Targeted Elk Brucellosis Surveillance Project
2016 Annual Report

Executive Summary

Montana Fish, Wildlife & Parks (MFWP) is conducting a multi-year targeted elk brucellosis surveillance project 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, and 3) evaluate the risk of seropositive elk shedding and potentially transmitting *Brucella abortus*. This report is an annual summary of the 2016 targeted elk brucellosis surveillance project. In January and February 2016, we sampled a total of 94 elk in 4 populations in the Big Timber and Red Lodge areas and screened blood serum for exposure to *B. abortus*. We found elk exposure to *B. abortus* in the area south of Red Lodge near the Wyoming border, but did not detect elk exposure to *B. abortus* in the Big Timber area elk populations. We collared a sample of elk in each study area and are currently collecting fine-scale elk movement information. To evaluate the risk of seropositive elk shedding *B. abortus* during abortion or birth events, we recaptured and assessed the pregnancy status of 24 seropositive elk originally captured and collared in southwest Montana elk populations during 2014 and 2015. We found that 12 of the 24 seropositive elk were pregnant. We outfitted these 12 pregnant elk with vaginal implant transmitters (VITs) to monitor birth events and sampled for *B. abortus* at birth sites. We identified and sampled 8 live birth events and *B. abortus* was not detected at any of the birth sites. We did not detect any abortion events. Three elk died prior to mid-April and 1 elk retained her VIT and no birth event was documented. Following 5 years of monitoring, we euthanized, necropsied, and sampled 3 seropositive elk to examine whether active *B. abortus* infections could be cultured. In addition, we sampled 4 elk that died during the winter for this purpose. We submitted tissue samples for culture testing and *B. abortus* was detected in 1 of these 7 elk.

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. 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.

In efforts to increase understanding of brucellosis in elk populations, MFWP initiated a targeted elk brucellosis surveillance project in the winter of 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, and 3) evaluate the risk of seropositive elk shedding and potentially transmitting *B. abortus*. In order to achieve these goals, MFWP has conducted intensive sampling efforts focused on 1 – 2 elk populations per year each year since 2011. Study areas are selected based on their proximity to the known distribution of brucellosis and/or significant livestock concerns. Surveillance areas are identified through collaborative discussions between MFWP, the Montana Department of Livestock (DoL), and landowners. Surveillance areas are both inside and outside of the State of Montana brucellosis designated surveillance area (DSA).

Study areas

Since 2011, we have sampled elk populations from 11 study areas (Figure 1). In January – February 2016, we sampled elk in 4 study areas in the Big Timber and Red Lodge areas. The 2016 study areas included Deer Creeks in hunting district (HD) 560, Greycliff and Work Creeks in HDs 560 and 575, Silver Run in HDs 575 and 520, and Border in HD 520.

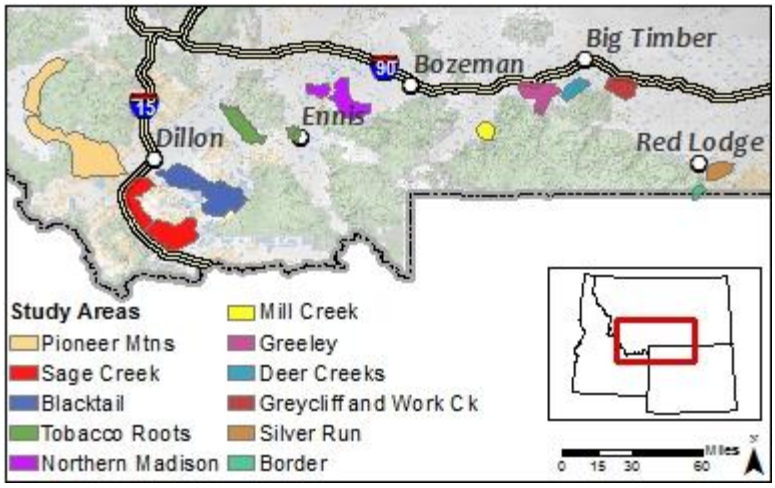

Figure 1. Study areas sampled during the 2011 – 2016 elk brucellosis surveillance project.

