



Montana Fish, Wildlife & Parks

2011-2012 Elk Brucellosis Survey and Research Summary

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Executive Summary:

In the winter of 2011-2012 Montana Fish, Wildlife and Parks initiated the second year of a multi-year project with the goals of delineating the geographical distribution of brucellosis in elk populations, enhancing our understanding of how brucellosis functions in elk populations and providing wildlife managers with information to inform elk management where brucellosis is present. Ninety-three adult female elk were captured in hunting district (HD) 325, and 30 adult female elk were captured in HD 329. Elk were initially tested for brucellosis in the field and again at the Montana Dept. of Livestock Diagnostic Laboratory at a later date. Elk that tested positive in the field were fitted with a GPS collar and, if pregnant, implanted with a vaginal implant transmitter (VIT). Seronegative elk were also collared bringing the total to 30 elk receiving GPS collars in HD 325. Five seropositive elk were detected in HD 325 and no elk tested positive for exposure to brucellosis in HD 329. Seven seropositive elk originally captured in the Blacktail/Sweetwater Hills (HDs 324 and 326) in 2010-2011 were recaptured, implanted with a VIT if pregnant, and tracked to collect samples at birth sites. Of the seven recaptured seropositive elk from HD's 324 and 326 and the five seropositive elk in HD 325, nine were pregnant and received VITs. Of those nine, one died from unknown causes, two had stillborn or aborted calves and the remainder carried their calves to full term, defined for this project as a birth event occurring May 15th or later with no evidence of an abortion event occurring. Samples collected from the birth site of the two stillborn/aborted calves were culture positive for Brucella abortus biovar 1. Samples from the remaining birth sites and the mortality were culture negative.

Brucellosis is a contagious disease caused by bacteria of the *Brucella* spp. Within the Greater Yellowstone Area (GYA), brucellosis, the result of infection with *Brucella abortus*, is known to exist in wild bison and elk and occasionally livestock. Brucellosis was first detected in

wildlife in the early 1900's and likely introduced into wildlife populations by contact with infected livestock. Eradication efforts have largely eliminated brucellosis in livestock within the contiguous United States leaving wildlife in the GYA as the last known brucellosis reservoir in the U.S. Recent livestock cases in the GYA have been linked to transmission from wildlife, with elk being the most likely source. Within Montana, surveillance efforts using blood tests to determine exposure rates (seroprevalence) to *B. abortus* began in the late 1980's. Seroprevalence estimates for GYA elk from the late 1980's and early 1990's were below 2%. Surveillance conducted within the last 10-15 years revealed what appeared to be increasing seroprevalence in some elk populations (Anderson and Williams 2008, Anderson et. al. 2009, Anderson et. al., 2010). Recent testing also detected brucellosis in elk populations where it had not previously been found. It is unclear if this is due to changes in the geographical distribution of the disease or increased sampling efforts in these areas. To date, brucellosis has only been detected in elk populations of southwestern Montana and the increase in brucellosis seroprevalence in some areas has not appeared to prevent elk population growth in the region.

Brucellosis is a concern and financial burden to livestock producers. The disease, which is transmitted primarily through contact with infected birth material, causes abortions in cattle. In 2007 Montana had its first case of brucellosis in cattle since gaining its brucellosis-free status in 1985. Montana lost its brucellosis-free status in 2008 when a second cattle case was detected and regained its class-free status in 2009. Changes in USDA-APHIS rules regarding brucellosis in livestock reduced the likelihood of entire states losing brucellosis-free status because of isolated livestock cases, but put increased focus on areas where brucellosis is known to exist in wildlife. As a result, the Montana Board of Livestock established a designated surveillance area (DSA) in 2010, which requires increased cattle testing and vaccination efforts by producers within the DSA (Montana Dept. of Livestock, 2010). Since 2007 there have been 5 cases of brucellosis in domestic livestock, three in cattle and two in domestic bison.

