Targeted Elk Brucellosis Surveillance Project 2022 Annual Report

EXECUTIVE SUMMARY

Montana Fish, Wildlife & Parks (MFWP) is conducting a multi-year targeted elk brucellosis surveillance project to evaluate 1) prevalence and spatial extent of brucellosis exposure in elk populations, 2) elk spatial overlap with livestock and interchange between elk populations, and 3) effects of serostatus on pregnancy rate, pregnancy outcome, shedding of *B. abortus* at event sites and the existence of live *B. abortus* in tissues of seropositive elk. This report is an annual summary of the 2022 targeted elk brucellosis surveillance project. In January 2022, we sampled 100 adult female elk from the northern Tobacco Root Mountains and screened blood serum for exposure to *B. abortus*. All northern Tobacco Root Mountains elk tested negative for exposure to *B. abortus* (prevalence = 0%, 95% CI: 0-3.7%, n = 100). In January and February 2022, we sampled 63 adult female elk from the southern Tobacco Root Mountains and screened blood serum for exposure to *B. abortus*. All southern Tobacco Root Mountains elk tested negative for exposure to *B. abortus* (prevalence = 0%, 95% CI: 0-6%, n = 63). We collared 40 elk in the northern Tobacco Root Mountains and potential overlap with livestock and interchange between elk populations is being monitored with GPS radio collars. Using data collected from 2011-2019, we completed an evaluation of the effects of brucellosis exposure on pregnancy rates, pregnancy outcome, shedding of *B. abortus* at birth sites and the existence of live *B. abortus* in tissues of seropositive elk. Pregnancy rates for seropositive animals were 9% lower in prime aged (2.5-15.5; 85%, 95% CI: 74-91%) elk and 26% lower in old age (>15.5; 43%, 95% CI: 19-71%) elk as compared to seronegative animals. To understand the risk of seropositive elk shedding *B. abortus* bacteria and the effects of exposure on elk reproductive performance, we monitored 30 seropositive elk for up to 5 years and monitored the fate of pregnancies using vaginal implant transmitters. Based on 82 elk-years of pregnancy monitoring, we documented 4 abortions, 61 live birth events, and 17 unknown events (e.g., failed VITs). We detected *B. abortus* at 3 abortions and 2 live births using a combination of culture and polymerase chain reaction (PCR) testing. We estimated the annual probability of a seropositive elk having an abortion as 0.06 (95% CI: 0.02-0.15). The 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 17 seropositive elk at the end of 5 years of monitoring, performed necropsies, and sampled tissues for *B. abortus* bacteria using culture and PCR testing. 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).

INTRODUCTION

Montana Fish, Wildlife & Parks (MFWP) has conducted surveillance for brucellosis in elk populations since the early 1980s. Surveillance consists of screening blood serum for antibodies signifying exposure to *Brucella abortus*, the bacteria that causes the disease brucellosis. Brucellosis may cause abortion in pregnant elk, typically from February through May (Cross et al. 2015) and is primarily transmitted through contact with infected fetuses, birthing fluids and material. 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 indicates the potential for elk to transmit the disease to livestock or other elk.

To increase understanding of brucellosis in elk populations, MFWP initiated a targeted elk brucellosis surveillance project in 2011. The goals of the project are to 1) evaluate the prevalence and spatial extent of brucellosis exposure in elk populations, 2) document elk movements to evaluate the extent of spatial overlap with livestock and interchange between elk populations, such as hazing and lethal removal, on elk distributions and spatial overlap with livestock, and 3) evaluate the effects of 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. In order to achieve these goals, MFWP has conducted targeted sampling and collaring efforts focused on 1 – 2 elk populations per year since 2011. Elk populations are identified through collaborative discussions between MFWP, the Montana Department of Livestock (DOL) and landowners. Selection is based on proximity to the known distribution of brucellosis and/or significant livestock concerns. Surveillance areas are both inside and outside the State of Montana brucellosis designated surveillance area (DSA, Figure 1).

SAMPLED POPULATIONS

Since 2011, we have sampled 21 elk populations (Figure 1). In January-February 2022, we sampled elk in the northern (formerly HD333; now part of HD320) and southern (HD320) Tobacco Root mountains.

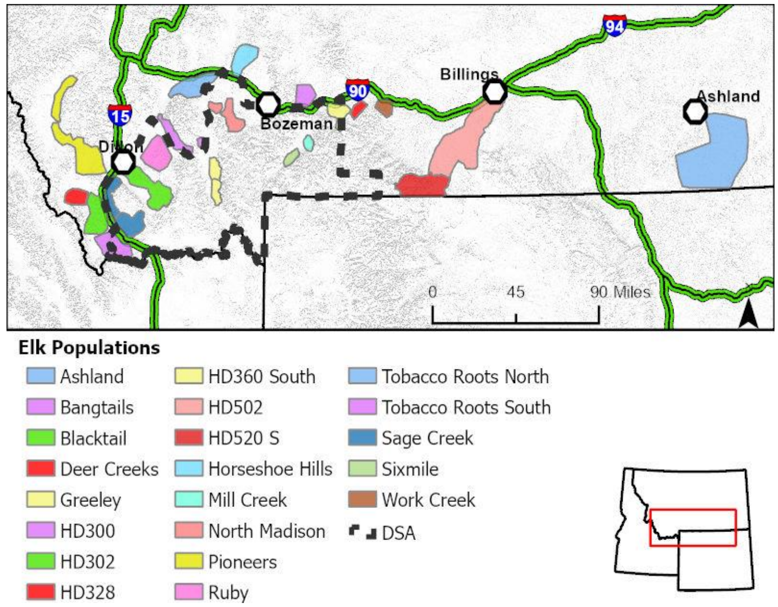


Figure 1. Elk populations sampled during the 2011 – 2022 targeted elk brucellosis surveillance project. The area inside the black dashed line is the Montana brucellosis DSA.

