Targeted Elk Brucellosis Surveillance Project

2011 – 2015 Comprehensive Report

Executive Summary

Montana Fish, Wildlife and Parks (MFWP) is conducting a multi-year targeted elk brucellosis surveillance project to 1) evaluate the prevalence and spatial extent of brucellosis exposure in southwest Montana elk populations, 2) evaluate the extent of elk interchange between infected and adjacent elk herds, and 3) evaluate the risk of seropositive elk shedding and potentially transmitting *Brucella abortus*. Since 2011, we have captured in areas adjacent to the previously documented distribution of brucellosis and tested elk for exposure to *B. abortus*. We have radiocollared a sample of elk in each study area to identify the timing and extent of herd interchange. We have outfitted seropositive, pregnant elk with vaginal implant transmitters to monitor birth events and sample for *B. abortus* at birth sites. We documented brucellosis in 4 areas beyond the previously documented distribution of the disease (Blacktail, Sage Creek, Northern Madison, and Greeley), found a higher exposure rate than previously documented in elk in the Mill Creek area, and found no exposure to *B. abortus* in elk in 2 areas (Pioneer Mountains, Tobacco Root Mountains). Levels of exposure to *B. abortus* ranged from 0% in the Pioneers and Tobacco Roots to a high of 53% in Mill Creek. We deployed radiocollars on a total of 38 seropositive and 144 seronegative elk. We monitored 51 seropositive elk pregnancies during 2011 – 2015 and documented 3 abortions, 45 live births, and 3 unknown events. *B. abortus* was detected at all 3 abortion sites, and 1 of the 45 live birth sites. This report is a comprehensive summary of the 2011 – 2015 surveillance, epidemiology and movement data collected as part of the targeted elk brucellosis surveillance project.

Introduction

Montana Fish, Wildlife and Parks (MFWP) has conducted surveillance for brucellosis in elk populations since the early 1980s. Surveillance consisted of screening blood serum for antibodies signifying exposure to *B. abortus*, the bacteria that causes the disease brucellosis. Elk that test positive for exposure to *B. abortus* (seropositive) may or may not be actively infected with the bacteria. Although not a true indicator of infection or the ability of an animal to shed *B. abortus* on the

landscape, detection of seropositive elk indicates brucellosis is present in the area and suggests that the disease could be circulating within the elk population, with the potential for elk to transmit the disease to livestock.

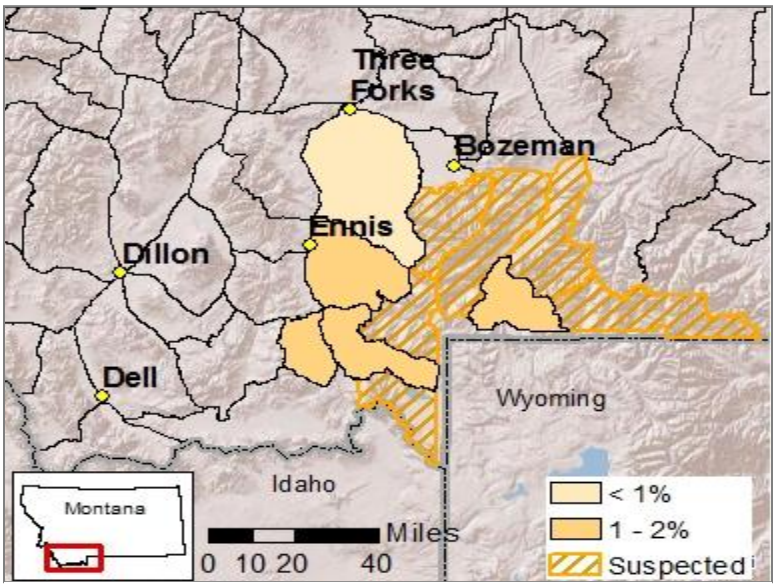

Figure 1. Distribution of brucellosis in elk by elk hunting district prior to 2000, based on documented (solid shading) and suspected presence (cross hatch).

Prior to 2000, brucellosis had been documented in 5 hunting districts (HDs) in the Greater Yellowstone Area (GYA). Based on elk movement patterns, the distribution of brucellosis in elk was believed to be limited to all or portions of 11 HDs in the GYA (Figure 1).

In response to detections of brucellosis in livestock in 2007 and 2008, MFWP expanded surveillance efforts to include 30 HDs within the GYA. Surveillance efforts focused on the collection and testing of blood from hunter-harvested elk and the opportunistic testing of animals captured and sampled as part of research projects. MFWP continued this effort until the winter of 2010 and then evaluated the effectiveness of the surveillance program. Across 3 years (2008 – 2010), sample sizes within the 30 individual HDs varied widely from 2 to 229. Although data were insufficient in some HDs to evaluate brucellosis presence/absence, additional insight on the distribution and prevalence of the disease was obtained. Prevalences appeared to be increasing in areas where adequate information was available and comparisons could be made to historical data. The disease was also detected in areas outside of its previously documented distribution (Anderson et. al. 2010). However, the small number of samples obtained in many hunting districts did not achieve the goal of delineating the geographical boundary of brucellosis in Montana elk populations.

In efforts to increase understanding of brucellosis in elk populations, MFWP initiated a 5-year targeted surveillance and research project in the winter of 2011. The goals of the project were to 1) delineate the geographical distribution and level of elk exposure to *B. abortus*, 2) assess the transmission risk seropositive elk pose to livestock and other elk populations, and 3) identify the potential movement pathways for brucellosis between elk populations. In order to achieve these goals, MFWP identified 7 priority study areas and conducted intensive sampling efforts in these areas during 2011 – 2015. Study areas were selected based on their proximity to the known distribution of brucellosis and/or significant livestock concerns. Surveillance areas were identified through

collaborative discussions between MFWP, the Department of Livestock (DOL), and landowners. Surveillance areas were both inside and outside of the State of Montana designated brucellosis surveillance area (DSA).

Study areas

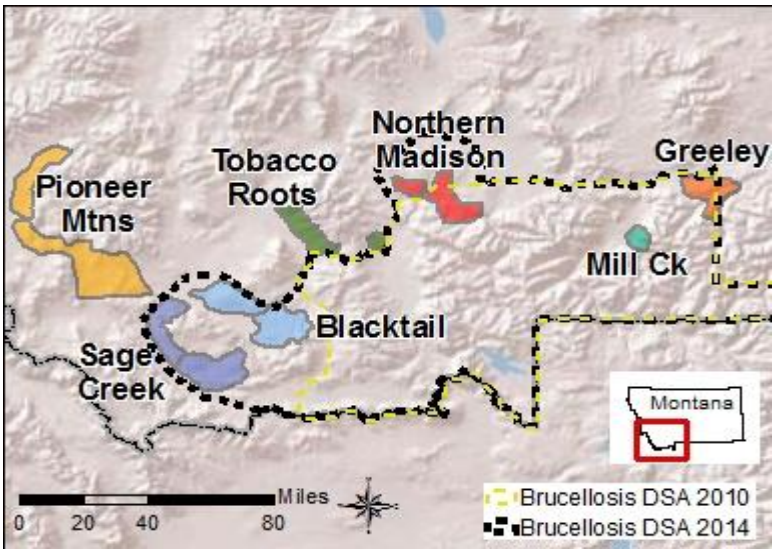

Figure 2. Study areas where elk brucellosis surveillance was conducted during 2011-2015.

