost of disease in a free-ranging ungulate: Brucellosis reduces mid-winter pregnancy in elk. *Ecol Evol* 8:10733–10742.

Creech TG, Cross PC, Scurlock BM, Maichak EJ, Rogerson JD, Henningsen JC, Creel S. 2012. Effects of low-density feeding on elk–fetus contact rates on Wyoming feedgrounds. *J Wildl Manage* 76:877–886.

Cross PC, Heisey DM, Scurlock BM, Edwards WH, Ebinger MR, Brennan A. 2010. Mapping brucellosis increases relative to elk density using hierarchical Bayesian models. *PLoS One* 5:e10322.

Cross PC, Maichak EJ, Rogerson JD, Irvine KM, Jones JD, Heisey DM, Edwards WH, Scurlock BM. 2015. Estimating the phenology of elk brucellosis transmission with hierarchical models of cause-specific and baseline hazards. *J Wildl Manage* 79:739–748.

Dadar M, Shahali Y, Fakhri Y, Godfroid J. 2021. The global epidemiology of *Brucella* infections in terrestrial wildlife: A meta-analysis. *Transbound Emerg Dis* 68:715–729.

Eacker DR, Lukacs PM, Proffitt KM, Hebblewhite M. 2017. Assessing the importance of demographic parameters for population dynamics using Bayesian integrated population modeling. *Ecol Appl* 27:1280–1293.

Ebinger M, Cross P, Wallen R, White PJ, Treanor J. 2011. Simulating sterilization, vaccination, and test-and-remove as brucellosis control measures in bison. *Ecol Appl* 21:2944–2959.

Etter RP, Drew ML. 2006. Brucellosis in elk of eastern Idaho. *J Wildl Dis* 42:271–278.

Ewalt DR, Bricker BJ. 2003. Identification and differentiation of *Brucella abortus* field and vaccine strains by BaSS-PCR. In: *PCR detection of microbial pathogens*, Sachse K, Frey J, editors. Humana Press, Totowa, New Jersey, pp. 97–108.

Gaillard JM, Festa-Bianchet M, Yoccoz NG. 1998. Population dynamics of large herbivores: Variable recruitment with constant adult survival. *Trends Ecol Evol* 13:58–63.

Godfroid J, Nielsen K, Saegerman C. 2010. Diagnosis of brucellosis in livestock and wildlife. *Croat Med J* 51:296–305.

Joint Food and Agriculture Organization/WHO Expert Committee on Brucellosis. 1986. *Joint FAO/WHO Expert Committee on Brucellosis: Sixth report*. World Health Organization, Geneva, Switzerland. https://apps.who.int/iris/handle/10665/40202. Accessed July 2022.

Kamath PL, Foster JT, Drees KP, Luikart G, Quance C, Anderson NJ, Clarke PR, Cole EK, Drew ML, et al. 2016. Genomics reveals historic and contemporary transmission dynamics of a bacterial disease among wildlife and livestock. *Nat Commun* 7:11448.

Klouche M, Schröder U. 2008. Rapid methods for diagnosis of bloodstream infections. *Clin Chem Lab Med* 46:888–908.

Leyla G, Kadri G, Umran O. 2003. Comparison of polymerase chain reaction and bacteriological culture for the diagnosis of sheep brucellosis using aborted fetus samples. *Vet Microbiol* 93:53–61.

Limmathurotsakul D, Jamsen K, Arayawichanont A, Simpson JA, White LJ, Lee SJ, Wuthiekanun V, Chantratita N, Cheng A, et al. 2010. Defining the true sensitivity of culture for the diagnosis of melioidosis using Bayesian latent class models. *PLoS One* 5: e12485.

Maichak EJ, Scurlock BM, Rogerson JD, Meadows LL, Barbknecht AE, Edwards WH, Cross PC. 2009. Effects of management, behavior, and scavenging on risk of brucellosis transmission in elk of western Wyoming. *J Wildl Dis* 42:398–410.

Merkle JA, Cross PC, Scurlock BM, Cole EK, Courtemanch AB, Dewey SR, Kauffman MJ. 2018. Linking spring phenology with mechanistic models of host movement to predict disease transmission risk. *J Appl Ecol* 55:810–819.

Montana Fish, Wildlife & Parks. 2018. *Biomedical protocol for free-ranging Cervidae in Montana: Capture, anesthesia, tagging, sampling and necropsy procedures*. Montana Fish, Wildlife & Parks, Helena, Montana.

O'Grady D, Byrne W, Kelleher P, O'Callaghan H, Kenny K, Heneghan T, Power S, Egan J, Ryan F. 2014. A comparative assessment of culture and serology in the diagnosis of brucellosis in dairy cattle. *Vet J* 199:370–375.

O'Leary S, Sheahan M, Sweeney T. 2006. *Brucella abortus* detection by PCR assay in blood, milk and lymph tissue of serologically positive cows. *Res Vet Sci* 81:170–176.

Paterson JT, Proffitt KM, Rotella JJ. 2022. Incorporating vital rates and harvest into stochastic population models to forecast elk population dynamics. *J Wildl Manage* 86:e22189.

Rayl ND, Proffitt KM, Almberg ES, Jones JD, Merkle JA, Gude JA, Cross PC. 2019. Modeling elk-to-livestock transmission risk to predict hotspots of brucellosis spillover. *J Wildl Manage* 83:817–829.

R Core Team. 2022. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Accessed June 2022.

Saytekin AM, Ak S. 2018. Direct diagnosis of *Brucella* species through multiplex PCR formed by a new method. *J Microbiol Methods* 154:86–94.

Smirnova EA, Vasin AV, Sandybaev NT, Klotchenko SA, Plotnikova MA, Chervyakova OV, Sansyzbay AR,

Kiselev OI. 2013. Current methods of human and animal brucellosis diagnostics. *Adv Infect Dis* 3:177–184.

Thorne ET, Morton JK, Blunt FM, Dawson HA. 1978. Brucellosis in elk. II. Clinical effects and means of transmission as determined through artificial infections. *J Wildl Dis* 14:280–291.

Thorne ET, Smith SG, Aune K, Hunter D, Roffe TJ. 1997. Brucellosis: The disease in elk. In: *Brucellosis, bison, elk, and cattle in the Greater Yellowstone Area: Defining the problem, exploring solutions*. Wyoming Game and Fish Department, Greater Yellowstone Interagency Brucellosis Committee, Cheyenne, Wyoming, pp. 33–44.

Truong QL, Kim K, Kim JT, Her M, Jung SC, Hahn TW. 2016. Isolation and characterization of *Brucella abortus* isolates from wildlife species in South Korea. *Korean J Vet Res* 56:147–153.

Vore JM, Schmidt EM. 2001. Movements of female elk during calving season in northwest Montana. *Wildl Soc Bull* 29:720–725.

Yang A, Gomez JP, Haase CG, Proffitt KM, Blackburn JK. 2019. Effects of brucellosis serologic status on physiology and behavior of Rocky Mountain elk (*Cervus canadensis nelsoni*) in southwestern Montana, USA. *J Wildl Dis* 55:304–315.

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