METHODS

To evaluate brucellosis presence and prevalence, we captured adult female elk using helicopter net-gunning and collected a blood sample to screen animals for exposure. Exposure was determined by the presence of antibodies to *B. abortus* in an animal's blood serum. Blood serum samples were tested at the Montana Department of Livestock Diagnostic Lab (Diagnostic Lab) utilizing the Rapid Automated Presumptive (RAP) and Fluorescence Polarization Assay (FPA) plate tests. Suspect or reactors to these screening tests were further tested with the FPA tube test. Final classification of serostatus (i.e., seropositive or seronegative) was based on test results received from the Diagnostic Lab.

We collared a sample of elk in the northern Tobacco Root Mountains population to track movements and evaluate risk of brucellosis transmission to livestock and other elk populations. We deployed satellite GPS collars. The collars are programmed to record locations every hour and have a timed-release mechanism that releases the collar after 62 weeks, allowing collars to be retrieved and redeployed. All collars have a mortality sensor that detects if the collar is stationary for > 10 hours.

This report also summarizes movement data from the Horseshoe Hills elk population that we monitored in 2021. Movement data collection was completed for the Horseshoe Hills in June 2022.

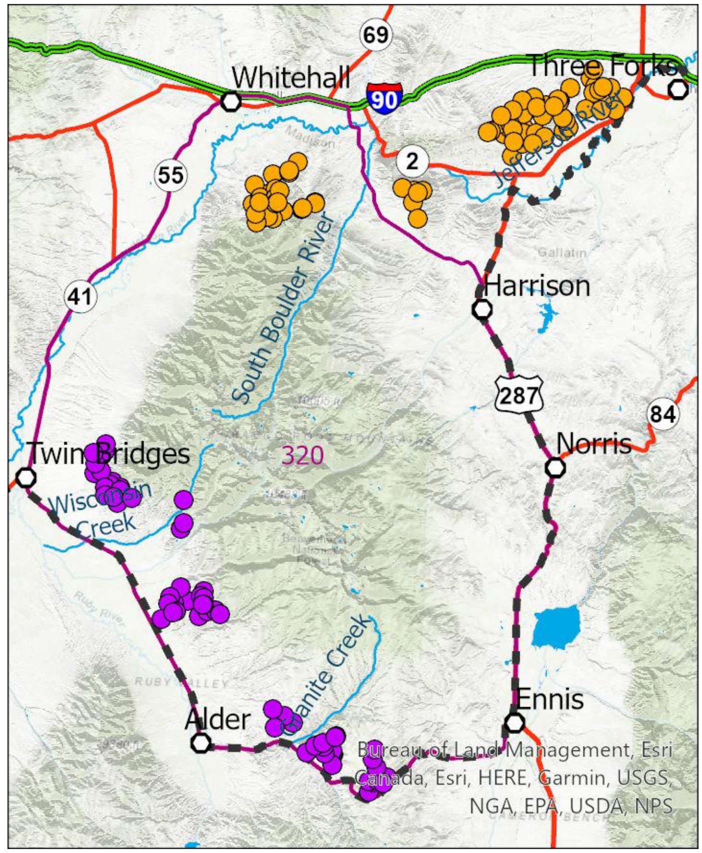


Figure 2. Capture and sampling locations of elk from the northern (orange) and southern (purple) Tobacco Root Mountains populations during January and February 2022.

To evaluate the effects of serostatus on pregnancy rates, pregnancy outcome, shedding of *B. abortus* at birth sites, and the existence of live *B. abortus* in tissues of seropositive elk we used capture and pregnancy monitoring data collected from 2011-2020. Data collection was completed in January 2019 and data analysis is complete. We are currently writing a manuscript detailing our research and hope to submit the manuscript for publication consideration at the Journal of Wildlife Diseases in late fall 2022.

RESULTS

Brucellosis surveillance

In January 2022, we sampled 100 adult female elk in the northern Tobacco Root Mountains and deployed collars on 40 elk (Figure 2, Table 1). All elk tested negative for exposure to *B. abortus*, giving the population an estimated seroprevalence of 0% (95% CI = 0-3.7%; Table 1).

In February 2022, we sampled 63 female elk in the southern Tobacco Root Mountains (Figure 3; Table 1). All elk tested negative for exposure to *B. abortus* resulting in an estimated seroprevalence of 0% (95% CI: 0-6%; Table 1). In 2014 we sampled 70 elk from this same population and all elk tested seronegative for brucellosis at that time. We re-sampled this area because 2 elk from the Ruby Mountains to the south tested seropositive in 2020 and movement data suggested some potential mixing between these two herds. We did not fit any elk with GPS collars during this capture because sufficient movement data was collected as part of the 2014 capture effort.

Table 1. The elk populations, number of elk sampled for *B. abortus* exposure, number of elk collared, number of elk testing seropositive for exposure, and the estimated seroprevalence with 95% confidence intervals (in parentheses).