Methods

To evaluate if *B. abortus* was present in the Big Timber and Red Lodge elk populations, we captured elk via helicopter netgunning 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 DoL Diagnostic Lab (Diagnostic Lab). Samples were screened utilizing the Rapid Automated Presumptive (RAP) and Flourescence 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 random sample of elk in the Red Lodge and Big Timber areas in order to track movements and evaluate risk of brucellosis transmission to livestock and other elk populations. Collars collect a GPS location every 30 minutes or 2 hours. Each collar has a timed release mechanism that releases the collar after 52 – 72 weeks so that collars may be retrieved and location data downloaded. Elk are relocated in the field using telemetry equipment every 6 – 8 weeks throughout the year. Collars have a mortality sensor that detects if the collar is stationary for > 6 hours.

Additionally, we recaptured the 24 seropositive elk found and collared during 2011 – 2015 to screen them for exposure to *B. abortus.* The purpose of monitoring serostatus and birth events for 5 years is to understand the epidemiology of the disease post infection, and determine the level of risk associated with exposed elk through time. Retesting seropositive elk annually for exposure is to determine if elk experience antibody titer loss following exposure. 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 and lymph nodes need to be collected in order for *B. abortus* bacteria to be cultured. We remove seropositive elk from the population following 5 years of testing to determine if they are infected with brucellosis. In February 2016, we recaptured and euthanized the 3 remaining seropositive elk from the Blacktail herd that were sampled in 2011 – 2015. The Diagnostic Laboratory performed necropsies and collected extensive tissue samples (e.g., lymph nodes, organs) and submitted tissue samples to the National Veterinary Services Lab (NVSL) for culture testing. In addition, 2 seropositive elk from N. Madison and 2 seropositive elk from Mill Creek died and we opportunistically conducted full necropsies and tissue sampling.

At each of the 24 seropositive elk recapture events, we also assessed pregnancy status and outfitted pregnant elk with a VIT to track seropositive elk birth events. VITs are programmed to emit

a slow pulse when the temperature is 32° C or higher (i.e., inside the body), and emit a fast pulse once the temperature cools below 28° 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° C. We monitored the pulse rate and PET code to determine if an implant had been expelled and the timing of expulsion. In addition, we field tested new VIT technology with 2 elk, where the collar sends a birth alert via text/email to researchers when the VIT is expelled and cools down. To identify birth events, we tracked elk outfitted with VITs nearly every day from time of capture until the VITs were expelled.

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, 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 and can detect bacteria that is no longer viable (i.e., died from exposure before sampling). The PCR test is a new method of detecting *B. abortus* that was unavailable before 2015. Detection of *B. abortus* from any sample, via culture or PCR, led to the classification of detected for that event. We categorized each birth site as *B. abortus* detected or not detected based on culture and PCR results. We considered elk that gave 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 observed 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 observed. Unknown events were restricted to cases where the VIT was lost due to a malfunction (i.e., stopped transmitting) or when no birth event occurred and the elk retained the VIT.

Results

Brucellosis surveillance and elk movements

In January 2016, we sampled a total of 94 elk from 4 study areas in the Big Timber and Red Lodge areas (Table 1). We found that 6 of 94 elk tested positive for exposure to *B. abortus* (Table 1). We deployed collars on a total of 5 seropositive and 26 seronegative elk. Location data from these collared animals is limited to flights once every 1 – 2 months (Figure 2), and fine-scale location data will be available after February 2017 when the collars release and can be collected and downloaded. Collared elk from the Deer Creeks population (green asterisks, Figure 2) moved east and south onto the Custer Gallatin National Forest. Collared elk from the Greycliff Creek (purple crosses) and Work Creek (red triangles) populations stayed relatively close to their capture sites, with Greycliff Creek elk moving west and Work Creek elk moving south. Collared elk from the Silver Run population (orange circles) generally moved west of their capture sites, with some elk only moving a short distance and others moving 20+ miles onto the Custer Gallatin National Forest. Collared elk from the Border population (blue diamonds) primarily moved south into Wyoming, but two collared elk moved northeast towards Belfry, MT.