We conducted brucellosis surveillance in 7 different study areas in western Montana from January 2011 through July 2015 (Figure 2). The study areas included the Blacktail area in hunting districts (HD) 324 and 326, the Sage Creek area of HD 325, the Pioneer Mountains area in HDs 329, 331 and 332, the Tobacco Root Mountains in HDs 320 and 333, the Northern Madison area of HD 311, the Mill Creek area of HD 317 and the Greeley area of HD 560.

Methods

We captured up to 100 elk per study area via helicopter netgunning and screened this sample of animals for exposure to *B. abortus*. Exposure was determined by the presence of antibodies to *B. abortus* in an animal's blood serum. We collected a blood sample and initially screened blood serum for exposure to *B. abortus* in the field utilizing the Card and/or the Fluorescent Polarized Assay (FPA) tests. Blood serum samples were then tested at the Montana Department of Livestock Diagnostic Lab (Diagnostic Lab). In 2011 – 2014, samples were screened for exposure to *B. abortus* in the lab utilizing the BAPA, Rivanol, Flourescence Polarization Assay (FPA), and Standard Plate Test (SPT). In 2015, samples were screened utilizing the Rapid Automated Presumptive (RAP) and FPA plate tests. Suspect or reactors to these screening tests were further tested with the FPA tube test. The change in testing protocol was the result of standardization of elk brucellosis testing protocols for Montana, Wyoming and Idaho, to ensure each state was using the most appropriate protocol and that results from these states are comparable to one another. Final classification of serostatus (i.e., seropositive or seronegative) was based on test results received from the Diagnostic Lab.

We assessed the pregnancy status of elk that field-tested positive for exposure to *B. abortus* and, if pregnant, outfitted the animal with a vaginal implant transmitter (VIT). VITs are programmed to

emit a slow pulse when the temperature is 32^{o} C or higher (i.e., inside the body), and emit a fast pulse once the temperature cools below 28^{o} C (i.e., expelled outside the body during an abortion or live birth). VITs have a precise event transmitter (PET) code which indicates the time since the VIT was expelled and cooled to a temperature below 28^{o} C. We monitored the pulse rate and PET code to determine if an implant had been expelled and the timing of expulsion. We tracked elk outfitted with VITs 2 – 4 times per week from time of capture until they were expelled to identify birth events.

We investigated each birth site to determine if an abortion or live birth occurred and sampled the birth site to determine if *B. abortus* bacteria were shed. We collected birth site samples from the VIT, soil, vegetation, and any available tissue or fluid. We also collected swabs of the VIT and any moist surface or material. All samples were submitted to the Diagnostic Lab to culture (i.e., grow) and identify any bacteria present in the sample. If bacteria cultured from the samples are suspected to be *B. abortus* they are forwarded to the National Veterinary Services Laboratory (NVSL) for final identification. In addition, during 2015 we submitted a swab of the VIT to the Wyoming State Veterinary Lab for a polymerase chain reaction (PCR) test that detects *B. abortus* DNA. The PCR test is a new method of detecting *B. abortus* that was unavailable in previous years. Detection of *B. abortus* from any sample, via culture or PCR, led to the classification of detected for that event. We considered elk giving birth on or after May 15 to have carried their calf to full term, unless evidence of an abortion event was detected at the birth site (Barbknecht et al. 2009, Cross et al. 2015). We monitored the adult elk post calving to confirm the presence of a live calf whenever possible. We categorized birth events as a confirmed abortion, suspected abortion, confirmed live birth, suspected live birth, or unknown. We defined a confirmed abortion as a birth event when the fetus was located and a suspected abortion as a birth event occurring outside of the normal calving period (May 15 – June 30) when no fetus was located at the birth site. We defined a confirmed live birth as a birth event where a live calf was located at the birth site or detected with the adult female and a suspected live birth as a birth event occurring during the normal calving period (May 15 – June 30) where no fetal material or live calf was detected. Unknown events were restricted to cases where the VIT was lost due to a malfunction (i.e., stopped transmitting). We then categorized each birth site as *B. abortus* detected or not detected based on culture results.

We radiocollared all elk that field-tested positive for exposure to *B. abortus*. In addition, we deployed radiocollars on a random sample of seronegative elk in order to track movements and evaluate risk of brucellosis transmission to livestock and other elk populations. All radiocollars collected a GPS location every 30 minutes or 2 hours for 52 – 72 weeks, and elk were relocated in the field using telemetry equipment every 4-6 weeks throughout the year. Radiocollars had a mortality sensor that detected if the radiocollar was stationary for > 6 hours. Radiocollars deployed on seropositive elk remained on the elk until it was recaptured and manually removed. Radiocollars

deployed on seronegative elk were built with a timed release mechanism that released the collar after 52 – 72 weeks. We retrieved radiocollars and downloaded the location data.

We recaptured and retested seropositive elk for exposure to *B. abortus* every year for 5 years. The purpose of retesting for exposure was to determine if elk experience antibody titer loss following exposure. At each recapture event, we assessed pregnancy status and outfitted pregnant elk with a VIT. The purpose of monitoring serostatus and birth events for 5 years was to understand the epidemiology of the disease post infection, and determine the level of risk associated with exposed elk through time. We will remove seropositive elk from the population following 5 years of testing to determine if they are infected with brucellosis. While testing blood serum annually determines if an elk has been exposed to *B. abortus*, lethal removal is necessary to determine if an elk is infected (i.e., capable of transmitting the disease brucellosis) because reproductive organs need to be collected in order for *B. abortus* bacteria to be identified.

Results

Overall summary of results, 2011 – 2015

We captured and sampled a total of 518 elk from 7 study areas (Table 1). Testing at the diagnostic laboratory revealed a total of 45 elk that tested positive for exposure to *B. abortus* (Table 1).

Table 1. The southwestern Montana study areas where elk were screened for exposure to *B. abortus* during 2011 – 2015, sample size of elk screened, number of elk testing positive for exposure, and the estimated seroprevalence with binomial confidence intervals.