Population	Number Sampled	Number Collared	Number Seropositive	Estimated Seroprevalence
Northern Tobacco Roots	100	40	0	0 (0, 0.037)
Southern Tobacco Roots	63	0	0	0 (0, 0.060)

Based on hunter harvest and targeted sampling data since the start of the Targeted Elk Brucellosis Surveillance Project (2010-2022), we estimate brucellosis seroprevalence in elk varies spatially across southwest Montana and ranges from 0 – 38% (Figure 4).

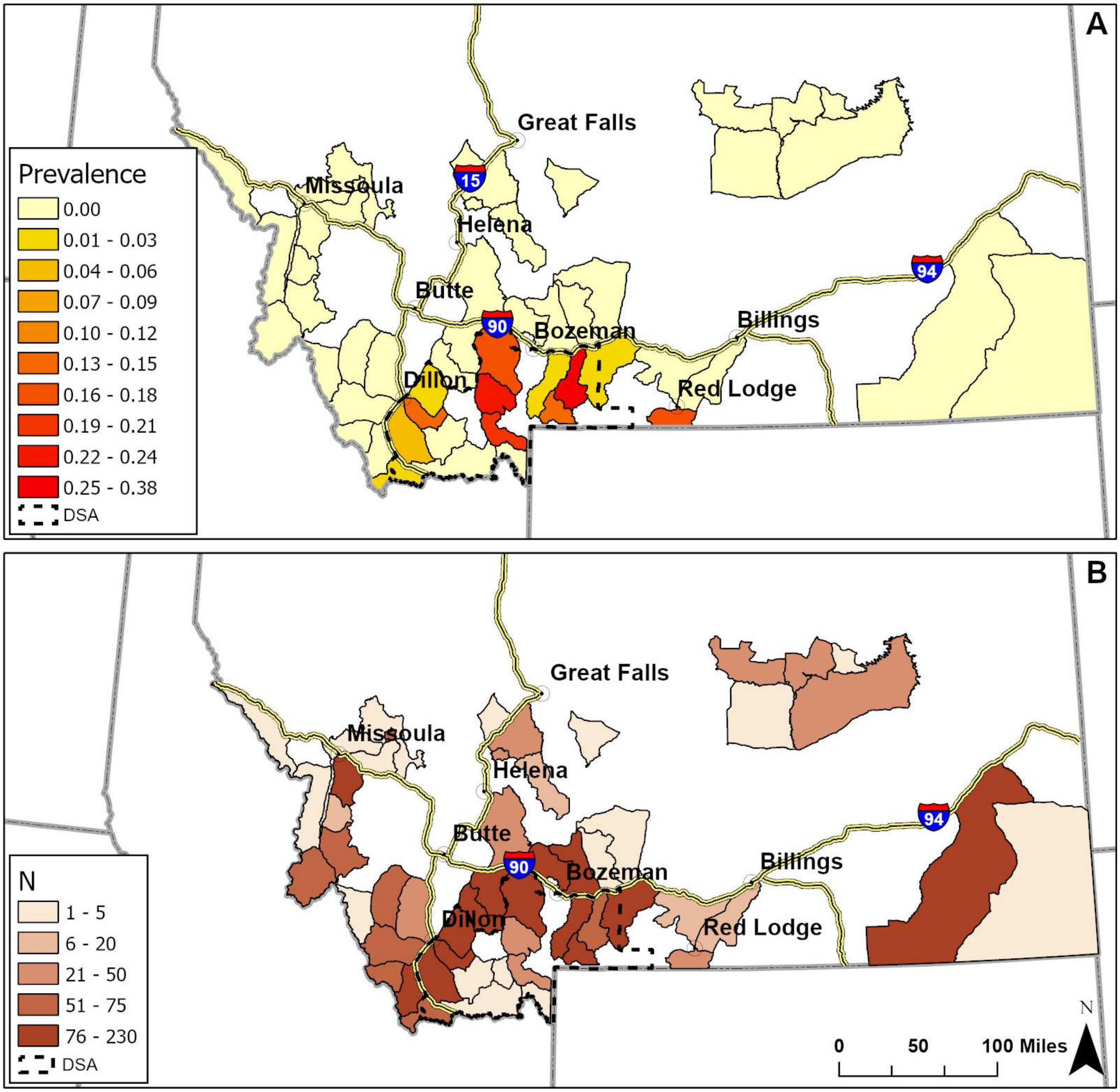


Figure 4. The estimated brucellosis seroprevalence (Panel A) and number of samples screened (n, Panel B) for adult female elk by hunting district* during 2010-2022. Samples include those collected opportunistically during fall hunter harvest and during targeted winter sampling. Note some seroprevalence estimates are derived from a low number of samples. The gray line denotes the boundary of the Montana brucellosis designated surveillance area (DSA). *Hunt district boundaries do not reflect the changes implemented in 2022. We are working to update the database. Hunt district 520, west of Red Lodge is divided in two along a legally defined sub-district boundary to reflect the limited sampling in the northwestern portion of the district.

Elk movements

In January 2022 we deployed 40 satellite upload collars in the northern Tobacco Root mountains (Figure 5). These collars are collecting hourly locations until April 2023. The drop on 1 collar malfunctioned allowing the collar to drop on 4/24/22. One collar failed in May and is no longer sending location data. We are monitoring the remaining 38 collars.

Elk captured in the Bone Basin Creek area (n=13) are residents remaining south of Parrot Bench primarily in the basin, along Mayflower Gulch, east to Cemetery Road and north/west of the South Boulder River with very limited use of Shaw Basin (Figure 5). Six elk spent some time wintering at the mouth of Perry Canyon. There was some use along the Jefferson River near Cardwell in late April, May and June.