Table 1. The study areas where elk were screened for exposure to *B. abortus* during 2016, sample size of elk screened, number of elk testing positive for exposure, and the estimated seroprevalence with binomial confidence intervals.

Study Area	Hunting Districts	Sample Size	Number Seropositive	Estimated Seroprevalence
Deer Creeks	560	30	0	0 (0, 0.11)
Greycliff/Work Creeks	560, 575	32	0	0 (0, 0.11)
Silver Run	575, 520	19*	0	0 (0, 0.17)
Border	520	16	6	0.38 (0.18, 0.61)

*Includes 3 hunter-harvest samples

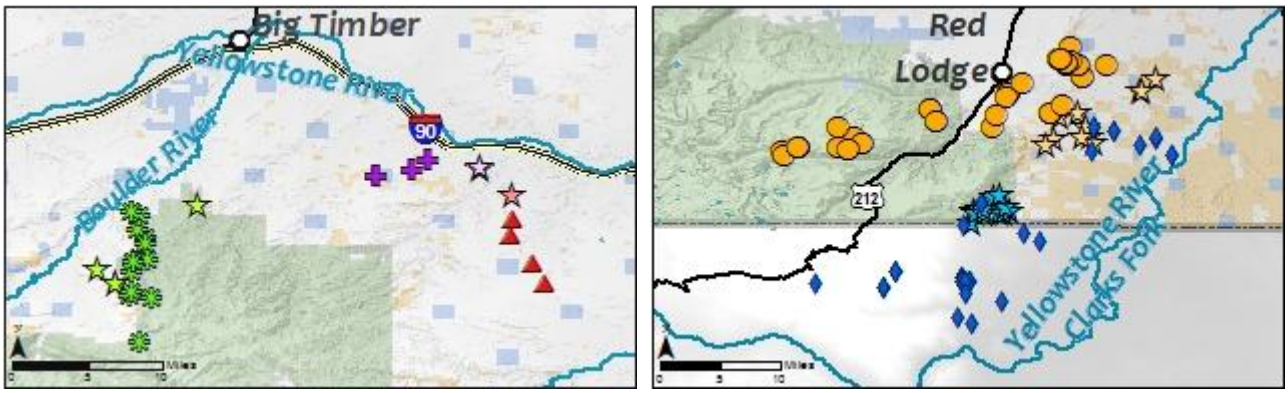

Figure 2. Flight locations of elk collared in January 2016 from Deer Creek (green asterisks), Greycliff Creek (purple crosses), Work Creek (red triangles), Silver Run (orange circles), and Border (blue diamonds). Capture locations are stars in a lighter shade of color for each study area.

Seropositive elk recapture and sampling

During January and February 2016, we recaptured 24 seropositive elk from Sage Creek (n = 1), Northern Madison (n = 7), Mill Creek (n = 14), and Greeley (n = 2; Figure 1). Pregnancy status was assessed and 12 pregnant elk received VITs. We monitored 9 seropositive elk pregnancies through the entire parturition season and documented 5 confirmed live births, 3 suspected live births, and 1 unknown (i.e., elk is still carrying the VIT and no birth event was detected; Table 2). The remaining 3 pregnant elk died prior to mid-April. The median number of hours for birth events to be detected was 1 hour (range 0 – 2 hr), and the median number of hours to investigate events was 4 hours (range 3 – 25 hr). Neither culture nor PCR tests detected *B. abortus* at any of these 8 birth sites.