Study Area	Hunting Districts	Year Sampled	Sample Size	Number Seropositive	Estimated Seroprevalence (%)
Blacktail	324, 326	2011	100	12	12.0 % (7 – 19.8)
Sage Creek	325	2012	93	5	5.4 % (2.3 – 12)
Pioneer Mountains	329, 331, 332	2013	100	0	0.0 % (0 – 3.7)
Tobacco Root	320, 333	2014	70	0	0.0 % (0 – 5.2)
N. Madison	311	2014	60	10	16.7 % (9.3 – 28)
Mill Creek	317	2015	30	16	53.3 % (36.1 – 69.8)
Greeley	560	2015	65	2	3.1 % (0.8 – 10.5)

We deployed radiocollars on a total of 38 seropositive and 144 seronegative elk. Six seropositive elk field tested negative for exposure to *B. abortus* and did not receive a collar. One seropositive elk died during capture and was never radiocollared. We monitored 51 seropositive elk pregnancies during 2011 – 2015. Of these 51 pregnancies, we documented 2 confirmed abortions, 1 suspected abortion, 23 confirmed live births, 22 suspected live births, and 3 unknowns (Table 2; Appendix A). *B. abortus*

was detected at all 3 confirmed or suspected abortion sites, and 1 suspected live birth site (Table 3; Appendix A).

Table 2. The total number of seropositive elk pregnancies monitored in each study area during 2011 – 2015, the number of confirmed or suspected abortions, the number of confirmed or suspected live births, and the number of unknown events. Elk that died prior to any birth event are not included.

Herd	Total Pregnancies Monitored	Confirmed Abortions	Suspected Abortions	Confirmed Live Birth	Suspected Live Birth	Unknown
Blacktail	18	1	0	9	7	1
Sage Creek	14	1	0	7	6	0
Pioneer Mountains	0	0	0	0	0	0
Tobacco Root	0	0	0	0	0	0
N. Madison	8	0	1	3	4	0
Mill Creek	9	0	0	4	4	1
Greeley	2	0	0	0	1	1
TOTAL	**51**	**2**	**1**	**23**	**22**	**3**

Table 3. The total number of abortion (confirmed or suspected) and live birth events (confirmed or suspected), and the number of cases per birth event category where *B. abortus* was detected in each study area during 2011 – 2015.

Herd	Abortions/ *B. abortus* detections	Live Births/ *B. abortus* detections
Blacktail	1/1	16/0
Sage Creek	1/1	13/0
N. Madison	1/1	7/0
Mill Creek	0/0	8/1
Greeley	0/0	1/0
TOTAL	**3/3**	**45/1**

The average number of days for birth events to be detected was 1 day with a range of 0 – 11, and the average number of days to investigate events was 2 days with a range of 0 – 20 (Table 4). Time to detection and sampling did not differ between abortions and live birth events.

Table 4. The median number of days to detect and investigate birth events for seropositive elk during 2011 – 2015.

Herd	2011 Days to Detect/Investigate	2012 Days to Detect/Investigate	2013 Days to Detect/Investigate	2014 Days to Detect/Investigate	2015 Days to Detect/Investigate
Blacktail	2.5/6.5	0/0.5	1/1	1/1	0.5/2
Sage		0/0	1.5/5	0/0	0/0
Black's				0.5/0.5	1/1
Greely					2/2
Mill					0/0.5

The average pregnancy rate for all 7 study areas, from 2011-2015, was 0.94 for seronegative (n = 153) and 0.75 for seropositive elk (n = 81, repeat testing of n = 38 individuals).

All seropositive elk maintained their positive serostatus throughout the duration of their monitoring, except for 1 Blacktail elk that cleared *B. abortus* antibodies in the fourth year of monitoring. This elk tested positive for exposure to *B. abortus* during the 2011, 2012 and 2013 screenings and tested negative for exposure during 2014 and 2015.

GPS movement data were collected from 19 of the 38 radiocollared seropositive elk, for 39 elk-years in 2011 – 2014 (Figure 3). GPS collars are still deployed and collecting data on 16 seropositive elk. Two collars failed and 1 elk died shortly after collar deployment. Additionally, we collected GPS movement data from 101 of the 144 radiocollared seronegative elk. GPS collars are still deployed and collecting data on 38 seronegative elk. Two collars failed and 3 collars released on private land with no access permission, and data could not be recovered.

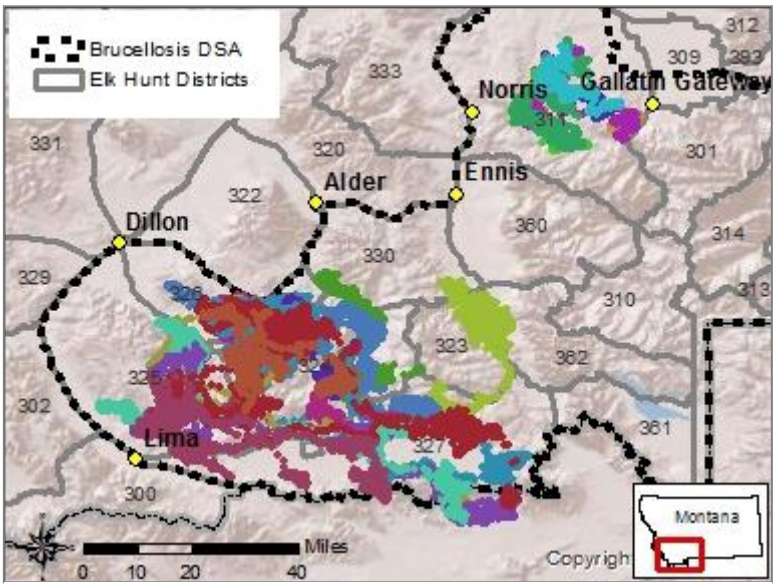

Figure 3. The movements of 19 seropositive elk during the January 1 – June 30th transmission risk periods of 2011 – 2014.

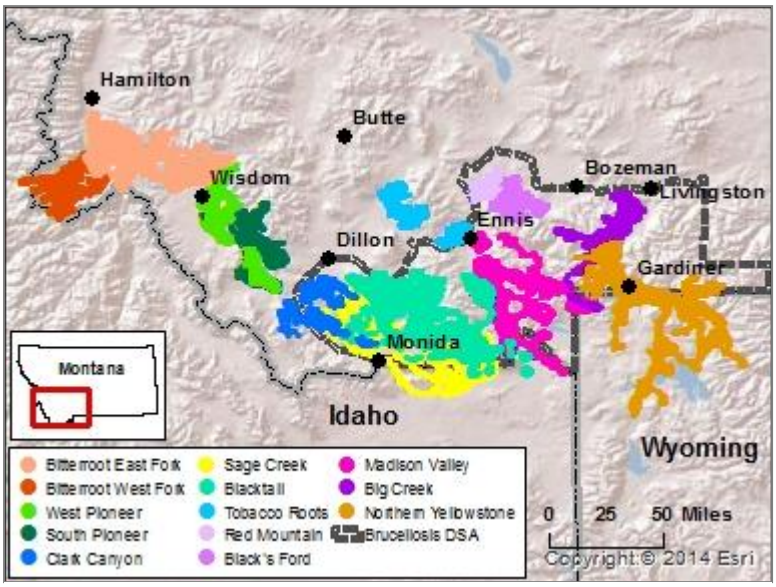

Figure 4. Movement of radiocollared elk within and adjacent to the brucellosis designated surveillance area (DSA) in southwest Montana during the January – June transmission risk period.