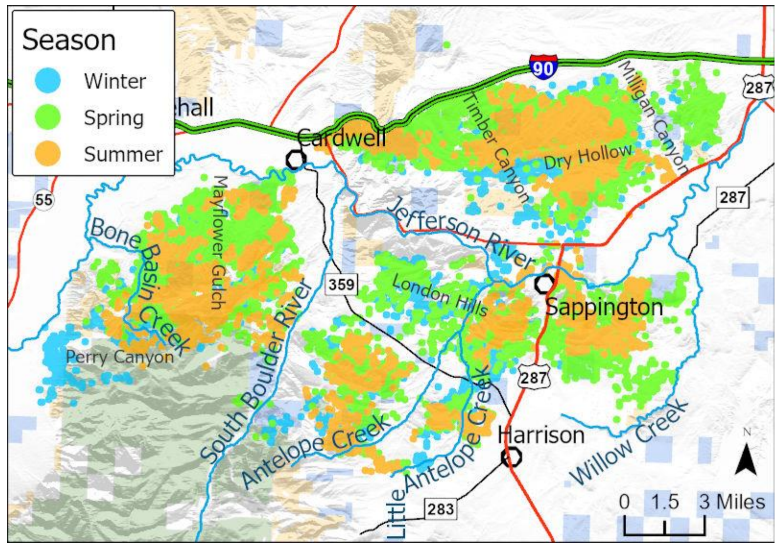


Figure 5. Annual locations (circles) of elk by season [Winter: Jan-Feb, Spring: Apr-Jun, Summer: July] from the northern Tobacco Roots population, February 2022 – July 2022.

Elk captured in the London Hills (n=4) are residents spending time both north and south of MT-359. These elk primarily wintered in the London Hills and Little Antelope Creek south of MT-359. Movement back and forth across MT-359 occurred March-April with more time being spent south of MT-359 and near Antelope Creek starting in April. Three of the elk stayed south of MT-359 by May and appear to be summering in the upper Antelope Creek area. One elk moved north in late May into the Milligan Canyon area for 13 days and then moved back to the London Hills in June where she has remained.

Elk captured in the Milligan Canyon area are primarily residents, with some minor movements to the London Hills and southeast of US-287. These elk typically wintered between Milligan Canyon and Timber Canyon, from Dry Hollow north to the foothills above I-90. Two elk moved south of MT-2 shortly after capture and spent time in the London Hills and on both sides of MT-359 near Little Antelope Creek before returning north to Timber Canyon in late March. One elk moved south to Little Antelope Creek in early February, moving back to Timber Canyon in late March as well. Another elk spent a few weeks around Sappington before returning north in mid-February. Most movement occurred from late March to late April, with 1 elk moving north to the Elkhorn Mountains in early June (Figure 6). Two elk are currently summering along Little Antelope Creek and a third is near Sappington just south of MT-2. Nine elk spent time in spring and/or summer south of the Jefferson River and east of US-287 to Willow Creek. Two elk moved west from Timber Canyon close to I-90 and west to just outside of Cardwell along MT-2. Only 2 elk spent time east of Milligan Canyon.

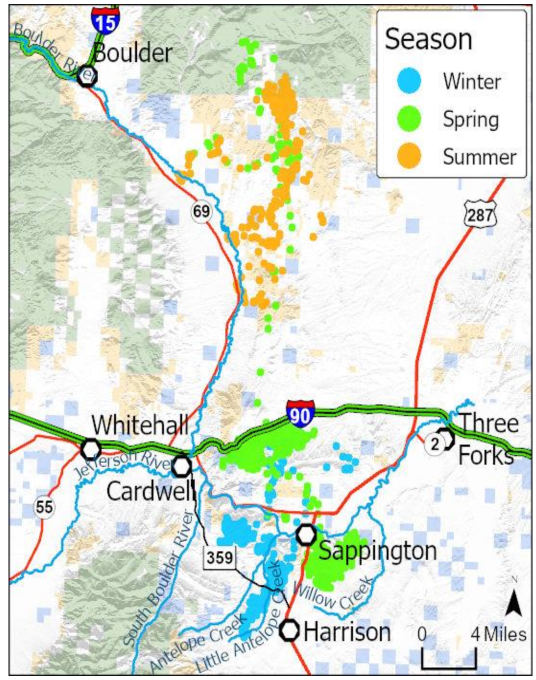


Figure 6. Annual locations (circles) of an elk from the northern Tobacco Roots that migrated to the Elkhorn Mountains by season [Winter: Jan-Feb, Spring: Apr-Jun, Summer: July], February 2022 – July 2022.

Movement into and use of the London Hills and Little Antelope Creek south of MT-2 and west of US-

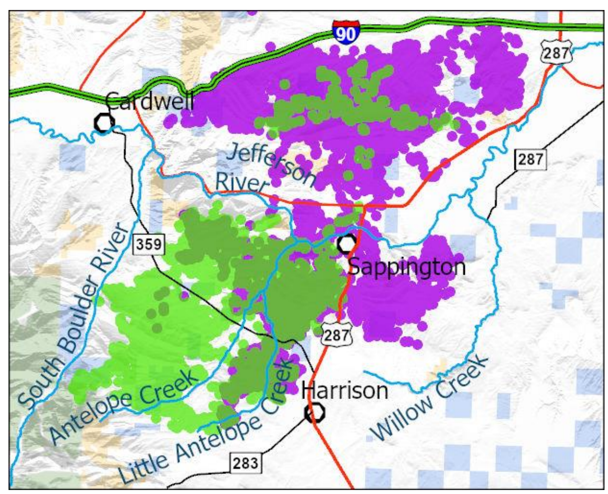


Figure 7. Locations highlighting the mixing of elk from the London Hills (green) with elk from Milligan Canyon (purple), February 2022 – July 2022.

