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Montana Fish, Wildlife & Parks

2008 Elk Brucellosis Surveillance Final Report



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Executive Summary:

Hunters returned 1288 blood samples from harvested elk for brucellosis testing during the 2008-09 surveillance period. Of these, 835 (64.8%) were considered suitable for testing. Forty-five elk captured for research in the Paradise Valley and two elk removed from the Gardiner area were also tested for brucellosis and included in the surveillance effort. A total of 882 useable samples were obtained and tested for exposure to Brucella abortus. The Rivanol, Standard Plate and Fluorescence Polarization tests were used to screen serum samples for possible exposure to brucellosis. Positive or suspect samples identified by the serologic screen were submitted to Louisiana State University and retested using a western blot assay to assess the potential for a cross-reaction with Yersinia enterocolitica resulting in false positives. The serologic screen identified 62 samples as being either suspect (5) or positive (57) for possible brucellosis exposure. Of the 62 potential positives, the western blot assay or culture results identified 12 as being positive for exposure to B. abortus and 49 as being cross-reactors to Yersinia enterocolitica. One sample from hunting district 313 tested positive for brucellosis on standard serology, but was not tested using western blot. This sample was considered to be positive for brucellosis exposure based solely on the serologic screen. In total, 13 of the 882 samples were identified as being brucellosis seropositive. Although western blot was used as a definitive test determining brucellosis exposure, readers are cautioned that none of the tests used are 100% accurate.

Tissue samples from 85 elk harvested during the general hunting season, 96 elk harvested during late and management hunts and two elk identified as being seropositive in 2008 and removed from the population in January 2009 were sent to the National Veterinary Services Laboratory for culture. B. abortus, biovar 1 isolates were found in tissues from four adult females harvested in HD 313 during the late hunt, two female elk harvested during the management hunt in the Madison Valley (HD's 360 and 362) and one adult male harvested during the general season in HD 324. Both elk removed from the population after initially testing positive on serologic tests and western blot assay in 2008 were culture negative.

Introduction

Brucellosis was detected in two Montana cattle herds, one in 2007 and the second in 2008, resulting in the loss of the state's brucellosis free status for the cattle industry. Both of the cattle herds were eliminated and testing of cattle herds associated with the brucellosis positive herds revealed no additional exposures. Based on a lack of evidence of brucellosis in associated cattle herds, the absence of bison migrating from Yellowstone National Park (YNP) and genetic information suggesting similarities between the cattle isolates and bison and elk isolates, the Montana Department of Livestock concluded that elk from the Greater Yellowstone Area (GYA) were the most likely source of infection (Montana Department of Livestock 2008).

As a result of concern over the potential for brucellosis transmission from elk to cattle and interest in determining the geographic distribution of brucellosis in elk

populations, Montana Fish, Wildlife and Parks (MFWP) initiated a large-scale surveillance project in southwestern Montana. Surveillance focused on areas surrounding YNP and near the Idaho border where brucellosis has been detected in free-ranging elk populations. Previous surveillance within Montana was concentrated in areas within the Madison and southern Paradise Valleys where brucellosis is known to exist. Existing data on elk populations adjacent to where brucellosis has been identified within the GYA was insufficient to evaluate the geographic area occupied by elk exposed to brucellosis. The goal of the 2008-09 surveillance effort was to enhance FWP’s understanding of the geographic distribution of brucellosis in elk, determine seroprevalence within a reasonable level of statistical certainty, and provide direction for future surveillance efforts.

Survey Area

The area surveyed consisted of 30 hunting districts within MFWP administrative regions 3 and 5 of southern and southwestern Montana (Figure 1). Hunting districts were selected based primarily on their proximity to YNP, the elk feedgrounds in Wyoming and areas in Idaho where brucellosis has been detected in free-ranging elk populations.

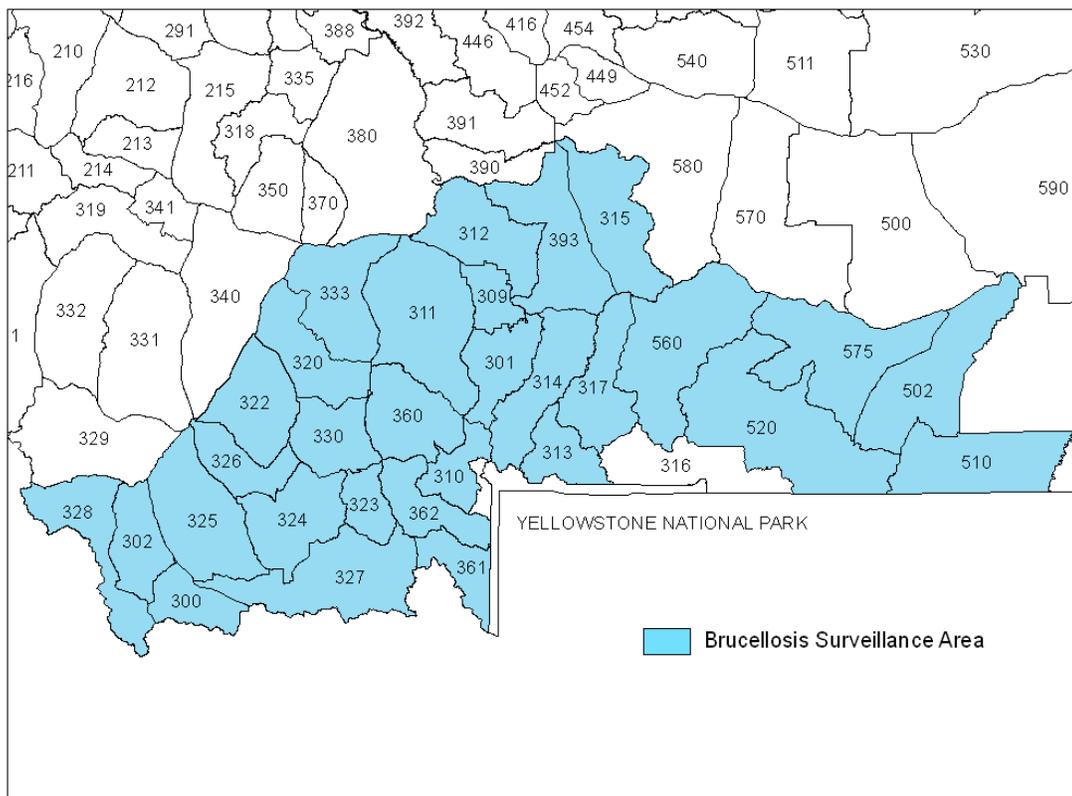


Figure 1. The 2008-09 elk brucellosis surveillance area.