Table 2. The total number of seropositive elk pregnancies monitored by study area during spring 2016, and the number and type of birth events documented. Elk that died prior to any birth event are not included.

Herd	Total Monitored	Abortion		Live Birth		Unknown
		Confirmed	Suspected	Confirmed	Suspected	
Sage Creek	0	0	0	0	0	0
N. Madison	2	0	0	1	1	0
Mill Creek	7	0	0	4	2	1
Greeley	0	0	0	0	0	0
TOTAL	**9**	**0**	**0**	**5**	**3**	**1**

Following euthanasia of the 3 Blacktail elk and opportunistic sampling of the 4 elk that died during winter 2016, *B. abortus* was detected in 1 lymph node of 1 elk (BF39). The annual serology results for these elk show that 2 of the Blacktail elk seroreverted (i.e., Positive to Negative, BT68 and BT83) and 1 of those elk seroconverted this past year (i.e., Negative to Positive, BT83; Table 3). From 2011 – 2015, we documented live births for these elk (Table 4). No abortions were documented for any of these elk. One Blacktail elk was never pregnant (BT55), but no physical abnormalities were detected during the necropsy.

Table 3. Annual serology results for all necropsied seropositive elk by individual and year, 2011 – 2016. *The only culture positive elk was BF39.

ElkID	Herd	2011	2012	2013	2014	2015	2016
BT55	Blacktail	Pos	Pos	Pos	Pos	Pos	Pos
BT68	Blacktail	Pos	Pos	Pos	Pos	Pos	Neg
BT83	Blacktail	Pos	Pos	Pos	Neg	Neg	Pos
BF02	Blacks Ford	---	---	---	Pos	Pos	Pos
BF39*	Blacks Ford	---	---	---	Pos	Pos	Pos
MC03	Mill Creek	---	---	---	---	Pos	Pos
MC07	Mill Creek	---	---	---	---	Pos	Pos

Table 4. Annual pregnancy and/or birth event results for all necropsied seropositive elk by individual and year, 2011 – 2016. *The only culture positive elk was BF39.

ElkID	Herd	2011	2012	2013	2014	2015	2016
BT55	Blacktail	Open	Open	Open	Open	Open	Open
BT68	Blacktail	Live Birth	Live Birth	Live Birth	Live Birth	Live Birth	Open
BT83	Blacktail	Live Birth	Live Birth	Live Birth	Live Birth	Live Birth	Open
BF02	Blacks Ford	---	---	---	Open	Live Birth	Preg
BF39*	Blacks Ford	---	---	---	Live Birth	Live Birth	Open
MC03	Mill Creek	---	---	---	---	Live Birth	Preg
MC07	Mill Creek	---	---	---	---	Live Birth	Preg

Movement

In January and February 2015 we deployed 23 collars in Mill Creek and 20 collars in Greeley. Collars collected a GPS location every 30 minutes or 2 hours. Those deployed on seronegative elk had a timed release mechanism that released the collar after 52 – 72 weeks. We traded out collars on seropositive elk when we recaptured them this past winter. We have recovered data from 20 collars from Mill Creek and 14 collars from Greeley. Two collared elk in Mill Creek died before May 2015 with limited movement data.

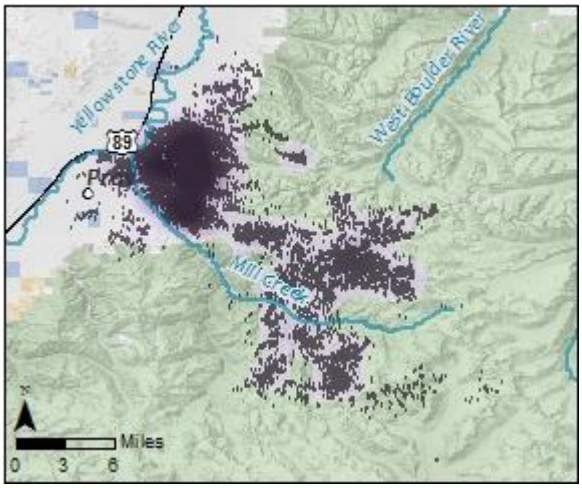

Figure 3. Locations and a 95% kernel utilization distribution (gray) of Mill Creek elk in 2015.