287 indicates potential mixing with elk from the London Hills (Figure 7). One of the London Hills elk also moved north of MT-2 in late May and spent 13 days along Dry Hollow before moving back to the London Hills.

In February 2021, we deployed 29 satellite upload collars in the Horseshoe Hills (Figure 8). These collars collected hourly locations for 1 year. Unfortunately, a hardware contamination issue caused 19 collars to drop off early and limited our movement data for summer range selection and fall migration. One collar failed in late June 2021 only providing data for the elk's spring movement from Cottonwood Gulch in April to Poison Hollow and the Maudlow area. All 9 remaining collars dropped or were deactivated at the end of June 2022. Elk captured in the north and northeast near Maudlow, MT (n=6) moved northeast to Haw Gulch in March shortly after captures and remained in that general vicinity. Three of these elk lost their collars early in April and May of 2021. One elk moved back south of Maudlow for most of the summer and returned north of Sixteenmile Creek in the fall where she remained until her collar dropped in May 2022. Another elk moved south of Sixteenmile Creek in late spring 2022. Elk captured in the southwest (n=23) generally wintered along Cottonwood Gulch just north of Logan, MT. One elk never left this area, but her collar dropped early in late June 2021. The remaining 22 elk migrated northeast in March and April towards Maudlow and the Blacktail Divide at the northern end of the Bridger Mountains. One elk began her

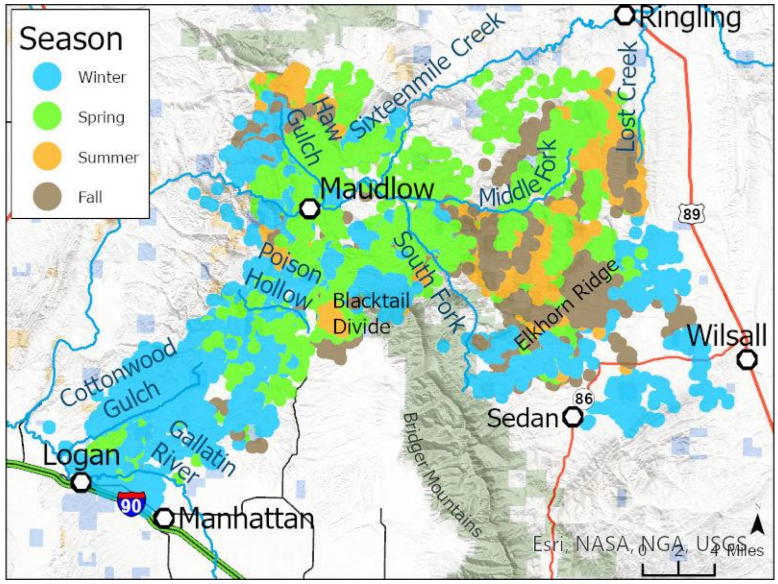


Figure 8. Annual locations (circles) of elk by season from the Horseshoe Hills population, February 2021 – June 2022.

summer in Haw Gulch, similar to the elk captured in the north, but her collar fell off in early July 2021. Most of the elk (n=8) summered between the Middle and South Forks of Sixteenmile Creek, with 2 elk travelling all the way to the Lost Creek area. Two additional elk traveled to the Lost Creek area, but their collars dropped early in April and May 2021, leaving their summer range unknown. Three elk summered in the Blacktail Divide area. Fall migration generally occurred in late November and early December, with most elk returning to the Cottonwood Gulch area.

During the February to June risk period (Figure 9), Horseshoe Hills elk were primarily on winter and spring ranges, from Cottonwood Gulch northeast to Haw Gulch. As the risk period progressed and migration progressed in April some elk migrated east in to Blacktail Divide and between the Middle and South Fork of Sixteenmile Creek. Most elk continued to use the same area from May – October.

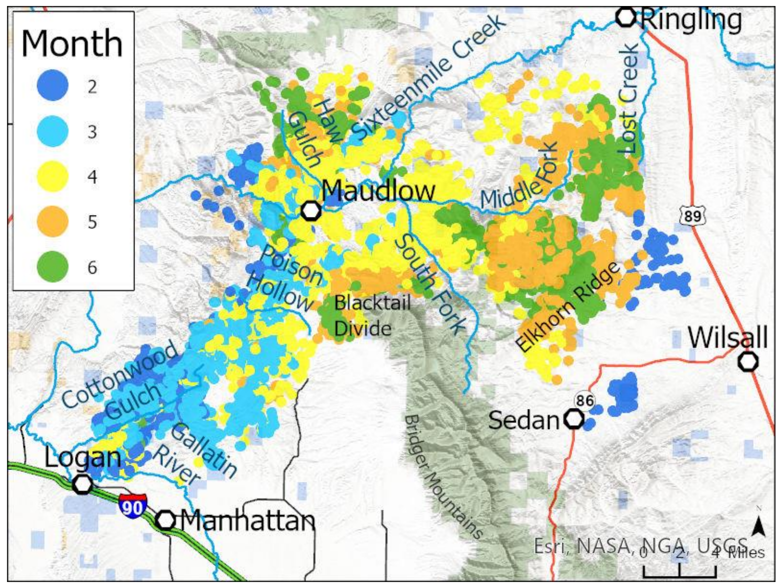


Figure 9. Risk period locations (circles) of elk by month from the Horseshoe Hills population, February 2021 – June 2021 and February 2022 – June 2022.