Methods

Blood collection kits (kits) containing a syringe, a plastic conical vial, latex gloves, a pencil, directions for collecting a blood sample and a data card were mailed to 2900 elk permit and antlerless elk license holders within the surveillance area. Kiosks containing kits were placed along roads frequently traveled by hunters. Several area businesses and cooperating agencies handed out kits to hunters or allowed kiosks to be placed nearby where hunters could access them during the hunting season. Prior to the start of general season wildlife laboratory personnel attended several Farm Bureau, Stock Growers and other landowner meetings and asked landowners offering elk hunting opportunities to hand out kits to hunters utilizing their property. Kits were also made available at the game check stations in southwestern Montana, handed out at regional offices near the surveillance area and were disseminated to hunters in the field by wildlife laboratory staff, game wardens, block management staff and area biologists. A hunt coordinator, hired to direct hunters to accessible private property during the Madison Valley management hunt, handed out blood collection kits to hunters partaking in the hunt. Kits were also provided to hunters at the required check-in for the late hunt near Gardiner. Successful hunters could either mail blood samples to the wildlife laboratory using the enclosed, postage-paid envelope or drop the sample off at various drop locations or game check stations within the survey area. Hunters returning blood samples were entered into a drawing for various prizes as an incentive to improve participation in the survey.

Retropharyngeal lymph nodes and, when available, supramammary lymph nodes were collected from harvested elk at game check stations and game processors in southwestern Montana during the general hunting season. Wildlife laboratory staff also collected tissues from hunter-harvested elk during the late hunt near Gardiner and the management hunt in the Madison Valley by backtracking to the site where the animal was field dressed. Tissues were collected from elk carcasses at the game check station during the Gardiner late hunt as well. During the late and management hunts, collected tissues consisted of retropharyngeal lymph nodes, supramammary lymph nodes, reproductive tracts from non-pregnant cow elk and amniotic fluid and cotyledons from pregnant cow elk. Not all tissues were available for collection dependant on scavenging activities and methods hunters used to field dress harvested animals. Tissue and blood samples were numbered and the location of kill, age class, sex and hunter name were recorded for each sample.

Two additional blood and tissue samples were obtained from elk previously captured as part of a research project near Gardiner, MT. The adult female elk were captured in February 2008 and determined to be seropositive for brucellosis both on standard serologic screens and the western blot assay. The elk moved back into YNP in the spring of 2008. The two seropositive elk were removed from the population in January of 2009 due to concerns of potential commingling with cattle after they migrated back out of YNP during the winter. Blood was collected and retested for brucellosis and tissue samples were submitted for culture. Samples from these animals were included in the analysis of the 2008 data.

Wildlife laboratory staff collected blood samples submitted by hunters and evaluated the quality of the sample to determine if it was suitable for submission. Serum from all suitable blood was submitted to the Montana Department of Livestock, Diagnostic Laboratory (MDLDL) and screened for possible exposure to *B. abortus* using standard serologic tests consisting of Standard Plate, Rivanol and Fluorescent Polarization tests. A sample was considered potentially suspect or positive if there was a reactor on any of the screening tests. Serum from positive or suspect samples were then submitted to Louisiana State University for additional testing using the western blot assay. The western blot was used to evaluate if potential cross-reactions to *Yersinia enterocolitica* had occurred on the serologic screen. Tissues collected during the general, late and management hunts were submitted to the National Services Veterinary Laboratory (NVSL) in Ames, Iowa for culture.

Serum samples were considered to be positive for exposure to brucellosis if western blot results indicated exposure to brucellosis had occurred or if *B. abortus* was cultured from tissues collected from the same individual. If western blot results indicated that a cross-reaction from *Y. enterocolitica* (*Yersinia*) had occurred, and if culture results from matching tissues were negative or unavailable the sample was identified as being negative for exposure to brucellosis.

For the purpose of evaluating serologic data adult (≥ 1 year of age) males, adult (≥ 1 year of age) females and calves (≤ 1 year of age) were evaluated separately due to potential differences in seroprevalence based on gender and age class. Adult females were the focus of the surveillance effort, as they are the most likely group to pose a threat of brucellosis transmission to other elk or cattle. Focusing surveillance efforts on adult females also will allow for comparison to prior surveillance efforts and for comparison to surveillance conducted in Idaho and Wyoming. Data obtained during the 2008-09 survey was evaluated at the hunting district level for adult males and calves and at the hunting district and elk population level for adult females. Elk populations were delineated on a map and based on information provided by MFWP area biologists on elk winter range use and movement patterns. Delineation of a population does not suggest that there is no movement in or out of that population, only that the majority of elk present are likely to utilize the designated area in the fall and winter. Estimated elk numbers reported in each population unit were based on 2008 flight observations. A sample was assigned to a population based on the reported location of harvest or capture.

Seroprevalence was reported for all areas where samples were collected. Binomial confidence intervals for seroprevalence rates were calculated using a 0.05 type error rate (binom.logit in program R). For the type of data collected in this survey, the 95% confidence bounds (i.e. the upper and lower limits), if applied to the new data repeatedly, would contain the true value 95% of the time. The confidence intervals do not address the value or accuracy of the 2008 sample, but suggest that if we were to conduct similar surveillance efforts 100 times we would expect the true seroprevalence to be within the bounds of the confidence interval 95 times. Confidence intervals do not suggest anything about the probability of the true brucellosis seroprevalence rate in 2008.

Instead they provide a standard measure of the precision in our knowledge of the seroprevalence rate in 2008 that can be compared to future results.

Results

Serology

A total of 1288 samples were obtained from hunter-harvested elk during the 2008 survey period. Thirty of the samples were returned from areas outside of the surveillance area, and the hunting district was not reported and could not be determined for three samples resulting in 1255 hunter-harvested samples being collected within the survey area. An estimated 8554 elk (ranging from 7742 to 9367 at an 80% Confidence Interval) were harvested from the survey area during the 2008-09 general, extended and management seasons (MFWP unpublished data 2009), indicating that approximately 14.7% of the successful hunters within the survey area participated in the surveillance program.

Eight hundred and thirty-five (64.8%) of the hunter-harvested samples were considered suitable and were submitted to MDLMD for testing using the serologic screening tests indicated above. An additional 45 samples were collected from adult female elk captured for research purposes in hunting district 314 in the Paradise Valley. The two samples obtained from the research animals removed from the population in January 2009 were also submitted for testing using the serologic screen. In total, 882 samples were submitted for testing. Sample sizes varied greatly by hunting district (Figure 2). Of those samples considered suitable for testing, but collected outside of the surveillance area, 19 were from hunting districts 321 (n = 3), 329 (n = 7), 331 (n = 1), 332 (n = 1), 340 (n = 3), 370 (n = 1), 380 (n = 1), 417 (n = 1) and 580 (n = 1). The gender or age class was not reported for an additional 37 individuals. All 56 of the samples from outside of the surveillance area or with insufficient age or gender information were considered to be negative for exposure to brucellosis based on serology or western blot assays, but were excluded from subsequent analysis.

A total 826 samples having associated gender and age information were collected from hunting districts within the 2008-09 elk brucellosis surveillance area. Adult females, adult males and calves comprised 602, 130 and 94 of the samples, respectively. No calves were considered positive or suspect for exposure to brucellosis based on the serology screen. Table 1 contains the hunting district of harvests for calves tested during the 2008 surveillance period.