As a result of an apparent increase in seroprevalence in some areas, finding brucellosis in areas it had not previously been found, the impact brucellosis presence has on livestock producers, and reduced tolerance of elk by some landowners, MFWP initiated an enhanced brucellosis surveillance effort in 30 hunting districts (HD) within and adjacent to the GYA in 2008. The goal of the enhanced surveillance was to better delineate the geographical distribution of brucellosis in elk populations of southwestern Montana and improve estimates of seroprevalence where the disease was detected. Like historical efforts, the enhanced surveillance focused on collecting blood samples from hunter-harvested elk. Although the efforts were successful in several HDs within the surveillance area, sample sizes were inadequate in most HDs where the presence of brucellosis in elk was not well understood. The lack of data provided MFWP with little ability to determine brucellosis presence or absence with a high level of confidence, therefore limiting knowledge about the actual distribution of

brucellosis in elk (Anderson et al 2010). In order to address the lack of information, MFWP initiated a multi-year project in the winter of 2010-2011 with the objective of identifying the geographical distribution of brucellosis in elk populations, furthering our understanding of how brucellosis functions within elk populations, and providing managers with information to inform elk management in southwestern Montana. The project reduces reliance on samples from hunter-harvested elk and shifts focus to capturing and testing a sufficient number of elk in areas where brucellosis may exist in an elk population, but serological testing information is lacking.

Study sites and methods:

Study sites are chosen based on the following criteria: location relative to known brucellosis presence, known elk movements (in general), need for increased sample size to assess brucellosis presence, priority for livestock concerns, and availability of elk through presence of public land and/or adequate landowner cooperation. Within chosen study sites, approximately 100 adult female elk are captured and tested in the field for exposure to *Brucella* utilizing blood tests. In addition, all blood samples are submitted to the Department of Livestock Diagnostic Laboratory (Diagnostic Lab) for further testing. Final designation of a brucellosis reactor is based on standard serologic tests performed at the Diagnostic Lab. Elk testing positive for exposure to *Brucella* via field tests are fitted with a GPS collar and, if pregnant, implanted with a vaginal implant transmitter (VIT). Elk receiving VITs are tracked from time of capture in January or early February until parturition to collect and culture samples from birth/abortion sites. Elk giving birth May 15 or later are considered to have carried their calf to full term, unless evidence of an abortion event is detected at the birth site. Environmental samples, swabs of the VIT, and available tissue samples are collected from each birth site and submitted to the Diagnostic Lab for culture. If bacteria cultured from the samples are suspected to be *Brucella* spp. they are forwarded to the National Veterinary Services Laboratory (NVSL) for identification. Additional seronegative elk will receive GPS collars until a total of 30 elk have been collared. Seropositive elk receiving GPS collars are recaptured, retested and implanted with a VIT (if pregnant) annually for a total of five years, after which the elk will be collected (removed) from the population for further testing. The purpose of testing and monitoring seropositive elk over a five-year period is to provide information on brucellosis dynamics in elk that are directly related to the elk-cattle and elk-elk transmission risk, including information regarding pregnancy and abortion frequencies, possible recovery of elk following initial infections, and shedding of the bacteria by elk that have been exposed to the disease.

Blood samples from hunter-harvested elk are also used to augment surveillance efforts within a larger geographic area of southwestern Montana. Blood samples from elk captured for research purposes and not associated with this project are also tested to help evaluate

brucellosis presence or absence outside of the GYA. In the past two years, these captures have been limited to the Bitterroot elk project in HDs 250 and 270.

The brucellosis surveillance and research project is slated to occur in five areas within southwestern Montana, contingent on funding. MFWP is currently in the second year of the project, and 2011-2012 surveillance work was focused in HD 325, south of Dillon, MT (Figure 1). Additional surveillance activities in 2011-2012 occurred in HD 329 west of Dillon and south of Bannack (Figure 1). Elk were captured via net-gun fired from a helicopter. Captured elk were hobbled, blindfolded and delivered to a ground crew for processing. Blood samples were collected and tested for exposure to *Brucella* on site utilizing the Card test and the fluorescent polarization assay (FPA). The Card test was performed on all samples collect in both years of the project. The FPA test was added to the field testing protocol in 2011-2012 to improve our ability to identify possible reactors in the field. Due to difficulties and time constraints in performing the FPA in the field, not all samples were tested with this assay. Elk that tested positive on at least one field test were considered to be potentially exposed to *Brucella*. These elk received a GPS collar and were checked for pregnancy by rectal palpation or ultrasound. If pregnant they received a VIT. Additional seronegative elk were selected and collared, bringing the total number of GPS collared elk to 30 in HD 325. Elk were released from the handling site after field test results were obtained and telemetry devices were fitted.