Two elk stayed near Elkhorn Ridge and Sedan, MT during their second winter in 2022, putting them in proximity to locations from Bangtails elk that approached Battle Ridge from the south (Figure 10). Bangtails elk collar data collected in 2019-2021 indicates they used Battle Ridge in August, September and May. Horseshoe Hills elk were in the same area December – February, indicating a spatial but not a temporal overlap.

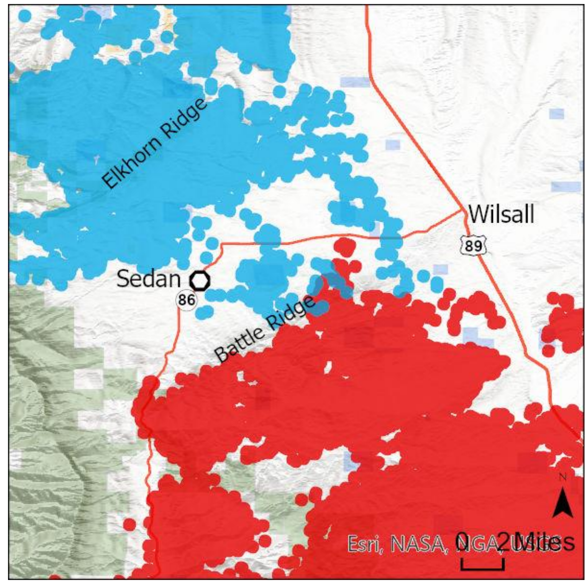


Figure 10. Locations from the Horseshoe Hills (blue) and the Bangtails (red) populations, showing potential mixing near Battle Ridge.

Data from elk collars has improved our understanding of elk movement and potential routes for the spatial spread of brucellosis or other diseases among elk populations (Figure 11). Elk movements have been and will continue to be used to determine the timing and degree of spatial overlap between elk and livestock in focused analyses.

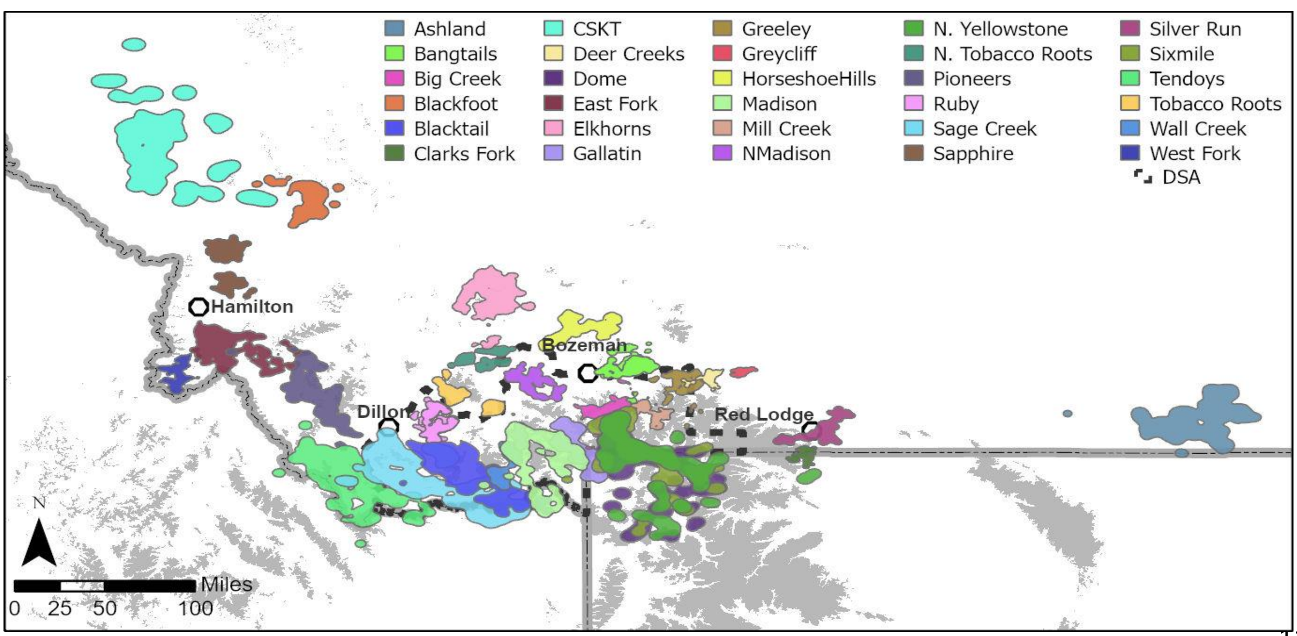
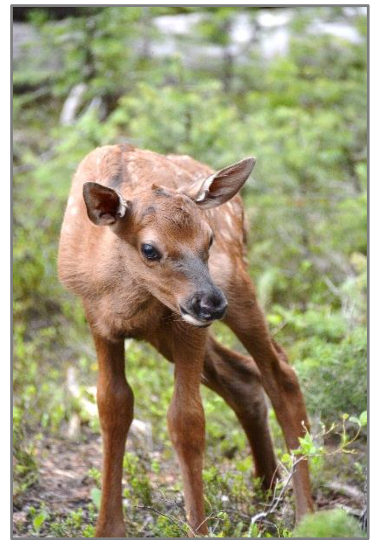


Figure 11. Annual kernel density distributions of sampled elk populations in Montana showing the potential overlap and interchange between populations. Gray polygons represent mountain ranges.