Adult Females

Fifty-two (8.6%) of the 602 adult female elk tested were considered to be either positive or suspect on standard serologic tests. Of the 52, 12 (23.1%) were identified as being exposed to brucellosis based on western blot and/or tissue culture. Positive samples were identified in hunting districts 313, 314, 360 and 362. Seroprevalence estimates ranged from 0 in most hunting districts to 6.0% in HD 313. Sample sizes in the

majority of hunting districts surveyed were not adequate for precise estimation of prevalence, as indicated by the large confidence interval (Table 2). Sample sizes ranged from 0 to 142, with the majority of hunting districts having fewer than 10 samples from adult female elk.

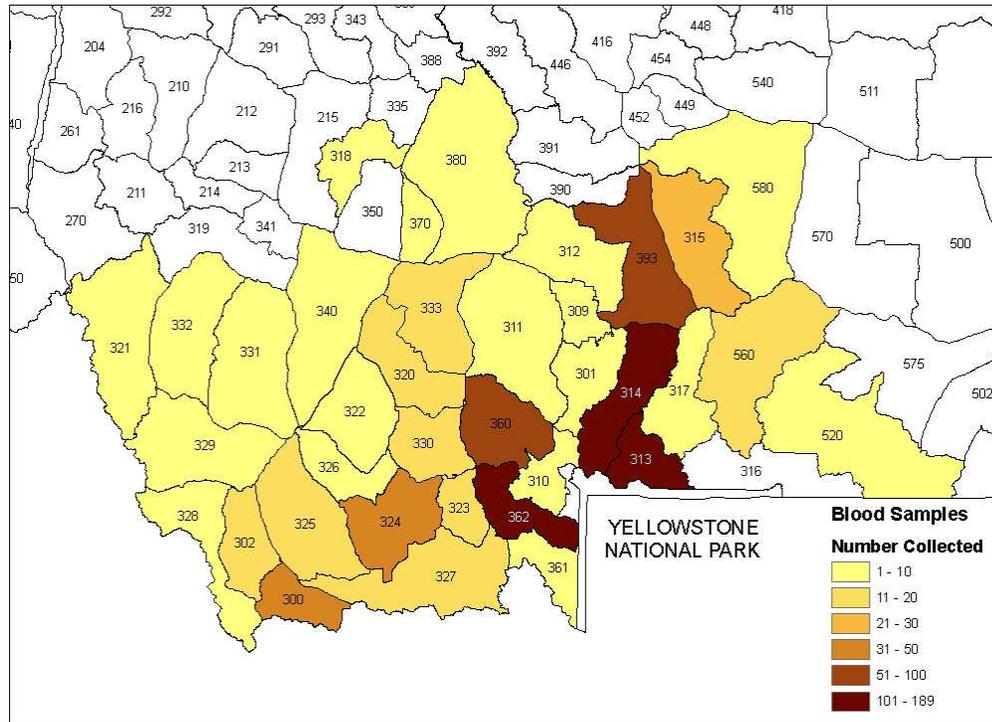


Figure 2. The total number of samples (cows, calves and bulls) obtained for hunting districts where useable blood samples were received during the 2008-09 elk brucellosis surveillance effort.

Table 1. Hunting district of elk calves tested during the 2008-09 elk brucellosis survey period. All samples were collected from hunter-harvested animals and were considered to be negative for exposure to *B. abortus* based on serologic tests.

| Hunting District | Calves tested | Hunting District | Calves tested |
|------------------|---------------|------------------|---------------|
| 300 | 5 | 327 | 1 |
| 302 | 2 | 330 | 5 |
| 313 | 8 | 333 | 3 |
| 314 | 15 | 360 | 15 |
| 315 | 3 | 361 | 1 |
| 320 | 2 | 362 | 16 |
| 323 | 1 | 393 | 6 |
| 324 | 1 | 520 | 1 |
| 325 | 6 | 560 | 2 |
| 326 | 1 | Total | 94 |

Table 2. Results, by hunting district, from adult female elk blood samples tested for exposure to brucellosis during the 2008-09 survey. Only districts within the designated surveillance area are presented. No samples from adult female elk were obtained in hunting districts 310, 502, 510 and 575.

| HD | Adult Female Sample Size | # Positive or suspect on serologic screen | Seropositive based on western blot and/or associated tissue culture | Seroprevalence (95% Confidence Interval) |
|--------------|--------------------------|---|---|--|
| 300 | 20 | 0 | 0 | 0 (0-16.8) |
| 301 | 1 | 0 | 0 | 0 (0-97.5) |
| 302 | 9 | 0 | 0 | 0 (0-33.6) |
| 309 | 1 | 0 | 0 | 0 (0-97.5) |
| 311 | 6 | 0 | 0 | 0 (0-45.9) |
| 312 | 2 | 0 | 0 | 0 (0-84.2) |
| 313 | 67 | 11 | 4 | 6.0% (2.3-14.9) |
| 314 | 142 | 9 | 2 | 1.4% (0.4-5.5) |
| 315 | 17 | 0 | 0 | 0 (0-19.5) |
| 317 | 5 | 1 | 0 | 0 (0-52.2) |
| 320 | 11 | 0 | 0 | 0 (0-28.5) |
| 322 | 8 | 0 | 0 | 0 (0-36.9) |
| 323 | 10 | 3 | 0 | 0 (0-30.8) |
| 324 | 21 | 3 | 0 | 0 (0-16.1) |
| 325 | 6 | 0 | 0 | 0 (0-45.9) |
| 326 | 2 | 0 | 0 | 0 (0-84.2) |
| 327 | 15 | 1 | 0 | 0 (0-21.8) |
| 328 | 6 | 0 | 0 | 0 (0-45.9) |
| 330 | 11 | 0 | 0 | 0 (0-28.5) |
| 333 | 8 | 0 | 0 | 0 (0-36.9) |
| 360 | 74 | 7 | 2 | 2.7% (0.7-10.2) |
| 361 | 4 | 0 | 0 | 0 (0-60.2) |
| 362 | 94 | 17 | 4 | 4.3% (1.6-10.8) |
| 393 | 48 | 0 | 0 | 0 (0- 7.4) |
| 520 | 5 | 0 | 0 | 0 (0-52.2) |
| 560 | 9 | 0 | 0 | 0 (0-33.6) |
| Total | 602 | 52 | 12 | 2.0% |

Samples obtained within the survey area were further evaluated at the elk herd unit level. Figures 3 and 4 contain a graphical representation of the herd units as described by MFWP area biologists, the estimated number of adult female elk in the unit and the number of adult female samples tested for exposure to brucellosis. Exposure to brucellosis was detected in adult female elk from six herd units: East of Ennis, North

Yellowstone-North Area, North Yellowstone-South Area, South of Big Creek, Sun Ranch Area, and Tom Miner Basin. Sample sizes ranged from 0 to 100 for individual herd units (Figures 3 and 4) and in most units were insufficient to determine seroprevalence for exposure to brucellosis. A summary of data for herd units having five or more samples is presented in Table 3. The herd unit could not be identified for nine of the adult female blood samples, all of which were considered to be negative for exposure to brucellosis based on the serologic screen. One of the two samples from the North Mill Creek herd unit tested positive on the serologic screen but was identified as being a cross-reactor to *Yersinia* on western blot.