Seropositive and seronegative elk had similar movement patterns. Elk distribution overlapped during the risk period among Blacktail, Sage Creek, and Wall Creek area elk. In addition, there were three cases of emigration by a single elk: from Sage Creek to the Tendoy Mountains, from Blacktail to Wall Creek, and from West Pioneer to the East Fork of the Bitterroot. Potential spatial overlap and interchange between Mill Creek and Greeley will be determined when collar data are retrieved in 2016. Overall, radiocollaring efforts in southwestern Montana document potential interchange between all infected and adjacent herds (Figure 4).

Blacktail Area

Twelve of 100 (12%) elk in the Blacktail area tested positive for exposure to *B. abortus* during January 2011 (Table 1). In the field, 8 seropositive elk were detected. These 8 animals were radiocollared and had pregnancies monitored annually through June 2015 to assess reproductive events and determine if *B. abortus* was actively shed through any birth events. We radiocollared an additional 22 seronegative elk and collected movement data for one year. The average pregnancy rate for the Blacktail area was 0.91 for seronegative (n = 23) and 0.63 for seropositive elk (n = 30; repeated sampling of n = 8 elk).

In 2011, 5 of the 8 seropositive elk were pregnant and outfitted with VITs to have their pregnancies monitored (Appendix A). Four birth events were documented and sampled. Each birth event was categorized as a suspected live birth and *B. abortus* was not detected at any of the birth sites. One collar malfunctioned and the elk has not been located since capture. In 2012, 5 of the remaining 7 seropositive elk were pregnant and outfitted with VITs. Four birth events were documented, including 1 confirmed abortion, 1 suspected live birth and 2 confirmed live births. *B. abortus* was detected at the abortion site only. The fifth elk with a VIT died in April prior to any birth event, likely due to mountain lion predation. The carcass was sampled 10 days postmortem and *B. abortus* was not detected. This elk was not included in the pregnancies monitored. In 2013, 4 of the remaining 6 seropositive elk were pregnant and outfitted with VITs. Three birth events were

documented, including 1 suspected and 2 confirmed live births, and *B. abortus* was not detected at any birth site. The fourth VIT malfunctioned and no birth event was documented resulting in an unknown event. In 2014, 4 of the remaining 6 seropositive elk were pregnant and outfitted with VITs. One of these elk tested seronegative in 2014, however we continued to include her in sampling because of her 3 prior seropositive test results. Four confirmed live birth events were documented, and *B. abortus* was not detected at any birth site. During the fall 2014 hunting season, 1 seropositive elk was harvested. In 2015, 3 of the remaining 5 seropositive elk were pregnant and outfitted with VITs. We documented two birth events, including 1 suspected and 1 confirmed live birth, and did not detect *B. abortus* at either birth site. The third VIT remained inside the elk through August, indicating the fetus was likely reabsorbed and no birth event will occur. This elk was not included in the total pregnancies monitored or any birth event category.

One of the seropositive Blacktail elk cleared *B. abortus* antibodies in the fourth year of monitoring. The date of her initial exposure to *B. abortus* is unknown. We estimated this elk to be 8 years old when initially captured in 2011. This elk tested positive for exposure to *B. abortus* during the 2011, 2012 and 2013 screenings and tested negative for exposure during 2014 and 2015. All other elk maintained their positive serostatus throughout the duration of their monitoring.

In general, Blacktail elk winter on the Blacktail and Robb-Ledford WMA along Blacktail Deer Creek, and migrate over the Snowcrest Range to calve and summer in the Gravelly Range north of Centennial Valley and the Centennial Range south of Centennial Valley (Figure 5). There is interchange with the Sage Creek elk herd year long, and likely interchange with the Sand Creek elk herd in Idaho during calving. One seropositive Blacktail elk dispersed in the fall of 2011 to winter on the Wall Creek Wildlife Management Area, and has wintered on the eastern side of the Gravelly Range ever since.

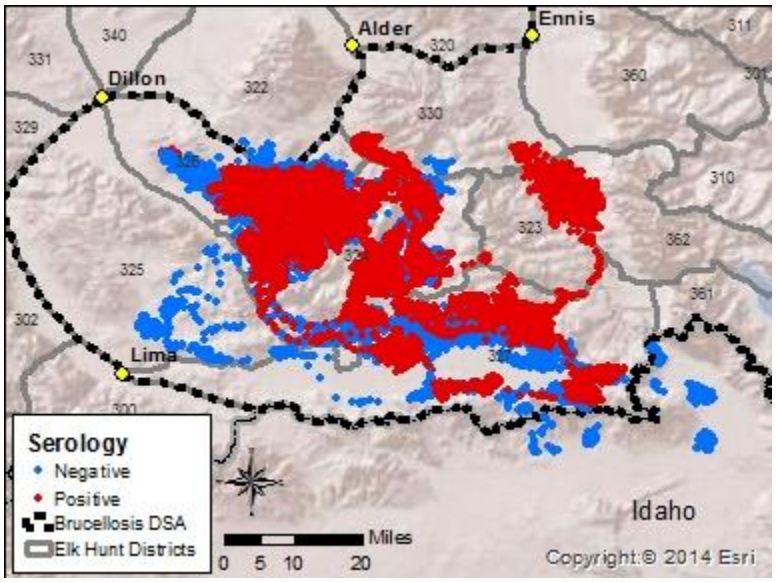

Figure 5. The locations of seropositive (red) and seronegative (blue) elk in the Blacktail area during the January 1 – June 30, 2011 – 2014 risk period.

Sage Creek Area

Five of 93 (5.4%) elk in the Sage Creek area (HD 325) tested positive for exposure to *B. abortus* during January 2012 (Table 1). Sampling occurred in two areas: Basin Creek in the SE and Clark Canyon in the NW. A total of 44 elk were sampled in the Clark Canyon area and no *B. abortus* was detected. A total of 49 elk were sampled in the Basin Creek area and five seropositive elk were detected. These 5 animals were radiocollared and had pregnancies monitored annually through June 2015 to assess reproductive events and determine if *B. abortus* was actively shed during birth events. We radiocollared an additional 24 seronegative elk and documented their movements for one year. The average pregnancy rate for the Sage Creek area was 1.00 for seronegative (n = 25) and 0.88 for seropositive elk (n = 17).