Additionally, during the winter of 2010-2011, 100 adult cow elk were captured and tested on winter ranges within hunting districts 324 and 326 in the Blacktail Creek/Sweatwater Hills area (Figure 1). From that effort, eight seropositive elk were identified in the field and received GPS collars. Results from the 2010-2011 field efforts were reported in the "2010-2011 Elk Brucellosis Surveillance" report (Anderson et al. 2011). We attempted to recapture and retest the eight seropositive animals in the winter of 2011- 2012.

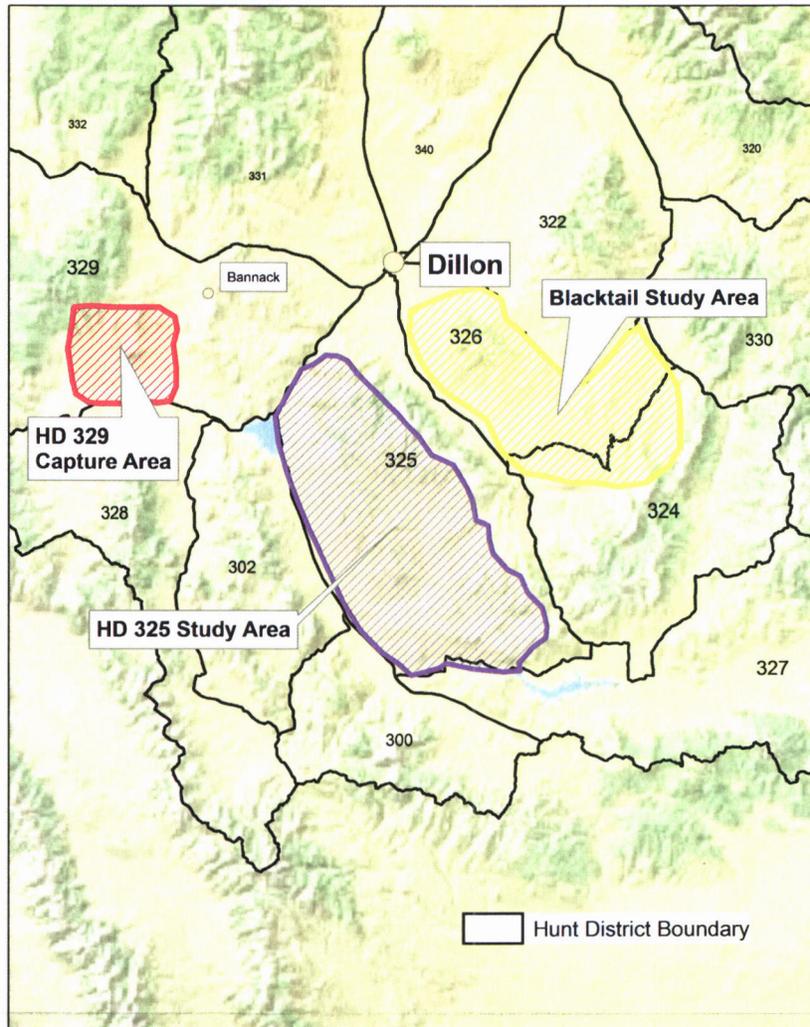


Figure 1. Study areas for the 2010-2011 and 2011-2012 brucellosis survey and research projects. Initial capture efforts in the Blacktail study area occurred in 2010-2011. Capture efforts for HDs 325 and 329 occurred in the winter of 2011-2012.