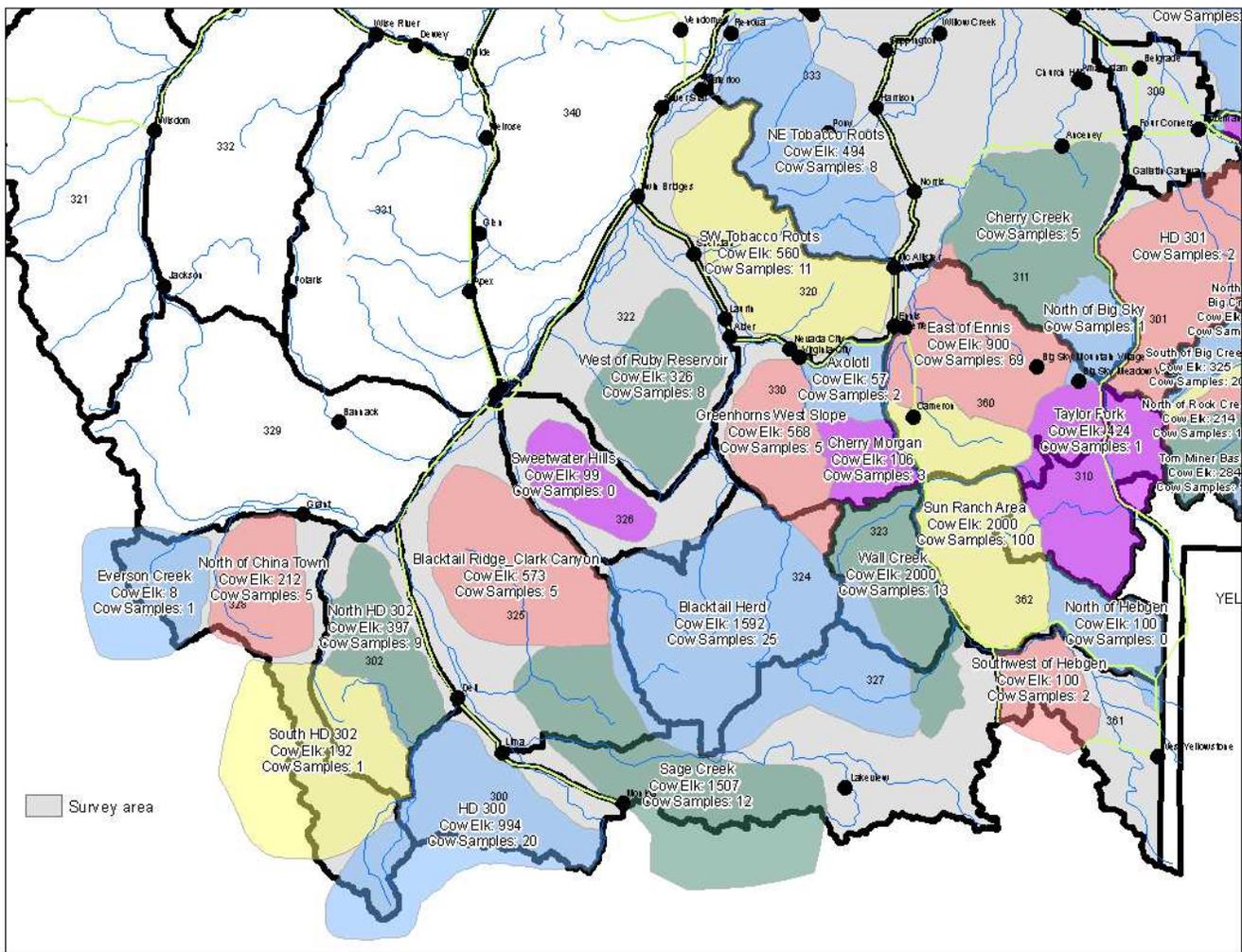


Figure 3. The western half of the 2008-09 survey area. The shaded areas depict different herd units as described by MFWP area biologists. The estimated number of adult female (cow) elk present within the herd unit and the number of useable samples obtained are listed below the unit name.