In 2012, 4 of the 5 seropositive elk were pregnant and outfitted with VITs to have their pregnancies monitored (Appendix A). Four birth events were documented, including 1 confirmed abortion, 2 suspected live births, and 1 confirmed live birth. *B. abortus* was detected at the abortion site only. In 2013, 1 of the seropositive elk died during capture operations. Of the remaining 4 seropositive elk, 2 were pregnant and outfitted with VITs. Two suspected live births were documented and *B. abortus* was not detected at either birth site. In 2014, all 4 remaining seropositive elk were pregnant and outfitted with VITs. Three confirmed live births and 1 suspected live birth were documented and *B. abortus* was not detected at any birth site. In 2015, all 4 seropositive elk were pregnant and outfitted with VITs. Three confirmed live births and 1 suspected live birth were documented, and *B. abortus* was not detected at any birth site.

Sage Creek elk herds wintered in the Blacktail Mountains and on the Blacktail and Robb-Ledford Wildlife Management Areas (Figure 6). Elk from the Clark Canyon area did not migrate a long distance and generally remained near the Clark Canyon Reservoir. Elk from the Basin Creek area migrated east to the southern end or over the Snowcrest Mountains to summer both north and south of the Centennial Valley in the Gravelly and Centennial Mountain Ranges. There were 18 elk from the Basin Creek area that moved south of the Centennial Valley and into Idaho during the risk period. In

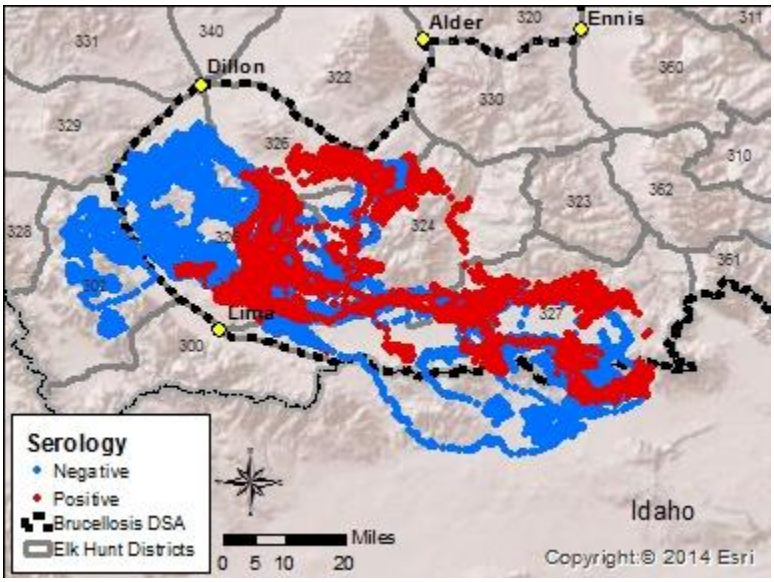

Figure 6. The locations of seropositive (red) and seronegative (blue) elk in the Sage Creek area during the January 1 – June 30, 2012 – 2014 risk period.

addition, 1 elk from the Clark Canyon area moved west across Interstate 15 outside of the risk period in November, but stayed on winter range at low elevation and returned to the Clark Canyon area after less than 48 hours. A second Clark Canyon elk was captured east of Interstate 15, but upon release, immediately moved west across the interstate and spent the remainder of the year in the Tendoy Mountains.

There was overlapping distribution between the Basin Creek and Clark Canyon area elk during the risk period. In addition, there was overlapping distribution between the Sage Creek and Blacktail elk herds year-long, and overlapping distribution during the risk period with both the Wall Creek elk herd and the Sand Point elk herd in Idaho.

Pioneer Mountains Area

Zero of 100 (0%) elk in the Pioneer Mountains area tested positive for exposure to *B. abortus* during February 2013 (Table 1). Sampling occurred in two areas: the southern and western portions of the Pioneer Mountains. We deployed radiocollars on 30 seronegative elk and documented their movements for 1-year (Figure 7). The pregnancy rate for the Pioneer Mountains area was 0.93 (n = 30).

Elk in the southern Pioneers wintered in the Grasshopper Valley and migrated north and northwest to summer range in the Pioneer Mountains. Elk in the western Pioneers wintered east of the Big Hole River in the foothills from Jackson north to Sawmill Creek and primarily migrated east and southeast to summer range in the Pioneer Mountains. There was interchange between elk in the western and southern Pioneer Mountains year round. One elk from the western Pioneers moved northwest to summer range in the upper Big Hole Valley, then emigrated to the East Fork elk winter range in the southern Bitterroot Valley.

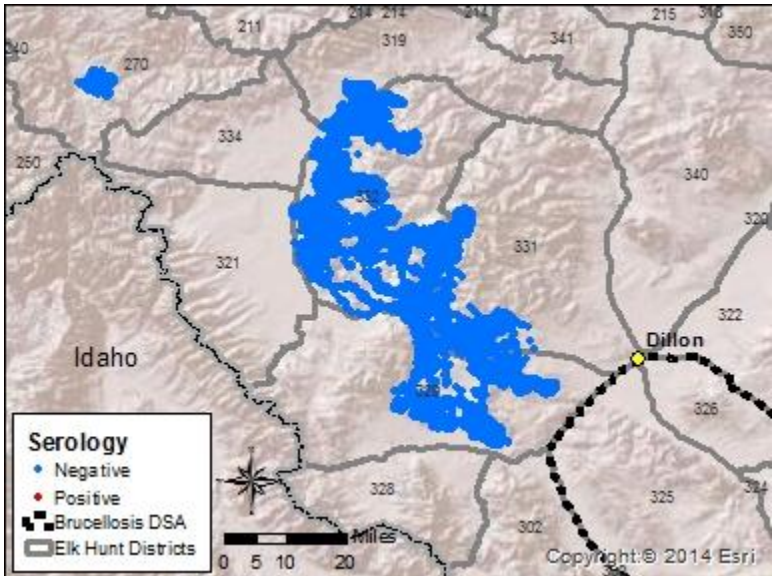

Figure 7. The locations of seronegative (blue) elk in the Pioneer Mountains area during the January 1 – June 30, 2013-2014 risk period.

Tobacco Root Area

Zero of 70 (0%) elk in the Tobacco Root Mountains area tested positive for exposure to *B. abortus* during February 2014 (Table 1). Sampling occurred in two areas: Wisconsin Creek in the SW and the Valley Garden area in the SE. We deployed radiocollars on 26 seronegative elk and documented their movements for 1-year (Figure 8). The pregnancy rate for the Tobacco Root area was 0.96 (n = 26).

