Montana Fish, Wildlife & Parks

2009-10 Brucellosis Surveillance in Elk

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Executive Summary:

A total of 829 adult female and 234 adult male blood samples were tested for exposure to brucellosis during the 2008-09 and 2009-10 survey periods. Blood samples were pooled for analysis and analysis focused on adult females. Adult female elk identified as reactors, based on standard serologic tests, were found in 12 elk herd units occupying nine hunting districts (HD's 313, 314, 317, 323, 324, 326, 327, 360 and 362) within the survey area. The western blot assay was performed on all reactors to identify possible cross-reactions due to Yersinia*. Adult female elk testing positive for exposure to brucellosis on the western blot assay (WB-seropositives) were detected in five herd units occupying four hunting districts(HD's 313, 314 360 and 362). WB-seroprevalence (based on the western blot assay) ranged from 0 to 8.2 % when data from the two years of surveillance was pooled, being highest in hunting districts 313 (8.2%) and 362 (4.3%). However, sample sizes in many hunting districts were small, reducing our ability to adequately assess seroprevalence.*

Elk tissues samples were collected and cultured for Brucella abortus *during the 2008-09 and 2009-10 survey periods. A total of 562 tissue samples consisting of both adult male and adult female elk were collected and submitted for culture at the National Veterinary Services Laboratory in Iowa.* B. abortus, biovar 1 *was cultured from 18 elk (13 female and 5 male) in 10 elk herd units occupying 10 hunting districts with the survey area. Culture positive elk (adult male and/or female) were found in hunting districts 313, 314, 317, 323, 324, 325, 326, 327, 360 and 362. Serology and culture data suggest that further evaluation of the western blot assay, and its use in determining seroprevalence in free-ranging elk populations is warranted.*

Introduction

Brucellosis is a contagious bacterial disease, transmitted primarily through birth tissues associated with abortion events. Brucellosis can cause abortions in cattle and some wildlife species, including elk and bison. The Greater Yellowstone Area (GYA) is the last known reservoir for the disease in the lower 48 States. Montana livestock were designated as being brucellosis free in 1984 and remained so for 24 years until 2008, when a second cattle case was detected in the state. The first case

occurred in 2007. In both cattle cases free-ranging elk were considered likely suspects for transmission to cattle.

Montana Fish, Wildlife and Parks (MFWP) has conducted surveillance for brucellosis in elk populations since the late 1980's. Surveillance has primarily been focused on elk in the GYA but herds from across the state have been tested. To date, MFWP has tested more than 8,300 elk for exposure to brucellosis through serologic analysis of blood samples. Within Montana, brucellosis has only been detected in elk in the GYA. Starting in the fall of 2008 MFWP entered into an enhanced surveillance strategy in an effort to improve our understanding of the distribution of the disease, and where found determine seroprevalence with a reasonable amount of statistical certainty. The surveillance area included 30 hunting districts in southwestern Montana near Yellowstone National Park (YNP) and the Idaho and Wyoming borders (Figure 1).