Seropositive elk and *B. abortus* shedding



To understand the impact of exposure on pregnancy rates for seropositive animals we captured 30-100 elk per year, testing their blood for serological exposure to brucellosis. To understand the risk of seropositive elk shedding *B. abortus* bacteria and the effects of exposure on elk reproductive performance, we enrolled seropositive elk captured during 2011-2015 in a pregnancy outcome study. Each year, we sampled these elk and monitored the fate of pregnancies using vaginal implant transmitters.

From 2011-2020, we captured and sampled 1062 adult (≥2 years old) female elk, assigned elk to a prime (2.5-15.5 years) and old (>15.5 years) age category. Pregnancy data were available for 67 seropositive (Prime = 63, Old = 4) and 497 seronegative (Prime = 485, Old = 12) individuals. Seropositive status and age category both significantly influenced pregnancy rate. Pregnancy rates for seropositive animals were 85% (95% CI: 74-91%) for prime (<15.5) and 43% (95% CI: 19-71%) for old (>15.5) elk compared to 94% (95% CI: 92-96%) in prime and 69% (95% CI: 44-87%) in old seronegative elk.

Although not a focus of our study we did document seroreversion, or a change from seropositive to seronegative, in 3 of our seropositive elk. One Blacktail elk was seropositive for 5 years and then tested seronegative at her necropsy when she was 12.5 years old. Another Blacktail elk was seropositive for 3 years, then seronegative for 2 years, and then seroconverted again to seropositive at her necropsy when she was 10.5 years old. One North Madison elk was seropositive for 4 years, seroreverted in the 5th year and then seroconverted again at her necropsy when she was 10.5 years old. The duration of an antibody response is very difficult to determine in free-ranging wildlife as the date of initial infection is often unknown. The probability of brucellosis antibody loss in elk was estimated to be 0.70 per year after 5 years of exposure (Benavides et al. 2017). Remission could cause a drop in antibodies and a seroreversion event and reinfection or resurgence of a latent infection could cause another seroconversion.

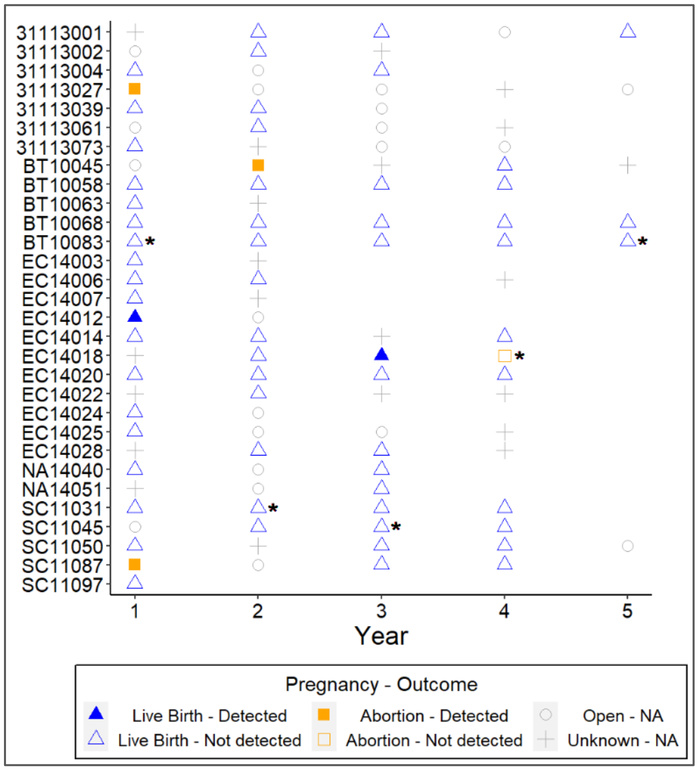


Figure 12. Elk enrolled in the pregnancy outcome study who had at least one known pregnancy outcome event (live birth, abortion) by year of monitoring from 2011-2018, Montana, USA. Pregnancy outcomes include open (circle), live birth (triangle), abortion (square) and unknown (cross). Events where *B. 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). *Sampling occurred more than 48 hours post-event, rendering the detection outcome unreliable due to environmental degradation.

For the pregnancy outcome study we monitored 82 elk-years of pregnancies and documented 4 abortions and 61 live birth events (Figure 12). We detected *B. abortus* at 3 abortions and 2 live births using a combination of culture and polymerase chain reaction (PCR) testing. We estimated the annual probability of a seropositive elk having an abortion as 0.06 (95% CI: 0.02-0.15). 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 opportunistically sampled (e.g., natural mortality) or euthanized 17 seropositive elk after 4-5 years of monitoring, performed necropsies, and sampled tissues for *B. abortus* bacteria using culture and PCR testing (Figure 13). *B. abortus* was detected in 3 seropositive individuals: by culture in the popliteal lymph node of 2 elk, and by PCR in the placentome and plasma of 1 of those elk and in the retropharyngeal lymph node of a third elk that was undetected by culture. 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).