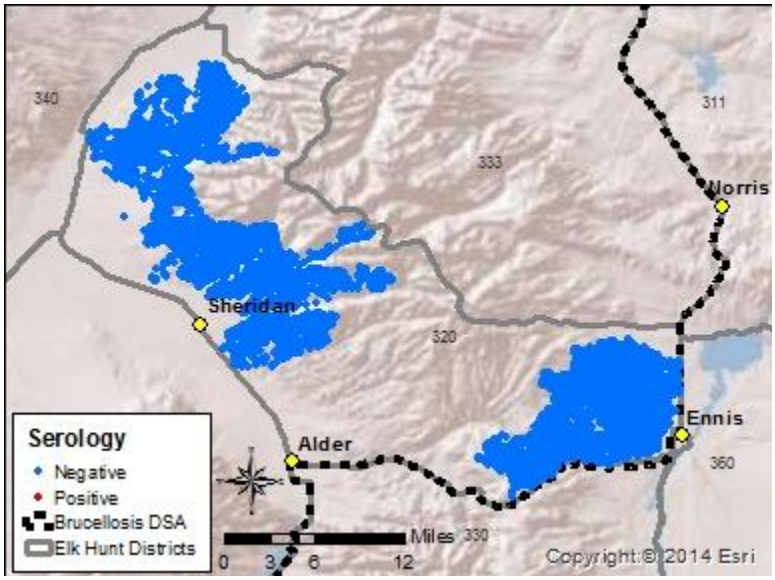

Figure 8. The locations of seronegative (blue) elk in the Tobacco Root Mountains area during the January 1 – June 30, 2014-2015 risk period.

Elk from the Wisconsin Creek area wintered along the foothills north and east of Sheridan and migrated east into the Tobacco Roots to summer. Elk from the Valley Garden area wintered in the foothills between McAllister and Ennis, west and north of Highway 287 and migrated short distances west to summer range in the Tobacco Root Mountains.

North Madison Area

Ten of 60 (16.7%) elk in the North Madison area of HD 311 tested positive for exposure to *B. abortus* during February 2014 (Table 1). Sampling occurred in two areas: the Black's Ford area east of the Madison River and the Red Mountain area west of the Madison River. A total of 20 elk were sampled in the Red Mountain area and one seropositive elk was detected. This elk tested seronegative in the field and did not receive a radiocollar. A total of 40 elk were sampled in the Black's Ford area and 9 seropositive elk were detected. Eight seropositive elk were detected in the field, radiocollared and had pregnancies monitored annually through June 2015 to assess reproductive events and determine if *B. abortus* was actively shed through any birth events. An additional 14 seronegative elk were radiocollared and their movements were documented for one year. The average pregnancy rate for the North Madison area was 0.86 for seronegative (n = 14) and 0.75 for seropositive elk (n = 16).

In 2014, 4 of the 8 seropositive elk were pregnant and outfitted with VITs to have their pregnancies monitored (Appendix A). Four birth events were documented, including 1 suspected abortion where *B. abortus* was detected, and 2 suspected and 1 confirmed live births where *B. abortus* was not detected. The suspected abortion occurred on March 30, 2014. The time of year indicates an abortion event, but the fetus could not be located, thus the event was classified as a suspected abortion. In 2015, 5 of the 8 seropositive elk were pregnant and outfitted with VITs. Four birth events were documented, including 2 suspected and 2 confirmed live births, and *B. abortus* was not detected at any birth site. The fifth elk was harvested in a damage hunt prior to any birth event.

We deployed GPS collars in both the Red Mountain and Black's Ford areas (Figure 9). Neither group of elk crossed the Madison River. Elk from the Red Mountain area remained near Red Mountain year round, with limited movement to the north in the spring. Elk from the Black's Ford area primarily wintered north of Highway 84, although some elk did spend time south of the highway. All Black's Ford elk migrated south in the spring and migrated to summer ranges from Pole Creek in the east to Highway 191 in the west and as far south as the Spanish Peaks.

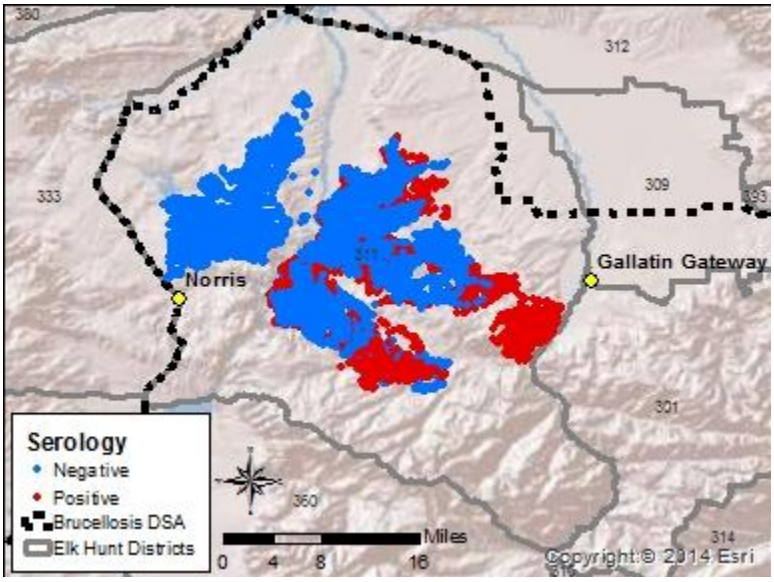

Figure 9. The locations of seropositive (red) and seronegative (blue) elk in the Black's Ford area during the January 1 – June 30, 2014-2015 risk period.

Mill Creek Area

Sixteen of 30 (53%) elk in the Mill Creek area tested positive for exposure to *B. abortus* during January 2015 (Table 1). One of the seropositive elk died during capture. The remaining 15 seropositive elk were detected in the field, radiocollared, and had pregnancies monitored through June 2015 to assess reproductive events and determine if *B. abortus* was actively shed through any birth events. We radiocollared an additional 8 seronegative elk and their movements are currently being documented. The pregnancy rate for the Mill Creek area was 0.92 for seronegative (n = 13) and 0.81 for seropositive elk (n = 16).

In 2015, 10 of 15 seropositive elk were pregnant and outfitted with VITs (Appendix A). Eight birth events were documented, including 4 suspected and 4 confirmed live births, and *B. abortus* was detected at one birth site. The *B. abortus* detection occurred at a suspected live birth site in mid-June. We submitted 3 samples for culture testing (VIT, swab of VIT, soil) and 1 VIT swab for PCR testing. PCR

was the only test to detect *B. abortus*. One VIT failed on June 2, 2015 resulting in an unknown event. One elk died from capture related injuries prior to any birth event.