In general, Mill Creek elk winter in the foothills of the Absaroka Mountains between Mill Creek and Strawberry Ridge and summer at higher elevations to the east (Figure 3). Sixteen of the 21 collared elk migrated east up into the mountains and summered on Custer Gallatin Forest Service lands. Drainages utilized as summer range included Passage Creek, East Fork of Mill Creek, Upper Mill Creek, Lambert Creek, Anderson Ridge and McDonald Creek. One of these elk migrated 15 miles southeast and spent time in the headwaters of the Hellroaring drainage. The remaining 5 elk behaved as residents, spending the entire year in the foothills and winter range area.

Eleven elk captured near Mount Greeley wintered between Mount Greeley and I-90. During the winter of 2015 – 2016, 2 of these elk also moved west along the foothills towards Livingston (Figure 4). In the spring, 6 of the 11 collared elk migrated south up the West Boulder River, Davis Creek and to the Mount Rae area. The other 5 elk behaved as residents, spending the entire year between Mount Greely and I-90. Nine elk captured on Coal Mine Rim and McLeod Basin wintered in those two locations. During the winter of 2015 – 2016, 3 elk also spent part of the winter north of the West Boulder River, primarily to the east of Mount Greeley. In the spring, most elk moved short distances south and east, summering around Baker Mountain and Mount Rae immediately south of McLeod Basin and southeast of the confluence of the Boulder and East Boulder Rivers. Exceptions included 2 elk that migrated long distances south, one 23 miles up the Boulder River and the other 14 miles to the head of the East Boulder River.

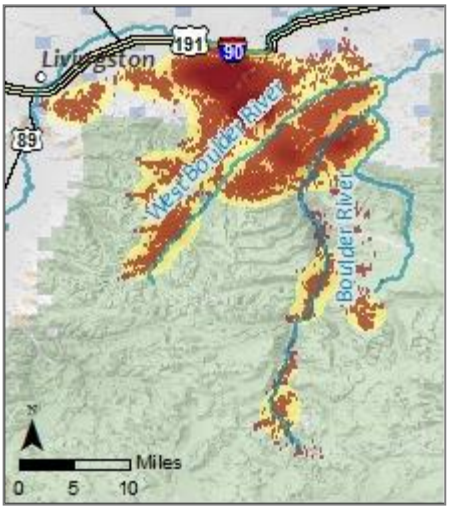

Figure 4. Locations and a 95% KUD (yellow) of Greeley elk in 2015.

While most elk from the Mount Greeley and Coal Mine Rim areas remain separated most of the year, we found two areas of overlap along the West Boulder River (Figure 5). The movement of 3 Coal Mine Rim elk north during the winter of 2015 – 2016 may have provided opportunity for mixing near Mount Greeley. In addition, 1 Mount Greeley elk summered around Mount Rae where Coal Mine Rim elk summered, and another Mount Greeley elk switched wintering grounds, spending the winter of 2015 – 2016 on Coal Mine Rim.

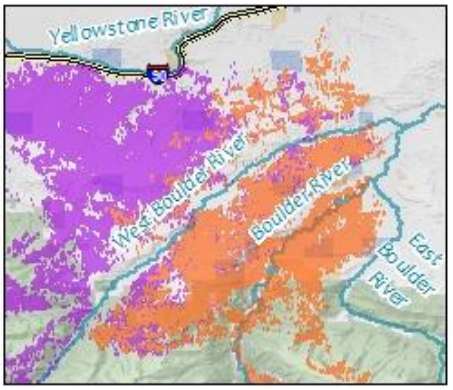

Figure 5. Locations showing overlap between elk originally captured north of Mount Greeley (purple) and elk captured in McLeod Basin and Coal Mine Rim (orange).