Results:

HD 325

Ninety-three adult female elk were captured in HD 325 via helicopter net-gun and delivered to ground crews for testing. Of the 93 elk captured, five died or were euthanized due to injuries associated with capture efforts. Examinations of the carcasses were conducted to evaluate general health and determine extent of injury. Blood samples were collected from the mortalities for testing at the Diagnostic Lab and meat from the five mortalities was donated to the food bank. An additional mortality occurred shortly after capture and is being attributed to

capture-related stress or injuries. Field testing of blood samples collected from captured elk indicated six potential brucellosis seropositives. However, one of the field positives was considered to be seronegative based on additional testing conducted at the Diagnostic Lab. Of the five remaining seropositives, four were considered to be pregnant and implanted with a VIT (Table 1). All seropositive elk were captured in the southeastern portion of HD 325.

The four seropositive and pregnant elk were located at least once weekly when possible, via fixed-wing aircraft and/or from the ground, and the status of the VIT was determined. All VITs were retained until late April when single abortion event occurred. The fetus and environmental samples were collected from the abortion site and submitted to the Diagnostic Laboratory for culture. Bacterial cultures from fetal tissues and a swab of the VIT suspect of being *Brucella* spp. were submitted to the National Veterinary Services Laboratory (NVSL) for identification. NVSL confirmed that *B. abortus* biovar 1 was present. The environmental samples associated with the abortion event were culture negative. The abortion occurred in the northern Centennial Valley and no cattle were present in the area at the time. The remaining three seropositive pregnant elk carried their calves to full term. *B. abortus* was not cultured from samples collected at their birth sites, and a live calf was observed at one birth site. All the birth sites occurred in the Centennial Valley, were found within two days of the birth event, and no cattle were not present in the general area. Information associated with seropositive elk captured in HD 325 is summarized in Table 2.

Recaptured seropositive elk from 2010-2011

One GPS radio collar failed and that individual could not be located for recapture. The remaining seven seropositive elk were recaptured. Blood samples were collected for *Brucella* testing at the Diagnostic Lab and their pregnancy status was evaluated. All seven elk remained seropositive for exposure to *Brucella*. Of the seven recaptured elk, five were pregnant and received a VIT (Table 2). All elk were relocated on average between 1-2 times per week from the air and/or ground to determine status of the VIT.

In mid April, a mortality signal from a GPS collar was received in a remote area of the Gravelly Mountains. A carcass was located several days later, but cause of death was inconclusive. Samples were collected and submitted to the Diagnostic Lab for testing. *B. abortus* was not cultured from submitted tissues. However, the carcass was in poor postmortem condition and had been scavenged, possibly influencing culture efforts. The VIT from another seropositive elk was expelled in mid May and a stillborn elk calf was found near the location of the expelled VIT. The calf carcass and birth site samples were collected and submitted to the Diagnostic Lab for culture. Bacterial isolates from environmental samples suspect of being *Brucella* spp. were submitted to NVSL for identification. Although tissues from the calf were culture negative, *B. abortus* biovar 1 was cultured from soil samples collected at

the birth site. The remaining five elk carried their calves to full term with two live calves being observed either at the birth site or at the cow's side. Samples collected at the birth sites for the remaining seropositive elk were culture negative for *Brucella*. No domestic livestock were observed near the abortion or birth site locations and all birth sites were located within the upper Ruby River drainage or the northern Centennial Valley. Additional birth/abortion site information is presented in Table 2.

HD 329

Thirty adult female elk were captured in HD 329 and delivered to ground crews for testing. No evidence of exposure to *Brucella* was detected in the elk. Seven elk were fitted with a VHF radio collar to provide the MFWP area biologist with general movement information for this herd, which is lacking.

Hunter-harvest samples

Eight hundred and fifty-six blood collection kits were mailed to hunters obtaining antlerless elk licenses in the Gravelly and Pioneer mountain ranges. Additional kits were handed out to hunters and landowners as requested. A total of 26 usable samples were obtained during the 2011-2012 hunting season. The samples were received from eleven different hunting districts: 300 (1), 311(8), 321 (2), 329 (3), 331 (5), 332 (1), 333 (1), 293 (2), 448 (1), 560 (1) and 621 (1). Three reactors were identified from the samples, all coming from HD 311, where brucellosis is already known to exist in elk. The remaining samples tested negative for exposure to *Brucella*.