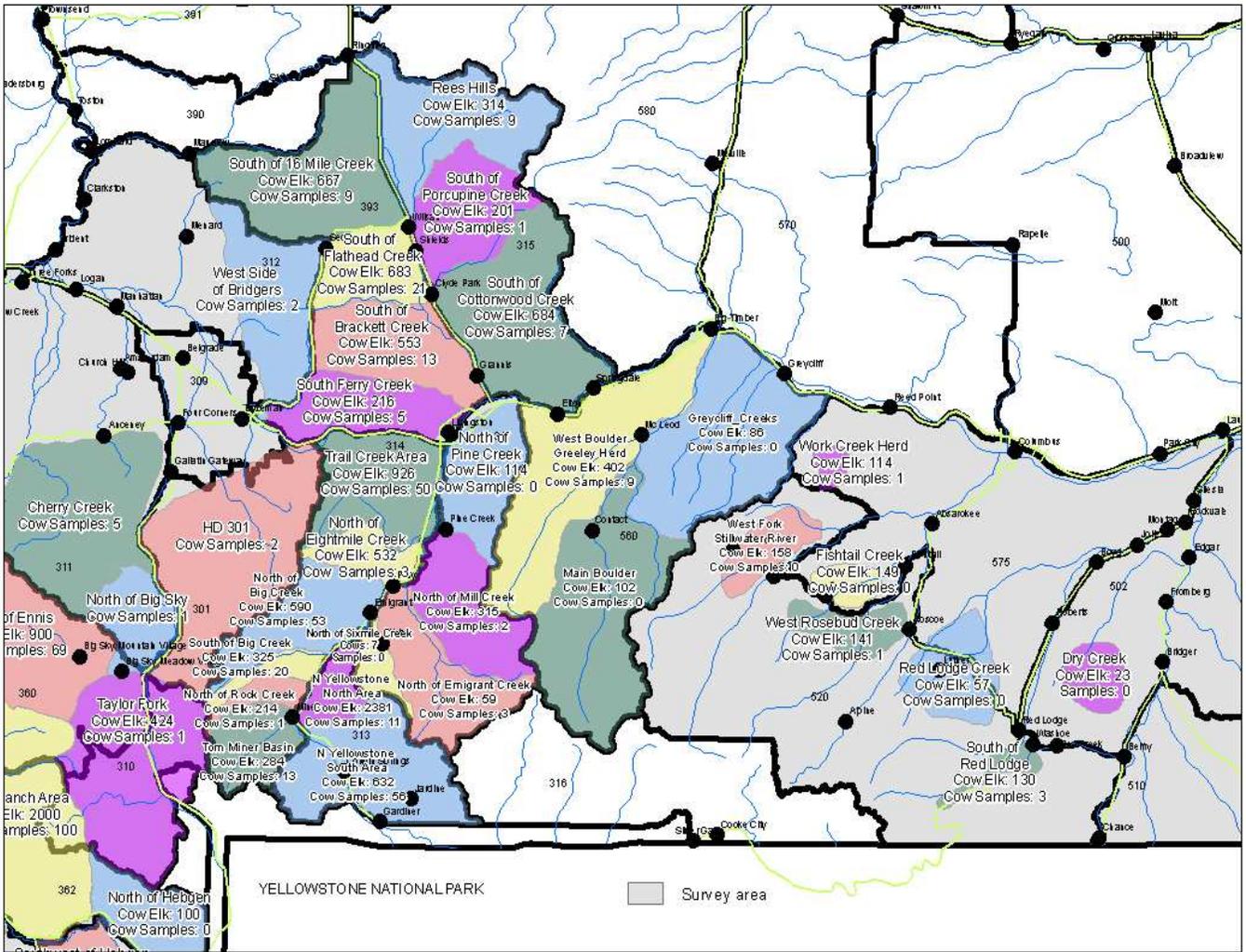


Figure 4. The eastern half of the 2008-09 survey area. The shaded areas depict different herd units as described by MFWP area biologists. The estimated number of adult female (cow) elk present within the herd unit and the number of useable samples obtained are listed below the unit name.

Table 3. Results, by elk herd unit, from adult female elk blood samples tested for exposure to brucellosis during the 2008-09 survey. Only units within the designated surveillance area and having five or more samples are presented.

| Herd Unit (Primary HD) | Adult Female Sample Size | # Positive or suspect on serologic screen | Seropositive based on western blot and/or associated tissue culture | Seroprevalence (95% Confidence Interval) |
|---------------------------------------|--------------------------|---|---|--|
| Blacktail (HD 324) | 25 | 3 | 0 | 0% (0-13.7) |
| Blacktail Ridge-Clark Canyon (HD 325) | 5 | 0 | 0 | 0% (0-52.2) |
| Cherry Creek (HD 311) | 5 | 0 | 0 | 0% (0-52.2) |
| East of Ennis (HD 360) | 69 | 6 | 1 | 1.4% |
| Greenhorns West Slope (HD 330) | 5 | 0 | 0 | 0% (0-52.2) |
| HD 300 | 20 | 0 | 0 | 0% (0 – 17.0) |
| N. Yellowstone –North Area (HD 313) | 11 | 7 | 3 | 27.3% (9.0-58.6) |
| N. Yellowstone –South Area (HD 313) | 56 | 4 | 1 | 1.8% (0.3-11.6) |
| NE Tobacco Roots (HD 333) | 8 | 0 | 0 | 0% (0-36.9) |
| North of Big Creek (HD 314) | 53 | 0 | 0 | 0% (0-6.7) |
| North of China Town (HD 328) | 5 | 0 | 0 | 0% (0-52.2) |
| North HD 302 | 9 | 0 | 0 | 0% (0-33.6) |
| Rees Hills (HD 315) | 9 | 0 | 0 | 0% (0-33.6) |
| Sage Creek (HD 327) | 12 | 1 | 0 | 0% |
| S. Ferry Creek (HD 393) | 5 | 0 | 0 | 0% (0-52.2) |
| S. 16 Mile Creek (HD 393) | 9 | 0 | 0 | 0% (0-33.6) |
| S. of Big Creek (HD 314) | 20 | 3 | 1 | 5.0% (0.7-28.2) |
| S. of Brackett Creek (HD 393) | 13 | 0 | 0 | 0% (0-24.7) |
| S. of Cottonwood Creek (HD 315) | 7 | 0 | 0 | 0% (0-41.0) |
| S. of Flathead Creek (HD 393) | 21 | 0 | 0 | 0% (0-16.1) |
| Sun Ranch Area (HD 362) | 100 | 18 | 5 | 5.0% (2.1-11.5) |
| SW Tobacco Roots (HD 320) | 11 | 0 | 0 | 0% (0-28.5) |
| Tom Miner Basin (HD 314) | 13 | 4 | 1 | 7.7% (1.1-39.1) |
| Trail Creek (HD 314) | 50 | 2 | 0 | 0% (0-7.1) |
| Wall Creek (HD 323) | 13 | 2 | 0 | 0% (0-24.7) |
| West Boulder-Greeley (HD 560) | 9 | 0 | 0 | 0% (0-33.6) |
| W. of Ruby Reservoir (HD 322) | 8 | 0 | 0 | 0% (0-36.9) |

Adult Males

Eight (6.2%) of the 130 adult male elk tested within the survey area were considered to be positive or suspect on the serologic screen conducted at the MDL DL. Of the eight positive or suspects, one (12.5%) was identified as being exposed to brucellosis by western blot. The one brucellosis positive blood sample came from a hunter-harvested bull within the Blacktail Herd Unit (Figure 3) within HD 324. Sample sizes within individual hunting districts ranged from 0 to 24 and seroprevalence in adult males ranged from 0 to 7.1%. A sample of adequate size was not achieved in any of the hunting districts to determine seroprevalence with reasonable statistical certainty, attributing to the large confidence intervals presented in Table 3.

Table 3. Sample size, number positive or suspect based on standard serologic screens, seroprevalence and 95% confidence interval (CI) for adult male elk tested for exposure to brucellosis during the 2008-09 survey period. No samples from adult female elk were obtained in hunting districts 322, 326, 333, 502, 510 and 575.

| HD | Adult Male Sample Size | # Positive or suspect on screen | Seropositive based on western blot and/or associated tissue culture | Seroprevalence and 95% CI () |
|--------------|------------------------|---------------------------------|---|-------------------------------|
| 300 | 5 | 0 | 0 | 0 (0-55.2) |
| 301 | 1 | 0 | 0 | 0 (0-97.5) |
| 302 | 1 | 0 | 0 | 0 (0-97.5) |
| 309 | 1 | 0 | 0 | 0 (0-97.5) |
| 310 | 1 | 0 | 0 | 0 (0-97.5) |
| 311 | 1 | 0 | 0 | 0 (0-97.5) |
| 312 | 1 | 0 | 0 | 0 (0-97.5) |
| 313 | 21 | 1 | 0 | 0 (0-16.1) |
| 314 | 24 | 2 | 0 | 0 (0-14.2) |
| 315 | 4 | 0 | 0 | 0 (0-60.2) |
| 317 | 3 | 1 | 0 | 0 (0-70.8) |
| 320 | 1 | 0 | 0 | 0 (0-97.5) |
| 323 | 3 | 0 | 0 | 0 (0-70.8) |
| 324 | 14 | 1 | 1 | 7.1% (1.0-37.0) |
| 325 | 1 | 0 | 0 | 0 (0-97.5) |
| 327 | 4 | 0 | 0 | 0 (0-52.2) |
| 328 | 2 | 0 | 0 | 0 (0-84.2) |
| 330 | 1 | 0 | 0 | 0 (0-97.5) |
| 360 | 4 | 0 | 0 | 0 (0-60.2) |
| 361 | 1 | 0 | 0 | 0 (0-97.5) |
| 362 | 12 | 2 | 0 | 0 (0-26.5) |
| 393 | 16 | 0 | 0 | 0 (0- 20.6) |
| 520 | 3 | 0 | 0 | 0 (0-70.8) |
| 560 | 5 | 0 | 0 | 0 (0-52.2) |
| Total | 130 | 7 | 1 | 0.8% |

The elk herd units where both brucellosis and *Yersinia* exposed elk were detected during the 2008-09 survey period are presented in Figure 5. When data from both adult females and adult males were pooled, only seven herd units within five hunting districts were identified as containing an elk exposed to *B. abortus* during the 2008-09 survey. Seven additional elk herd units within HD's 314, 317, 323 and 327 initially tested positive or suspect on serologic screening tests but were identified as negative for exposure to brucellosis due to cross-reactions with *Yersinia* as indicated by the western blot assay.