Discussion

Our targeted brucellosis surveillance efforts documented the presence of *B. abortus* in elk from the Montana – Wyoming border area south of Red Lodge. Our epidemiological results from 2016 are similar to results from 2011 – 2015 and suggest that only a small proportion of seropositive elk are shedding *B. abortus* bacteria and pose a risk for transmitting the disease to livestock or other elk. Pregnancy rates affect seropositive elk brucellosis transmission risk because elk are not always pregnant and able to have an abortion or live birth. Since 2011, the estimated pregnancy rate for seropositive elk is 62%, leaving nearly 40% of the seropositive elk not pregnant and thus posing no transmission risk in a given year. We have observed 3 abortion events out of a total of 56 (5.4%) known-fate birth events, and *B. abortus* was present at each of these 3 abortion sites. The abortion events occurred on March 30th, April 20th and May 14th. These dates fall within the riskiest time of year, in March through mid-May (Cross et al. 2015). Additionally, *B. abortus* was detected at 1 of 53 live birth events (2%) since 2011, suggesting that live births do pose a potential risk for transmission, although these cases are rare. The Wyoming Game and Fish Department (WGFD) have similarly detected *B. abortus* at 5 out of 118 (4%) live birth events attributed to seropositive elk (B. Scurlock, personal communication, August 2016). Although time to detection and sampling efforts did not differ between abortions and live birth events, female elk behavior during live birth events (i.e., consumption of birth material and vegetation) may remove some of the *B. abortus* shed at a live birth event. It should also be noted that predation of aborted fetuses or weak calves prior to site investigation may cause a misclassification of the birth event, but with our rapid response times we think this is highly unlikely.

Repeated annual serology sampling on seropositive elk revealed two cases of seroreversion, where the elk cleared *B. abortus* antibodies from their bloodstream and tested seronegative in subsequent years of sampling. Both elk were part of the Blacktail herd originally sampled in 2011 and both were estimated to be 8 years old in 2011. One elk seroreverted (i.e., Positive to Negative) on the final 2016 serology test. The other elk seroreverted in 2014, tested seronegative in 2015, and then tested seropositive again in 2016. Possible explanations for the seroconversion include re-exposure to *B. abortus*, causing a resurgence of antibodies, or false negative tests in 2014 and 2015, perhaps driven by antibody levels that were too low to detect. Culture testing from the necropsy in 2016 did not detect *B. abortus* in tissues collected from this individual. Research concerning seroreversion, or titer loss, is difficult because it requires long term, repeated sampling of individuals to monitor serostatus. Two seroreversions out of 36 seropositive elk sampled in multiple years represents 6% of the seropositive population. This represents a similar rate found by the WGFD who has been sampling elk at feedgrounds since the 1990's. Of the 839 feedground elk sampled at least twice, 38 (4.5%) seroreverted (B. Scurlock, unpublished data). In addition, the WGFD documented only 2 (0.2%) seroreversions followed by a seroconversion (Pos to Neg to Pos).

The necropsy sampling and culture testing of 7 seropositive elk resulted in *B. abortus* being detected in 1 lymph node of 1 elk. A total of 22 samples were collected from this individual, and *B. abortus* was only detected in 1 of the 22 samples, confirming that *B. abortus* is difficult to detect using culture. Thus the lack of *B. abortus* detections in other seropositive elk may either represent failed detection of *B. abortus,* or individuals that truly do not harbor *B. abortus*. Given the large number of samples collected and tested from these elk, however, at least some of the seropositive elk were likely not actively infected at the time of their death. It should be noted that this does not mean these elk posed no transmission risk over the previous 5 years. They could have been actively infected in previous years.