Bitterroot Study

Over the last two years, 83 blood samples from adult female elk captured in HD 250 (n = 41) and HD 270 (n=43) as part of a research project in the Bitterroot Mountains were tested for exposure to brucellosis. All samples were considered to be negative for exposure to brucellosis (MFWP unpublished data).

GPS Collar Locations

GPS collars deployed on elk captured in HDs 324 and 326 in 2010-11 were recovered during the winter-spring of 2012, after dropping off in January 2012. Collars used on seropositive elk did not contain blow-off mechanisms and were retrieved when these animals were recaptured. All collars were programmed to obtain a location every two hours. A summary of the data obtained from the GPS collars will be presented in a separate report (Proffitt et al. 2012).

Table 1. Serology results from adult female elk captured in southwestern Montana and tested as part of a brucellosis surveillance and research project in the winters of 2010-2011 and 2011-2012. The Wilson's exact test was used to calculate 95% confidence intervals.

Location	Year	Sample Size	Field Positive	Lab Positive	Seroprevalence	95% Confidence Interval
Blacktail Study Area (HD's 324 & 326)	2010-2011	100	8	12	12.0%	7.0% – 19.8%
HD 325	2011-2012	93	6	5	5.4%	2.3% - 11.9%
HD 329	2011-2012	30	0	0	0%	0% -11.3%

Table 2. Pregnancy status (open indicates non-pregnant) and birth site culture results for seropositive elk captured in the Blacktail study area, originally captured in HD 324/326 in 2010-2011 and recaptured in 2011-2012, and elk captured in HD 325 in 2011-2012.

Elk ID #	Original Capture Location	2011 Pregnancy Status	2011 Birth Site Culture Results	2012 Pregnancy Status	2012 Birth Site Culture Results
BT10055	HD 324/326	Open	N/A	Open	N/A
BT10045	HD 324/326	Open	N/A	Pregnant	<i>B. abortus</i> (soil sample)
BT10068	HD 324/326	Pregnant	Negative	Pregnant	Negative
BT10075	HD 324/326	Open	N/A	Open	N/A
BT10058	HD 324/326	Pregnant	Negative	Pregnant	Negative
*BT10063	HD 324/326	Pregnant	Negative	Pregnant	*Negative
BT10083	HD 324/326	Pregnant	Negative	Pregnant	Negative
SC11097	HD 325	N/A	N/A	Pregnant	Negative
SC11050	HD 325	N/A	N/A	Pregnant	Negative
SC10087	HD 325	N/A	N/A	Pregnant	<i>B. abortus</i> (fetal tissue)
SC11031	HD 325	N/A	N/A	Pregnant	Negative
SC11045	HD 325	N/A	N/A	Open	N/A

*Mortality – culture results for samples collected from the remaining carcass and fetus.

Discussion

In 2010-2011, field testing for exposure to *Brucella* utilized a single serologic test, the Card test. Although considered to be a highly sensitive test, only eight out of 12 brucellosis reactors were identified in the field. In order to maximize field detection of brucellosis reactors, the FPA was added to the testing protocol. The FPA utilized a machine that requires a stable, heated environment and frequent calibration. At this time it is the only additional test that can be performed in the field. However, due to the requirements stated above, it could not be used to test all the samples collected. Environmental conditions in the field did not allow for consistent use of the assay. Conducting the FPA also adds several minutes to handling times, potentially allowing for increased animal temperatures and stress. Consequently, the FPA was performed on only 55 of the 93 samples collected in HD 325 and 9 of the 30 samples collected in HD 329. Despite its limited use, field application of the FPA identified two seropositive elk (as determined by the Diagnostic Lab) that the Card test would have missed. One elk that was identified as being a potential positive on the Card test and in the suspect range on the FPA was later identified as being seronegative by tests performed at the Diagnostic Lab. Overall, the ability of field crews to detect potential reactors was improved from the 2010-2011 season. Developing a protocol for the FPA that reduces the testing time would be beneficial for field application. The possibility of modifying the existing FPA protocol to reduce testing time will be investigated prior to future captures.