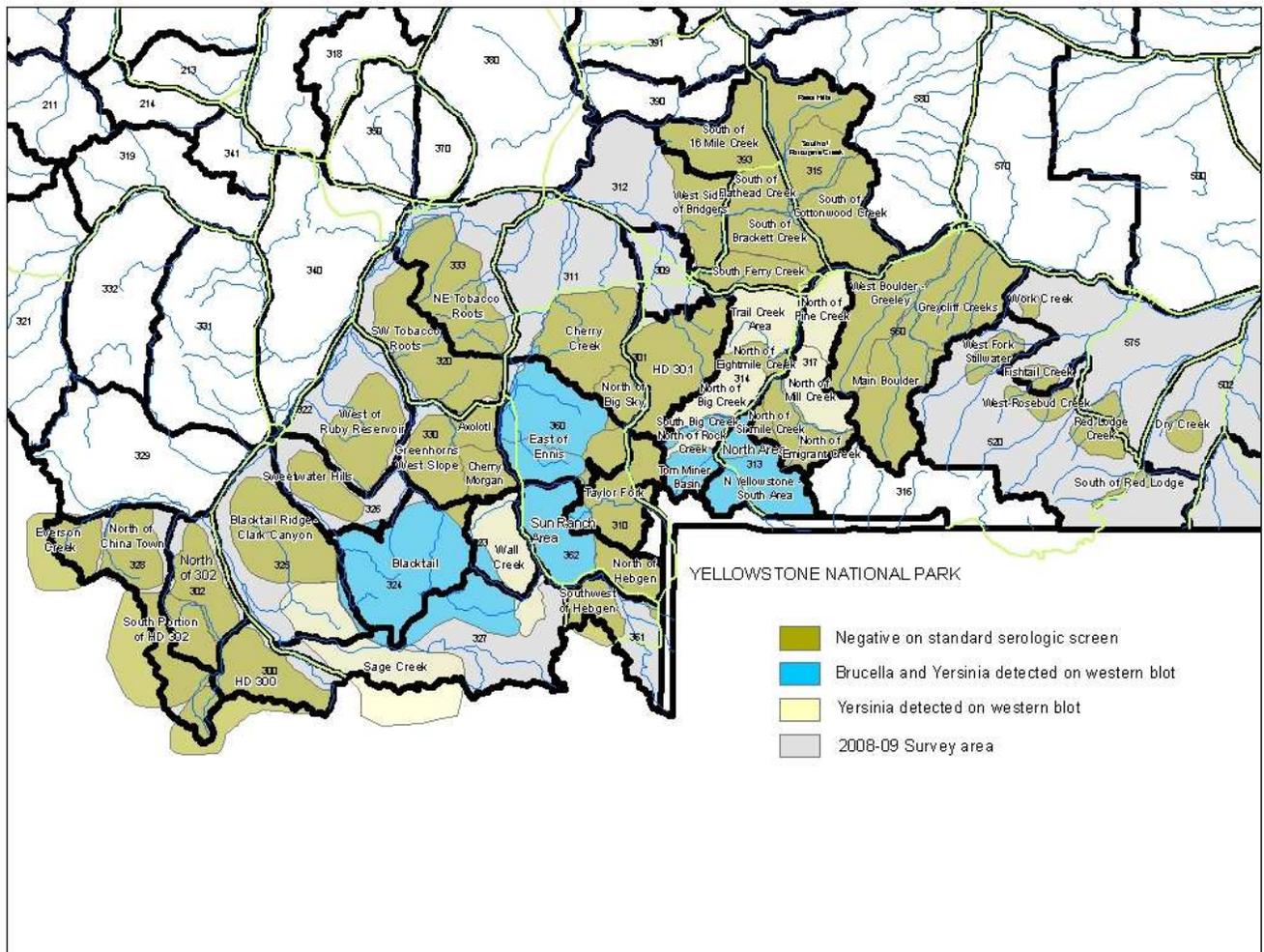


Figure 5. Brucellosis seropositive and *Yersinia* positive elk herd units based on the 2008-09 surveillance data.

Culture Results

Tissue samples from 85 hunter-harvested elk were collected during the general hunting season. Tissue samples were collected in HD's 300 (n = 1), 301 (n = 1), 302 (n = 1), 313 (n = 2), 314 (n = 2), 319 (n = 3), 321 (n = 2), 322 (n = 1), 323 (n = 7), 324 (n = 21), 325 (n = 9), 326 (n = 1), 327 (n = 2), 328 (n = 1), 330 (n = 6), 332 (n = 1), 341 (n = 1), 393 (n = 3), 360 (n = 8), 362 (n = 6), 421 (n = 2), and 580 (n = 1). The hunting district could not be confirmed for three samples. *B. abortus*, biovar 1 was cultured from one (1.2%) of the 85 samples. The culture positive animal, a four-year-old bull, was harvested in HD 324.

Tissue samples were also collected during the Gardiner area late hunt in HD 313 (n = 60) and the management hunt in HD's 360 (n = 22) and 362 (n = 14) of the Madison Valley. *B. abortus*, biovar 1 was cultured from four adult female elk from HD 313, one female of unknown age in HD 360 and one adult female from HD 362. Additional tissue samples collected from two elk captured during 2008 research efforts near Gardiner were also submitted for culture. Both adult females were captured in February of 2008 and tested positive for exposure to brucellosis on standard serology and western blot at that time. When removed from the population in 2009, one tested elk negative on standard serology and the other positive on standard serology but negative for brucellosis exposure on western blot. Both were culture negative.