Fine-scale movement data for the Mill Creek area elk will be available after the seropositive elk are recaptured in January/February 2016 and the GPS collars on seronegative drop off in April 2016. One seronegative elk died in April 2015. Ground and aerial telemetry during parturition monitoring and collar relocation flights provides a coarse idea of movement in this area (Figure 10). In general, Mill Creek elk winter in the foothills of the Absaroka Mountains north of Mill Creek. In spring, 62% of the elk migrated east up the Mill Creek drainage, and 33% of the elk remained in the foothills between Elbow Creek and Strawberry Ridge. One elk migrated north along the foothills towards Pine Creek.

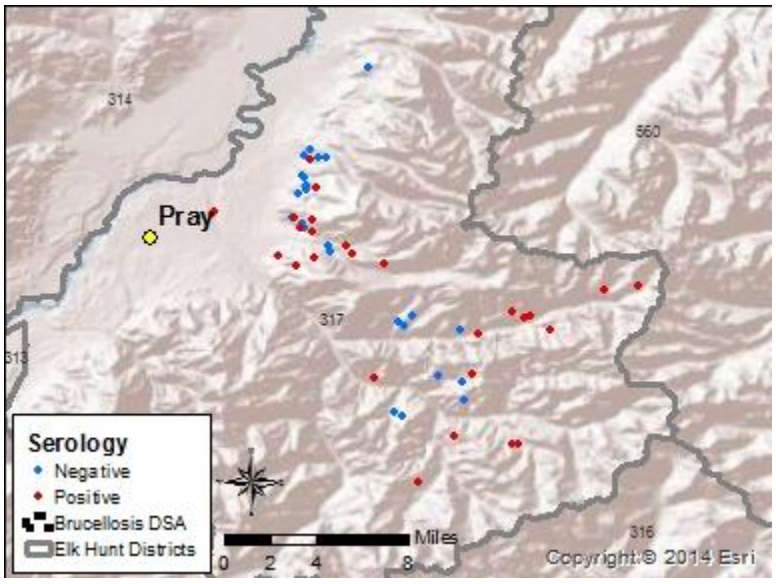

Figure 10. The locations of seropositive (red) and seronegative (blue) elk in the Mill Creek area during the January 1 – June 30, 2015 risk period.

Greeley Area

Two of 65 (3.1%) elk in the Greeley area tested positive for exposure to *B. abortus* during January 2015 (Table 1). A total of 65 elk were sampled in the Greeley area and two seropositive elk were detected. Both seropositive elk were detected in the field, radiocollared and had pregnancies monitored through June 2015. We radiocollared an additional 18 seronegative elk and their movements are currently being documented. The pregnancy rate for the Greeley area was 0.95 for seronegative (n = 22) and 1.00 for seropositive elk (n = 2).

In 2015, both seropositive elk were pregnant and outfitted with VITs to have their pregnancies monitored (Appendix A). One suspected live birth was documented, and *B. abortus* was not detected at the birth site. The second VIT failed on May 18, 2015 resulting in an unknown event.

Fine-scale movement data for the Greeley area elk will be available after the GPS collars drop off in April 2016. Ground telemetry while monitoring the two seropositive elk and GPS collar relocation flights have provided a coarse idea of movement in this area (Figure 11). Elk wintered north of Greeley Mountain, on Coal Mine Rim and in McLeod Basin. In spring, approximately 50% of the elk migrated south into the West Fork and the Main Boulder River.

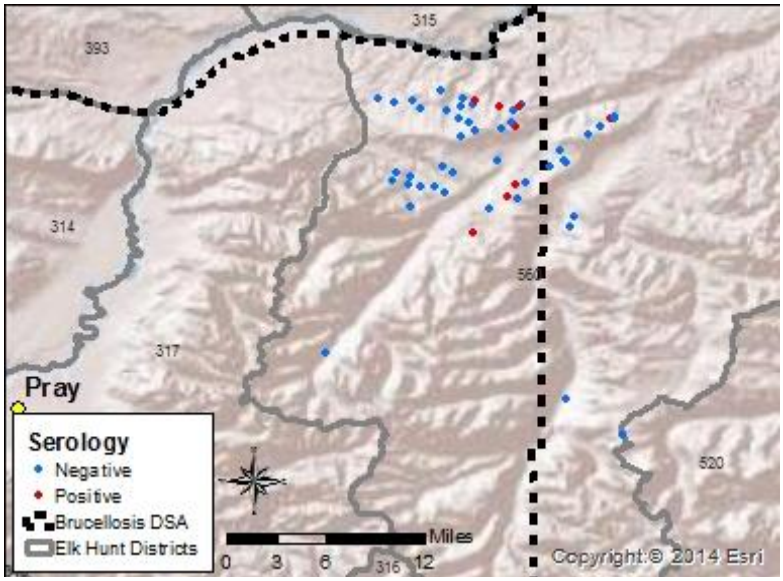

Figure 11. The locations of seropositive (red) and seronegative (blue) elk in the Greeley area during the January 1 – June 30, 2015 risk period.

Discussion

Our targeted brucellosis surveillance efforts documented the presence of the disease in elk from the Blacktail, Sage Creek, Black's Ford, Mill Creek and Greeley areas. We detected no evidence of the disease in elk from the Pioneer Mountains or Tobacco Root areas. This surveillance effort included 10 hunt districts (HDs 317, 320, 324, 236, 325, 329, 331, 332, 333, and 560) where sampling had previously been absent or inadequate and accurate brucellosis presence/absence was unknown. The documented seroprevalence in HD 311 of 16.7% was a marked increase over the <1% reported prior to 2000. In general, brucellosis has expanded its' range and seroprevalence has increased. In response to this information, Montana Department of Livestock has expanded the DSA three times, most recently in 2014 (Figure 2).

Our results suggest that only a small proportion of seropositive elk are shedding *B. abortus* bacteria. We documented only 3 abortion events out of a total of 48 (6.3%) known-fate birth events. The abortion events occurred on March 30th, April 20th and May 14th. These dates fall within the riskiest time of year identified by Cross et al. (2015) who showed that abortions peak in March through May. Additionally, *B. abortus* was only detected at 1 live birth event, suggesting that live births are not significant sources of brucellosis transmission. While time to detection and sampling did not differ between abortions and live birth events, cow elk behavior during live birth events (i.e., consumption of birth material and vegetation) may remove most of the *B. abortus* shed at the site.

Annual serology on all radiocollared seropositive elk revealed one case of seroreversion, where the elk cleared *B. abortus* antibodies from her bloodstream and tested seronegative in the fourth and fifth year of sampling. This elk was estimated to be 8 years old when she was first captured and tested in 2011. Research concerning seroreversion, or titer loss, is difficult because it requires long term, repeated sampling of individuals to monitor serostatus. One seroreversion out of 26 seropositive elk captured at least twice represents 4% of the seropositive population.