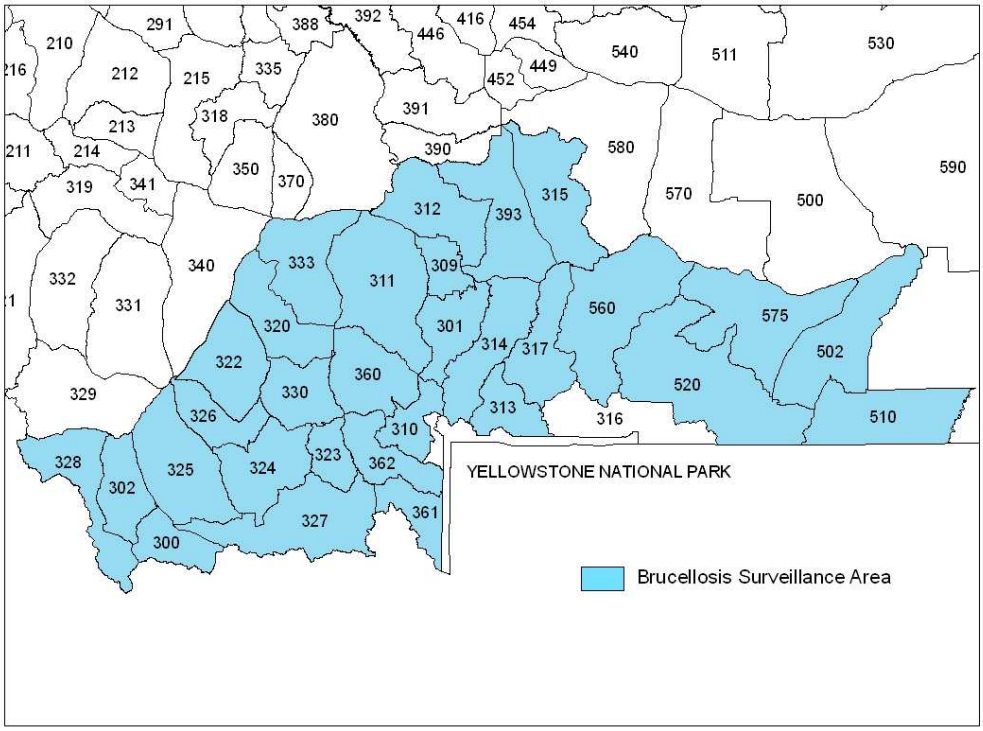

Figure 1. The survey area for brucellosis in elk populations of southwestern Montana. The survey area remained the same for both the 2008-09 and 2009-10 surveys.

Methods

Blood samples were collected in similar ways during the 2008 and 2009 survey periods. In both years, blood samples collected from hunter-harvested elk were the primary samples obtained. Blood collection kits were disseminated to hunters by mailing the kits to antlerless elk license and permit holders. Kits were also made available through kiosks located at primary travel routes and were dispensed to hunters by participating landowners. Blood collection kits were available at US Forest Service offices and MFWP Regional offices within the survey area, and were handed out to hunters at weekend game check stations within the survey area. Game check stations were located at Columbus, Livingston, Cameron and near the Ruby Reservoir in southwestern Montana. The 2009 hunting season was the first time a game check station had been operated in Livingston. The check station was available to hunters three weekends during the five week season. Elk hunters were asked to collect blood samples immediately after harvesting an elk and submit the sample through the use of the postage paid envelope provided or by dropping the kit off at designated drop stations

Blood samples were centrifuged at the MFWP laboratory and the serum collected for testing. Samples that were extremely hemolyzed, contaminated, or would not separate when centrifuged were deemed unsuitable for testing. Serum samples were submitted to the Montana Department of Livestock Diagnostic Laboratory for initial testing for antibodies, indicating exposure to *Brucella abortus*. Samples were initially screened using the Buffered Acidified Plate Antigen (BAPA) test, the Rivanol test, Fluorescent Polarization Assay (FPA) and the Standard Plate test. Samples classified as being suspect or reactors on these screening tests were also tested using the Compliment Fixation (CF) test, Card test and Western Blot assay. The Western Blot assay was conducted at Louisiana State University in efforts to determine whether a potential cross-reaction with other bacteria, primarily *Yersinia enterocolitica* O:9, may have resulted in false positives on standard serologic tests. For the purpose of this report a **reactor** is based solely on standard serologic testing and is defined as an elk that was considered positive on at least two standard serologic tests. A seropositive elk is defined as a reactor that also tests positive for exposure to brucellosis on the western blot assay and will be labeled as **WB-seropositive**. Due to the potential of cross-reactions on standard serologic tests and to maintain consistency in reporting, results based on the use of western blot assay were considered to the final estimate of seroprevalence. Results for animals considered reactors and WB-seropositives based on the above definition are presented for comparison purposes.

In addition to blood samples, tissue samples from harvested elk were collected opportunistically. Retropharyngeal lymph nodes were removed from elk heads collected at game check stations and cooperating meat processors. Tissue collections were conducted in tandem with chronic wasting disease surveillance that was also taking place in southwestern Montana during the 2009 general hunting season. Additional tissues were collected by backtracking to hunter kill sites in the northern Paradise Valley during the general season and near Gardiner, MT during the late hunt. At kill sites, retropharyngeal lymph nodes, supramammary lymph nodes, amniotic fluid, cotyledons from pregnant elk, reproductive tracts from non-pregnant elk and ileocecal material were collected for culture from adult female elk. All tissue, with the exception of ileocecal material, was submitted to the

National Veterinary Services Laboratory in efforts to culture *B. abortus*. Ileocecal material was submitted to the Wyoming Game and Fish Diagnostic Laboratory in efforts to culture *Yesinia enterocolitica*.