Discussion

Blood samples from 835 hunter-harvested elk, 45 elk captured for research and two elk removed from the population and were tested for brucellosis exposure using a standard serologic screen conducted at the MDL DL. Sixty-two (7.0%) were identified as suspect or positive on serologic screens and 61 were submitted for retesting using western blot. One sample was not initially submitted for western blot testing and due to the length of time it takes to get results back would have delayed completion of this report. Since sample came from HD 313, an area where brucellosis is known to exist in elk populations and therefore would not change the known distribution of the disease a decision was made to forgo western blot testing on the sample. The one sample was considered to be seropositive based solely on the serologic screening test results. Of the 62 potential seropositives, 13 (20.9%) were considered to be positive for brucellosis exposure by either western blot, a positive culture result or, in the case of one sample, serology alone. Although 882 blood samples were tested, sample sizes in many hunting districts and elk herd units were insufficient to determine the presence or absence of brucellosis in elk populations with any level of statistical confidence.

Upper and lower limits for confidence intervals calculated for hunting districts and herd units were directly related to the number of samples obtained. Small sample sizes relate to large differences between the upper and lower limits. Readers are cautioned not to conclude that seroprevalence must be somewhere midway between the upper and lower limits of the confidence interval for samples where brucellosis was not

detected. Brucellosis may not be present in these populations and the true seroprevalence may be 0% rather than somewhere between zero and the upper confidence interval.

Elk serum was tested using standard serologic tests to initially screen samples for potential exposure to brucellosis. *Yersinia enterocolitica*, as well as other bacteria, has been known to cross-react with these tests causing potential false positives. In an effort to define when that occurs, MFWP for the last four years has routinely submitted any potential positives for additional testing. Western blot has been used to discern between possible brucellosis and *Yersinia* exposure in serum samples. Sensitivity (the ability of a test to identify true positives) and specificity (the ability of a test to identify true negatives) are not well defined or known for the western blot assay. Using multiple serologic screening tests in conjunction increases our confidence that the tests are able to detect possible brucellosis exposed animals. However, the presence of *Yersinia* on the landscape complicates interpretation of those results. Western blot was used to aid in the interpretation of serologic test results, but since the specificity and sensitivity of the western blot assay have not been quantified, identifying samples as being *Yersinia* or *Brucella* positive cannot be done with 100% accuracy. Decisions made on classification of samples as being either positive or negative for exposure to brucellosis are, however, made using the best available information. For example, tissue samples from seven elk collected during the surveillance period were culture positive for *B. abortus*, biovar 1. Of these seven elk, three had matching blood samples which were identified as being seropositive based on standard serology but *Yersinia* positive only on western blot. These samples were ultimately identified as being seropositive based on serologic screening tests and culture results. Methodologies used for determining seroprevalence when only blood samples were obtained are consistent with previous surveillance efforts. The western blot was first used in 2005 to address concerns over test results that suggested brucellosis exposed elk were present in the Pioneer Mountains, an area where elk movement data and general distance from Yellowstone National Park suggests it would be highly unlikely that brucellosis was present. Additionally we observed what appeared to be a threefold increase in seroprevalence in the Madison Valley in the course of a single year with no drastic changes in elk populations or management during that time. Both of these instances suggested that an error had occurred in the standard serologic tests. Western blot was used to address potential cross-reactions with other bacteria that may have resulted in false positives on standard serologic tests. Results from western blot indicated that a cross-reaction had occurred explaining the potential positive samples in the Pioneer Mountains and the observed increase in seroprevalence in the Madison Valley. Incorporating western blot into testing methodology has and continues to be used in Idaho as well.

Although culture results were used during the 2008-09 surveillance to identify an animal as being seropositive, culturing tissues may not identify all infected individuals. Isolation of *B. abortus* from tissue confirms that the bacteria is present in an individual, but a negative culture implies one of several things: the animal is not infected, the animal is infected but the bacteria was not present in the tissues tested or the bacteria was present in the tissues but could not be grown (isolated) in cultures. Culturing tissues

may be used to reinforce findings of serologic surveys but negative culture results do not indicate that infection has not occurred.

Brucellosis exposed elk were found in five hunting districts and seven elk herd units within the survey area. However, the only areas not previously defined as being positive for brucellosis exposure was the Blacktail herd unit of HD 324 and the South of Big Creek herd unit in HD 314. Although brucellosis had not been detected in elk in HD 324 in prior surveillance efforts, few animals were tested. Based on our understanding of elk movement patterns in the Gravely Mountains, it was not surprising to find a seropositive male elk in HD 324. The positive cow elk in the South Big Creek herd unit was a research animal that frequented the Paradise Valley until mid-May and has since migrated into YNP (MFWP unpublished data 2009). Prior to detection of the seropositive elk in the South Big Creek herd in the 2008-09 survey, the farthest north a seropositive was detected in HD 314 was the Tom Miner Basin. However, few samples had previously been collected north of Tom Miner Basin in HD 314.

Culture results identified brucellosis-infected elk in four hunting districts within the survey area. Brucellosis was known to exist in three of those hunting districts (HD's 313, 360 and 362). Isolation of *B. abortus* from a bull elk in HD 324 indicates that the animal was infected but does not tell us where the animal was exposed or if brucellosis is well established in the Blacktail herd unit. Both the seropositive and culture positive elk in HD 324 were males. To date, no seropositive or culture positive female elk, capable of transmitting brucellosis to cattle or other elk, have been detected in the hunting district. Additional testing is needed to assess the presence of brucellosis in the female segment of the population.

The goal of the 2008-09 surveillance effort was to improve our understanding of where brucellosis exists in Montana elk populations and to aid in our determination of where additional surveillance was needed in the future. It was not expected that enough samples would be tested in a single year to meet the ultimate goal of determining, with a high level of statistical confidence, where brucellosis is present or absent within the elk populations surveyed. The greatest numbers of samples were received in areas having high numbers of elk and districts that had antlerless elk licenses or permits available. In these areas we were able to send hunters blood collection kits through the mail and utilize active landowner participation to disseminate kits to hunters accessing their property. Even in areas where the greatest number of samples were received, additional surveillance will be required to address the presence or absence question. In some hunting districts, it may take several years to achieve an adequate sample size if the number of elk tested in a given year is similar to the 2008-09 surveillance effort. The results of the 2008-09 surveillance do indicate that a consistent, long-term surveillance plan is needed to assess brucellosis exposure in elk populations of southwestern Montana.

The difficulty we and other states have in evaluating serologic test results for elk points to the need for a better understanding of how the tests perform for this species. Tests are generally evaluated under laboratory conditions that may or may not be indicative of free-ranging animals. Although several serologic tests have been validated

by USDA for elk, the validation process may not have accounted for potential cross-reactions with *Yersinia*. Currently western blot is not a validated test for elk and sensitivity and specificity are not known. The lack of validation raises uncertainty in the application of western blot to identify true brucellosis seropositives. Additional research and funding is needed to validate the western blot test and improve the overall diagnostic tools we have for detecting brucellosis in free-ranging elk.

2009-10 Survey Recommendations

Based on the known presence of brucellosis exposure in elk and the results of the 2008-09 survey, following are three recommended options for the 2009-10 elk brucellosis surveillance effort. Each option is ranked by priority. The option selected depends largely on available funding.

Option 1. Conduct surveillance in hunting districts and elk herd units adjoining areas containing known brucellosis exposed elk.