Data from GPS radiocollars has improved our understanding of elk movement and potential routes for the spatial spread of brucellosis or other diseases. Interchange and overlap between 8 different herds during the risk period was documented. There was interchange and overlap of elk from the Blacktail, Sage Creek and Wall Creek areas, as well as overlap between the western and southern Pioneer herds. Interchange in the form of emigration occurred with one elk from the Clark Canyon area of Sage Creek dispersing west into the Tendoy Mountains, one elk from the Blacktail area dispersing east to the Wall Creek area, and one elk from the western Pioneer area dispersing to the East Fork area of the Bitterroot Valley. In addition, elk movements will be used to determine the timing and degree of spatial separation between elk and livestock in future focused analyses.

Over the next five years, we plan to continue the targeted brucellosis surveillance efforts in the areas north and northeast of the current DSA. The focus of the next 5 years of effort will be to 1) continue to document the spatial extent of the disease, 2) to integrate the exposure, movement and epidemiology data to predict the risk of transmission from elk to livestock, and 3) to evaluate the effectiveness of elk management actions at reducing transmission risk within the DSA designed to affect elk distribution and elk-cattle spatial overlap. We will also continue to monitor seropositive elk birth events and remove seropositive elk after 5 years of monitoring. After five years, seropositive elk will be euthanized and tissues cultured to determine if they are actively infected with brucellosis. The first cohort to be euthanized and sampled will be the 5 seropositive elk from the Blacktail area in February 2016. Seropositive elk in the remaining areas will be euthanized in 2017 (Sage Creek), 2019 (Black's Ford) and 2020 (Mill Creek, Greeley). This effort will establish the individual's infection status, allow us to calculate the proportion of seropositive elk that may be infectious, and provide information on the persistence of antibodies following exposure to *B. abortus.*

The primary goal of this project is to provide wildlife managers and livestock producers and authorities with information useful for designing strategies to reduce the risk of brucellosis transmission from elk to livestock. Transmission risk is a complex combination of elk seroprevalence, the proportion of infected elk, associated abortion risk, and the spatial overlap of elk and livestock during the risk period. Seroprevalence, epidemiology and elk movement data collected during the first five years of this project will be integrated with livestock distribution maps to develop a risk model that will quantify the actual risk of transmission across space and time within the DSA. With this model, the

riskiest areas based on spatial and temporal overlap between elk and livestock can be identified. Management actions can then target these risky areas for more effective resource allocation.

The elk brucellosis working group recommended that MFWP focus management on reducing the risk of elk to livestock transmission by managing elk distribution within the DSA. Following that recommendation, a new phase of the project aims to evaluate the effectiveness of management actions at reducing transmission risk, by deploying radiocollars in elk herds subject to elk brucellosis management hunts, hazing efforts, or other actions. The risk model and elk movements associated with each management action will be used to quantify the change in predicted risk of transmission. This aspect of the project also addresses the working group's recommendation to evaluate management performance, maximize cost effectiveness and focus effort.

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Literature Cited

Anderson, N. J., J. M. Ramsey, K. Hughes, K. Lackey and S. Olind. 2010. 2009-10 brucellosis surveillance in elk. MT Fish, Wildlife and Parks, Bozeman, MT.

Barbknecht, A. E., W. S. Fairbanks, J. D. Rogerson, E. J. Maichak, and L. L. Meadows. 2009. Effectiveness of vaginal-implant transmitters for locating elk parturition sites. Journal of Wildlife Management 73: 144–148.

Cross, P. C., E. J. Maichak, J. D. Rogerson, K. M. Irvine, J. D. Jones, D. M. Heisey, W. H. Edwards, B. M. Scurlock. 2015. Estimating phenology of elk brucellosis transmission with hierarchical models of cause-specific and baseline hazards. Journal of Wildlife Management 79(5):739-748.

Appendix A. Abortion (A) and live birth (LB) events and the associated sampling result that did detect (+) or did not detect (-) *B. abortus* at a birth site for seropositive elk during 2011 – 2015. Unknown (U) events do not have a sampling result because there was no site to sample. NA denotes the animal field-tested not pregnant and was not outfitted with a VIT. x denotes the animal died or was lost prior to parturition season. Herd association for individual elk is given: Blacktail (BT), Sage Creek (SC), Northern Madison (NM), Mill Creek (MC), and Greeley (GR).

Elk ID	Herd	2011 Birth Event Result	2012 Birth Event Result	2013 Birth Event Result	2014 Birth Event Result	2015 Birth Event Result
BT10032	BT	U	x	x	x	x
BT10045	BT	NA	A +	U	LB -	U
BT10055	BT	NA	NA	NA	NA	NA
BT10058	BT	LB -	LB -	LB -	LB -	x
BT10063	BT	LB -	U	x	x	x
BT10068	BT	LB -	LB -	LB -	LB -	LB -
BT10075	BT	NA	NA	NA	NA	NA
BT10083	BT	LB -	LB -	LB -	LB -	LB -
SC11031	SC		LB -	LB -	LB -	LB -
SC11045	SC		NA	LB -	LB -	LB -
SC11050	SC		LB -	NA	LB -	LB -
SC11087	SC		A +	NA	LB -	LB -
SC11097	SC		LB -	x	x	x
31113001	NM				NA	LB -
31113002	NM				NA	LB -
31113004	NM				LB -	NA
31113009	NM				NA	x
31113027	NM				A +	NA
31113039	NM				LB -	LB -
31113061	NM				NA	LB -

31113073	NM	LB -	NA
NA14-051	GR		U
NA14-040	GR		LB -
EC14-002	MC		NA
EC14-003	MC		LB -
EC14-006	MC		LB -
EC14-007	MC		LB -
EC14-012	MC		LB +
EC14-014	MC		LB -
EC14-015	MC		NA
EC14-018	MC		NA
EC14-020	MC		LB -
EC14-022	MC		NA
EC14-024	MC		LB -
EC14-025	MC		LB -
EC14-028	MC		U
EC14-029	MC		NA
EC14-030	MC		x

Appendix B. Birth events where B. abortus was detected, samples tested and whether B. abortus was detected (+) or not detected (-) for each sample. Blanks denote that a sample was not collected, and thus not tested. All samples were culture tested with the exception of the VIT Swab PCR test, which was only available in 2015.

Sample	Abortion Confirmed 4/20/2012	Abortion Confirmed 5/14/2012	Abortion Suspected 3/30/2014	Live Birth Suspected 6/16/2015
VIT			+	-
VIT Swab Culture	+	-	+	-
VIT Swab PCR				+
Fetus	+	-		
Placenta Swab		-		
Fluid Swab		-		
Soil		+		-
Soil Swab	-	-		
Vegetation		-		
Vegetation Swab	-	-		
Fecal	-	-		