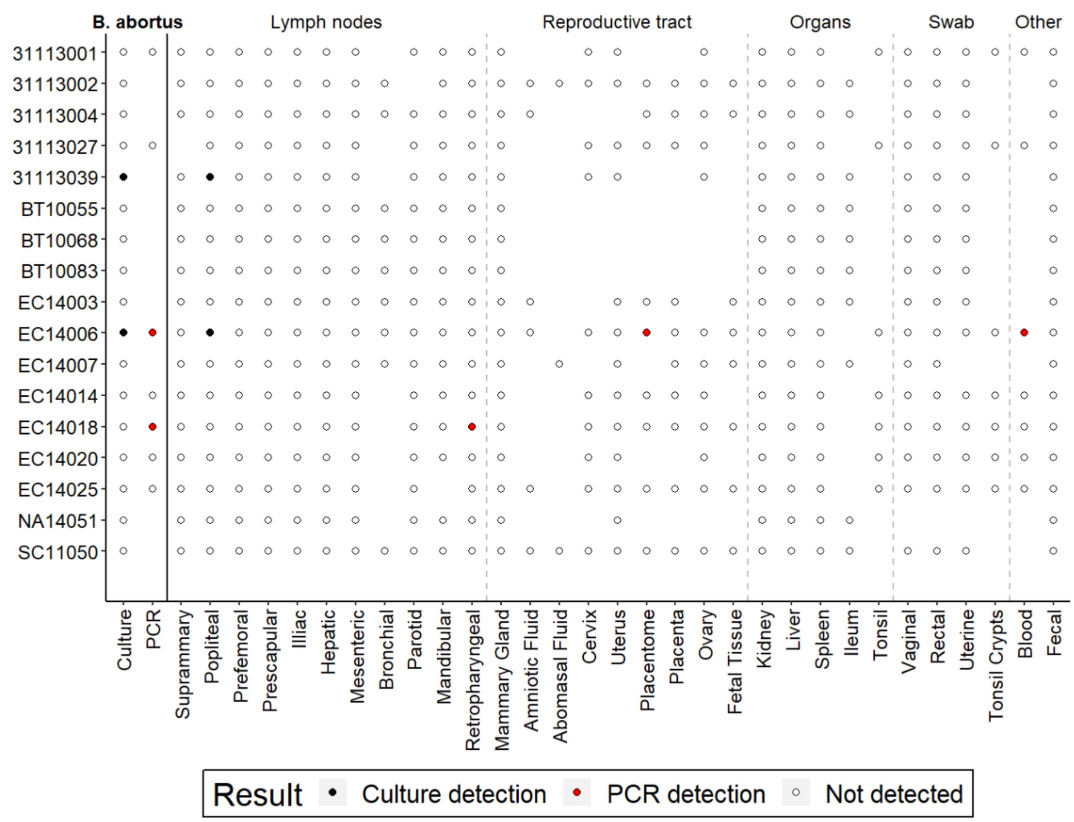


Figure 13. Samples from necropsied seropositive elk grouped by test result for *B. abortus*, and then sample type: lymph nodes, reproductive tract, organs, swabs, and other collected from 2016-2019, Montana, USA. Detection of *B. abortus* is indicated by solid circles: black for culture and red for PCR. 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).

While abortions are likely still the primary mode of transmission, *B. abortus* was detected at live births and this should be considered when making management decisions to reduce transmission risk. Detection of *B. abortus* via culture has long been the “gold-standard” for diagnosis, but PCR should be considered in conjunction with culture testing because it improves detection rates and helps address the problem of non-viable bacteria from environmental degradation. 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 active infections as evidenced by *B. abortus* in tissues. A manuscript detailing the methods and results of this study will be submitted to the Journal of Wildlife Diseases for consideration as a publication.

Next Steps

In 2023, we plan to capture 100 elk in the Pioneer Mountains (HD331; Figure 14) northwest of Dillon, MT. We sampled 100 elk from the southern and western portions of this area in 2013. The northeast portion of the Pioneer Mountains has never been sampled. The Pioneer Mountains are just outside the brucellosis DSA and movement data from Tendoy Mountains elk shows potential for interchange in the southern Pioneer Mountains.

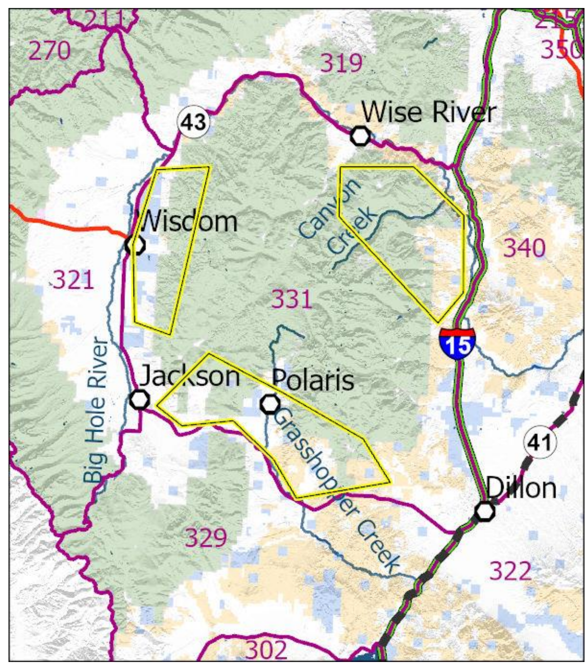


Figure 14. Planned sampling area (yellow & black polygons) for 2023 in the Pioneer Mountains near Dillon and Wisdom, MT.

The focus of next year's effort will be to 1) continue to document the spatial extent of the disease, and 2) to publish our results regarding pregnancy rate, pregnancy fate, shedding of *B. abortus* at birth sites, and retention of *B. abortus* in seropositive elk tissues.

Acknowledgements

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