The greatest risk of brucellosis transmission occurs during the third trimester of pregnancy, which extends from mid January through parturition in mid June when elk are on winter range or calving grounds. Interpreting transmission risk when utilizing samples from hunter-harvested elk collected in the fall is difficult due to our limited understanding of where individual elk typically winter and calve. A cow elk may not winter and calve in the same area from which it was harvested. Cross et.al. (2010) noted a nearly two-fold difference in seroprevalence within hunting units in Wyoming when comparing results from hunter-harvested samples to results from samples collected from research animals later in the winter. As noted by Proffitt et al. 2012, many of the radio collared elk in this project had not migrated to winter ranges by the end of the fall hunting season. Although additional hunter-harvested samples were collected in 2012, due to the small number of usable samples and difficulty in assessing where harvested elk winter, seroprevalence estimates reported here were based solely on captured animals.

Prior to this project, no evidence of brucellosis exposure had been detected in elk captured or harvested in HD 325 (n = 92; MFWP, unpublished data) and a seroprevalence of 0.44% was noted from elk captured in the Gravelly-Snowcrest Mountains from 1984-1995 (Hamlin and Ross 2002). The findings of this project suggest that seroprevalence is now

approximately 12% and 5.4% in the Gravelly-Snowcrest Mountains and HD 325, respectively. The cause for this increase is unknown, but increased elk density on winter range may play a role (Cross et al. 2010). Finding brucellosis in elk herds occupying the two study areas was not surprising given the potential overlap they have with other Gravelly Mountains elk. Also, cow elk movements from areas known to have elk exposed to *Brucella* (such as HD 323) to HDs 324, 325, and 326 during the transmission risk period have been previously documented (Hamlin and Ross 2002). One of the seropositive elk originally captured in the Blacktail study area in 2010-2011 was recaptured on the Wall Creek Wildlife Management Area (HD 323) in the eastern Gravelly Mountains in 2012, further indicating how elk and potentially brucellosis might move between elk populations. The finding of seropositive elk in both the 2010-2011 and 2011-2012 study sites resulted in changes to the boundaries of the Montana Department of Livestock DSA.

VITs are used to improve a researcher's ability to determine when a birth or abortion event has happened and locate the site. During the spring of 2012 field crews were able to locate all but one VIT within 2 days of the device being expelled. On at least two occasions a live calf was observed near the VIT suggesting the calf had been born recently. One VIT was located within 5 days of the being expelled. Of the nine seropositive pregnant elk being tracked, one died from undetermined causes, two had stillborn calves, and the remaining elk carried to full term. In both abortion/stillbirth cases, the elk had migrated to calving grounds in the upper Ruby River drainage or Centennial Valley prior to losing their calves. *B. abortus* was cultured from tissue or environmental samples in both cases. *B. abortus* was not cultured from birth sites where calves were carried to full term and no evidence of an abortion or stillbirth was evident for any of the full-term elk. However, caution should be taken when interpreting culture negative data as the bacteria may have been present but died prior to collection or could not be grown for some reason. The environmental conditions and the length of time from the VIT being expelled to sample collection can have a significant impact on the survival of the bacteria in the environment (Aune et al. 2012). The time between the VIT being expelled and samples being collected was greatly reduced in 2012, partially due to better weather conditions and elk staging in less remote and rugged country. No livestock were observed within the vicinity of abortion or birth sites suggesting that the potential of elk-livestock transmission was limited.

Elk captured in 2010-2011 will be recaptured and retested three more times before they are collected (removed) from the populations. Elk captured in 2011-2012 will be recaptured four additional times, and then collected from the population. Tissues from these elk will be cultured for *Brucella* bacteria, providing information on the relationship between exposure status and actual brucellosis infections. Areas being considered for future surveillance and

research efforts include the southern Pioneer Mountains and the southern Tobacco Root Mountains.