Elk populations are not evenly distributed throughout a hunting district. They may cross hunting district lines and have varying levels of comingling among adjacent populations. As a result, brucellosis exposure is not likely to be evenly distributed throughout a hunting district and may not be present in some herd units. In order to gain better insight into brucellosis presence and absence on the landscape, elk populations were divided into herd units based on elk movement and distribution information obtained through research efforts and expert opinion from area biologists (Figure 2). A goal of surveillance over the past two years has been to determine the presence or absence of brucellosis within both hunting districts and ultimately within herd units and, if brucellosis is found, to estimate the seroprevalence at the herd unit and larger hunting district scales.

Statistical analysis was conducted using both Statgraphics© (2006) and Program R© (2009). Binomial confidence intervals were calculated for adult female and adult male elk using a 0.05 error rate for hunting districts where reactors and seropositive elk were detected during the 2009-10 survey season (Statgraphics© binomial proportions test) and for data pooled from the 2008-09 and 2009-10 surveillance periods (binom.logit in Program R©). For the type of data collected in this survey, the 95% confidence bounds, if applied to new data repeatedly, would contain the true value 95% of the time. The confidence intervals do not address the accuracy of the data but suggest that if similar surveillance was conducted 100 times we would expect the true seroprevalence to be within the bounds of the confidence interval 95 times.