Objective: Verify the spatial distribution of brucellosis in elk in areas adjacent to but not currently known to have brucellosis exposed elk populations. This option would limit surveillance to only those areas described below.

- a. Focus efforts on hunting districts and elk herd units near areas of documented exposure and where standard serologic screens indicated potential exposure to brucellosis but western blot indicated cross-reactions to *Yersinia* had occurred.
- b. The area of high priority would include all herd units within hunting districts 301, 311, 314, 317, 320, 325, 326, 327 and 330 and the South Ferry Creek and South of Brackett Creek herd units of HD 393, the South of Cottonwood Creek herd unit of HD 315 and the West Boulder-Greeley and Main Boulder herd units of HD 560 (Figure 6).
- c. Efforts would include sending blood kits to antlerless elk license and elk permit holders within the high priority hunting districts, contact landowners allowing elk hunting opportunities in herd units within the high priority area and ask them to help disseminated blood collection kits, place kiosks at access roads and businesses within the high priority area, work with block management personnel to place kiosks at block management areas and encourage MFWP personnel working in the high priority area to distribute blood kits to hunters.
- d. Focus tissue collection efforts in the high priority area to improve detection of brucellosis and enhance interpretation of serology results.

Option 2. Conduct surveillance in the high priority area indicated above (1b) and the Madison Valley. This option is similar to option 1 but includes monitoring seroprevalence in the Madison Valley.

Objective: Monitoring brucellosis seroprevalence in the Madison Valley where brucellosis is known to exist and verify the spatial distribution of brucellosis. This option would allow for monitoring the change in seroprevalence, if any, over time and may allow for evaluation of the effect proposed elk management activities in the Madison Valley have on seroprevalence in elk populations and the potential risk of transmission to cattle.

- e. Conduct surveillance in the high priority area as described above in option 1b (Figure 6).
- f. Send blood kits to antlerless elk license and elk permit holders in the Madison Valley.
- g. Work with landowners allowing elk hunting opportunities within the high priority area and the Madison Valley and ask them to help disseminate blood collection kits.
- h. Work with block management personnel within these areas to disseminate kits and place kiosks at block management areas.

Option 3. Conduct surveillance in the high priority area as described in 1b above and the remaining areas outlined for the 2008-09 survey. This option is a continuation of the 2008-09 surveillance strategy with the addition of collecting tissue samples in the high priority areas described above.

Objective: The objectives of this option would be to assess the spatial distribution of brucellosis in elk, monitor seroprevalence in brucellosis endemic areas and allow for some level of surveillance in the remaining surveillance area outlined in 2008.

- i. Conduct surveillance in the high priority area as described above in option 1b (Figure 6).
- j. Send blood kits to antlerless elk license and elk permit holders.
- k. Work with landowners allowing elk hunting opportunities within the general survey area and ask them to help disseminate blood collection kits.
- l. Work with block management personnel to place kiosks at block management areas.
- m. Kiosks would not be distributed in the general surveillance area
- n. Tissue collections would focus on high priority areas and be limited or not occur within the general surveillance area.

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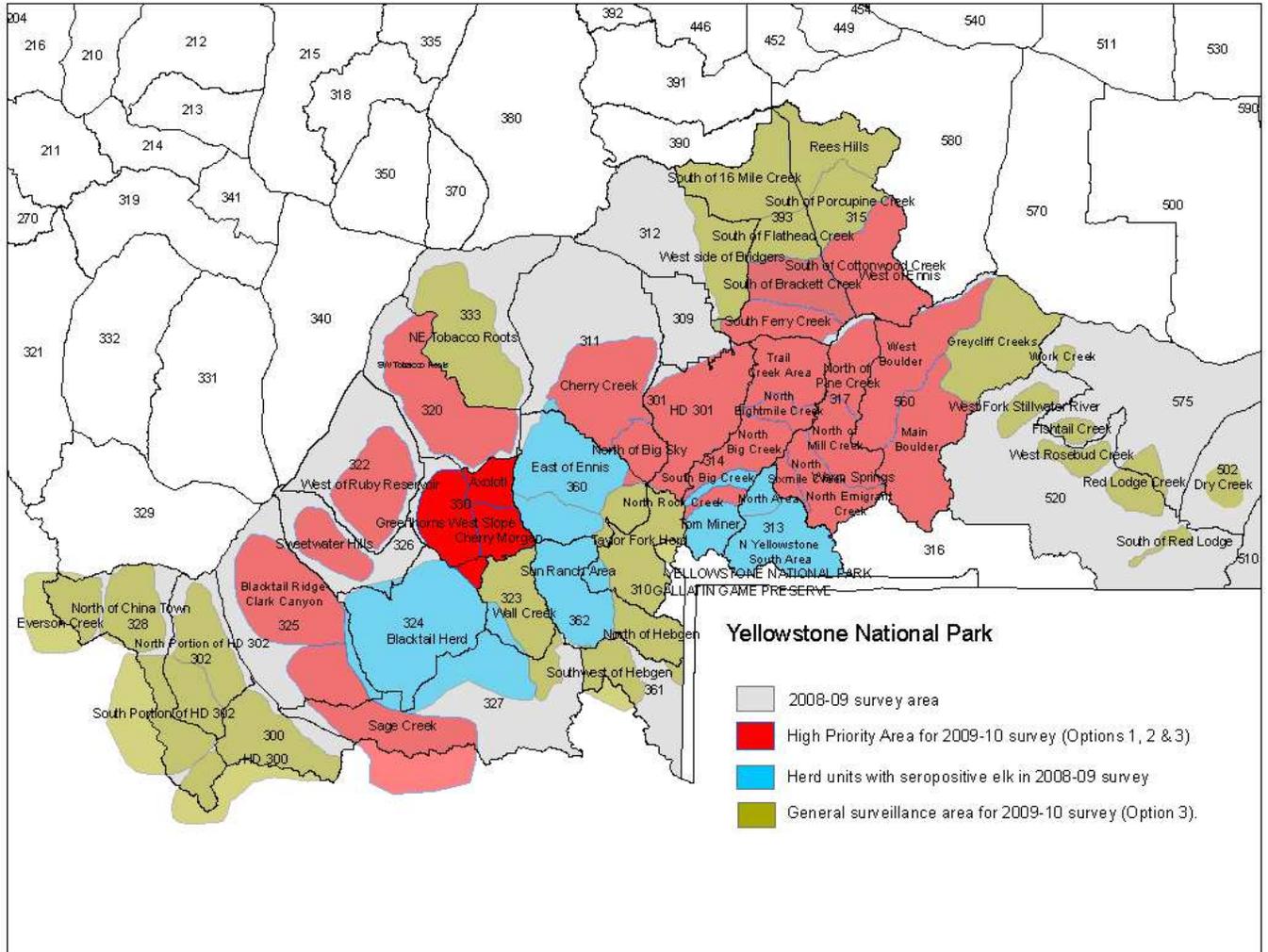


Figure 6. Proposed areas for the 2009-10 elk brucellosis surveillance survey, with areas recommended in options 1-3.