Acknowledgements

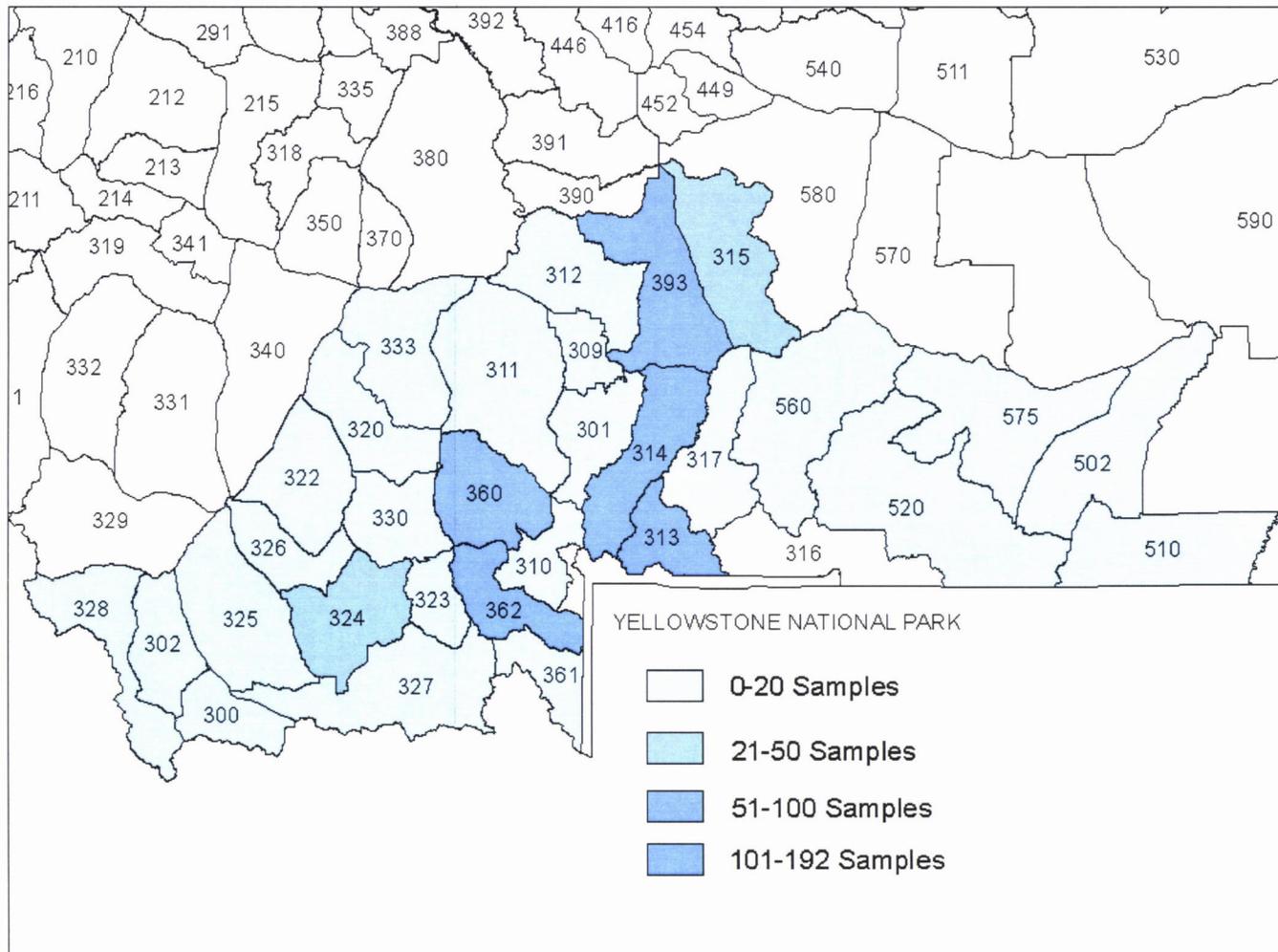
We would like to thank the landowners and sportsmen of southwestern Montana for supporting this project. Without landowner cooperation this project would not be possible. Funding for the project was supplied by USDA-APHIS and MFWP. We would also like to thank the MFWP area biologist and wardens for their efforts in helping with landowner contacts, capture and field operations, and continued support of the project. Ryan Clarke and Brent Thompson provided valuable training in performing the FPA and in the use of an ultrasound to evaluate pregnancy status. A special thanks to MFWP technicians, Julee Shamhart and Torrey Ritter, for vigilant tracking of elk during the spring which often required working in extreme weather conditions.