Data from GPS collars has improved our understanding of elk movement and potential routes for the spatial spread of brucellosis or other diseases between herds (Figure 6). Elk movements will be used to determine the timing and degree of spatial separation between elk and livestock in future focused analyses. While no overlap was detected between radiocollared elk from Mill Creek and Greeley, elk from both herds were on opposite sides of a ridgeline and within 1.2 and 1.7 miles (Figure 7). Transmission risk is extremely low in summer when these elk were in close proximity, but there is concern that a seropositive elk from Mill Creek (0.53 seroprevalence) might disperse and follow Greeley elk back to their winter range.

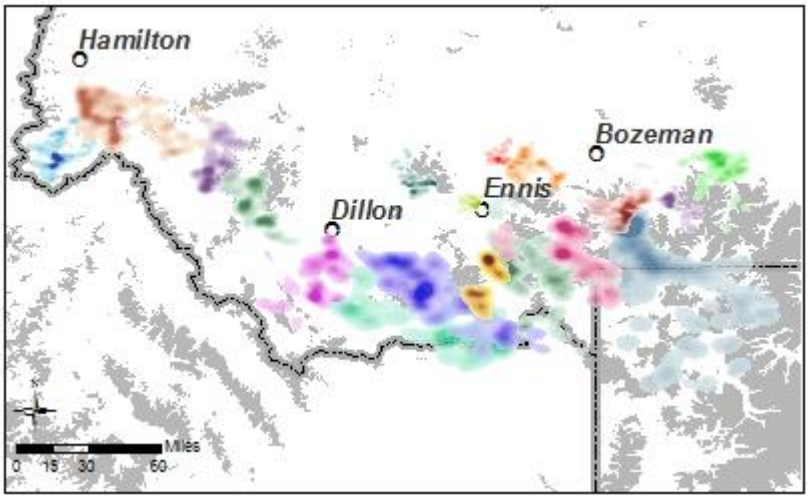

Figure 6. 95% KUDs of elk herds in SW Montana with GPS collar data showing the potential overlap and interchange between herds.

Over the next four years, we plan to continue the targeted brucellosis surveillance efforts in the areas north and east of the current DSA. The focus of the next 4 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 designed to affect elk distribution

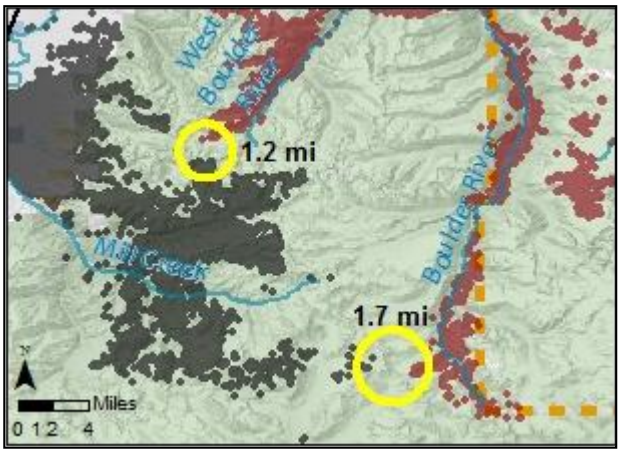

Figure 7. Locations of Mill Creek (green) and Greeley (red) elk coming within 2 miles of each other.

and elk-cattle spatial overlap at reducing transmission risk within the DSA. For seropositive elk captured prior to 2016, we will continue to monitor their serology, movement and birth events. After five years, seropositive elk will be euthanized and tissues cultured to determine if they are actively infected with brucellosis. Seropositive elk in the remaining areas will be euthanized in 2017 (Sage Creek), 2019 (N. Madison) 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 and livestock managers 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, population size, pregnancy rates, associated risk of shedding from abortions and live births, 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 and prioritized for management. 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 beginning in 2017 will aim to evaluate the effectiveness of management actions at reducing transmission risk. We plan to deploy collars and collect elk movement data in areas with 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.

Acknowledgements

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