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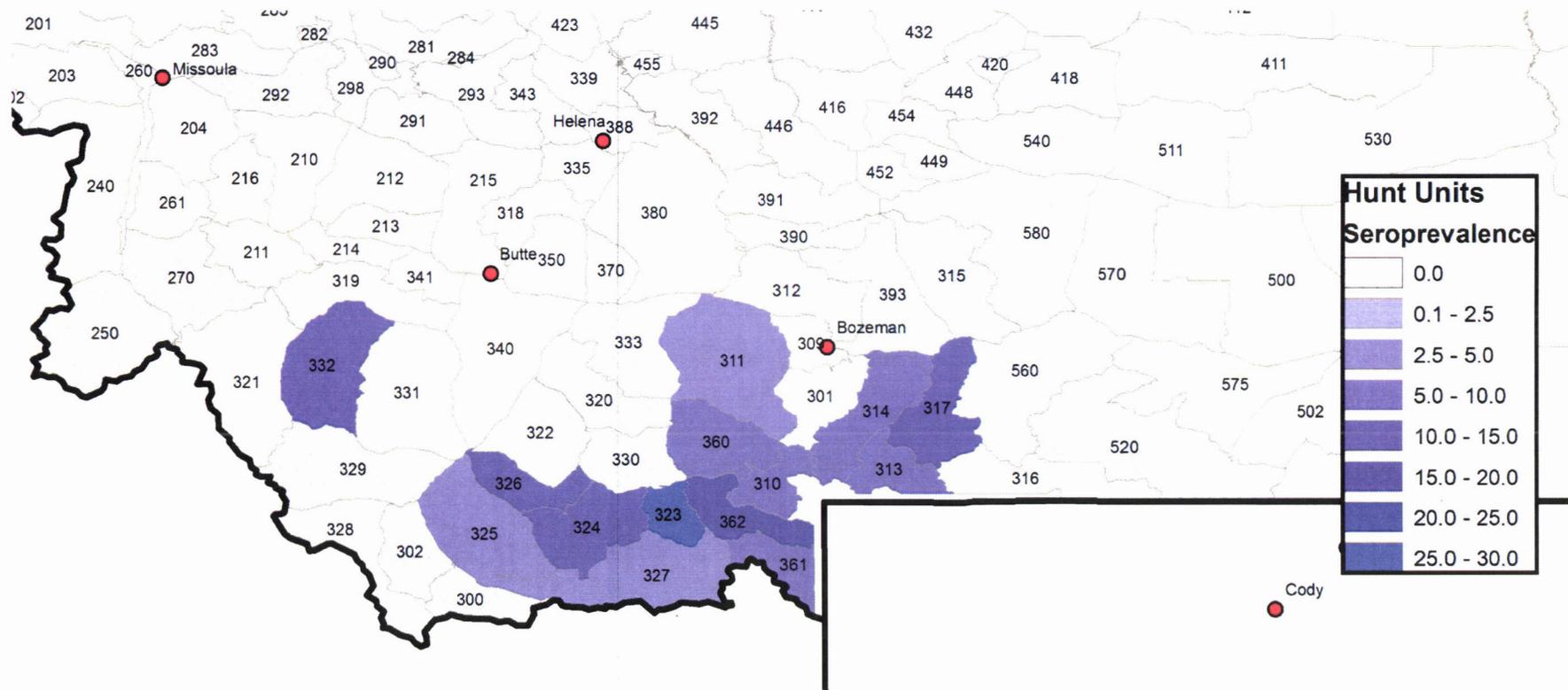
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Map 1. Sample sizes from the 2008-2010 enhanced survey efforts to detect *Brucella* exposure in elk, by hunting district. Samples were collected primarily from hunter-harvested elk although research animals are included in hunting districts 313 and 314.



Map 2. Estimated seroprevalence for Montana elk populations, 2001-2011. Only hunting districts with at least 20 samples collected from 2001-2011 are included.