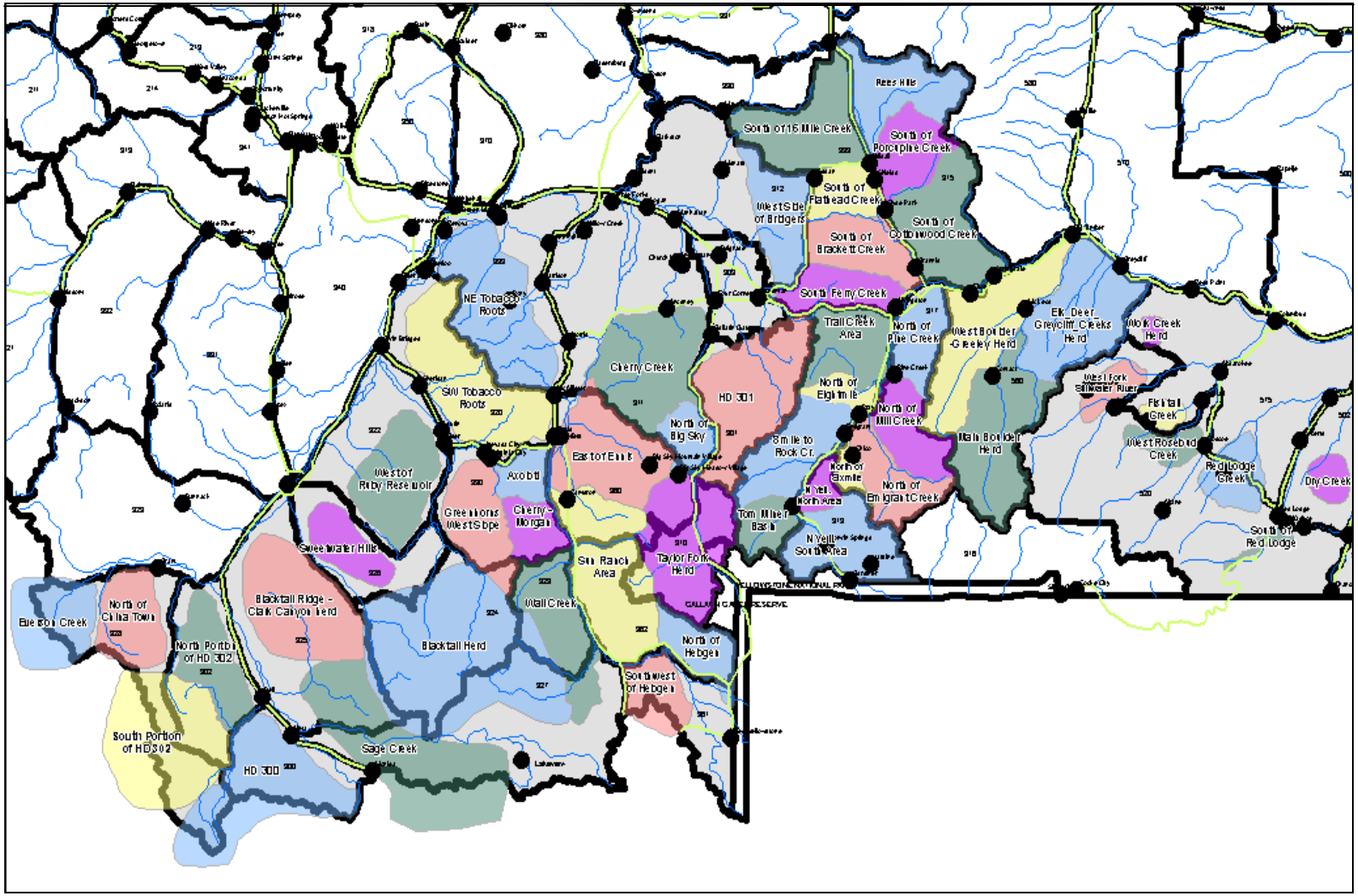

Figure 2. Elk herd units as defined by area biologists and research information for the brucellosis surveillance area of southwestern Montana.

2009-10 Survey Summary

Hunters harvested approximately 5,590 elk within the survey area during the 2009-10 general and late hunting seasons (MFWP unpublished data, 2010). A total of 455 elk blood samples were received during the 2009-10 survey period. Of those samples, 448 were from hunting districts within the survey area, resulting in about an 8% hunter participation rate. Three hundred and ninety-seven (397) of the 455 samples were considered to be of suitable quality for testing. Adequate information on age, sex and harvest location was available for 376 of the samples. The remaining 21 samples tested, all of which were negative for exposure to brucellosis, were excluded from further analysis. Calves, adult (yearling and older) females and adult (yearling and older) males comprised 43, 236 and 97 of the samples with adequate information, respectively. A summary of the sample size, serology results and final designation of WB-seroprevalence based on western blot results for each age and gender group is presented in Tables 1, 2 and 3.

Table 1. Calf samples received during the 2009-10 surveillance period. One calf in hunting district 327 was considered to be a reactor on standard serology but negative for exposure to brucellosis on western blot. All remaining samples were considered to be negative on standard serologic tests for exposure to brucellosis.

HD	Samples	HD	Samples	HD	Samples
300	2	315	5	333	1
301	1	322	1	360	5
302	1	324	1	361	2
309	1	325	2	362	7
311	1	327	1	393	2
313	4	328	1	520	1
314	3	330	1	Total	43

Sample sizes for both adult female and adult male elk within individual hunting districts were not adequate to assess overall WB-seroprevalence. Tables 2 and 3 provide relative information on the samples obtained and the number of reactors and WB-seropositive samples but, due to small sample sizes, 95% confidence intervals were not calculated. Samples from adult female elk considered reactors to brucellosis based on standard serology were detected in seven hunting districts within the survey area in the fall/winter of 2009-10. Of these samples three (HD 313, HD 314 and HD 362) were considered to be WB-seropositive. WB-seropositive adult male elk were detected in HD 313, HD 323 and HD 360.

Table 2. 2009-10 serologic test results from adult female elk. Sample sizes, the number and proportion (%) of adult female elk considered to be reactors to brucellosis on standard serology and the number and proportion (%) testing positive for exposure to brucellosis based on western blot assay (WB-seropositive) are reported. The 95% confidence interval was calculated for HD's where reactors or WB-seropositive elk were detected.

HD	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-Seropositive 95% CI
300	12	0		0	
301	1	0		0	
302	3	0		0	
310	1	0		0	
311	9	0		0	
312	3	0		0	
313	44	6 (13.6%)	5.2% - 27.3%	5 (11.4%)	3.8% - 24.6%
314	50	3 (6.0%)	1.3% - 16.5%	2 (4.0%)	0.5% - 13.7%
315	9	0		0	
317	3	1 (33.3%)	8.4% - 90.6%	0	
319	1	0		0	
320	2	0		0	
321	1	0		0	
322	4	0		0	
323	2	0		0	
324	5	1 (20.0%)	0.5% - 71.6%	0	
325	3	0		0	
326	4	2 (50.0%)	6.8% - 98.2%	0	
327	5	0		0	
328	2	0		0	
329	1	0		0	
330	5	0		0	
333	4	0		0	
340	1	0		0	
360	15	1 (6.7%)	0.2% - 31.9%	0	
361	2	0		0	
362	23	2 (8.7%)	1.1% - 28.0%	1 (4.3%)	0.1% - 28.9%
380	1	0		0	
393	9	0		0	
520	4	0		0	
560	2	0		0	
570	1	0		0	
575	4	0		0	
Total	236	16 (6.8%)	3.9% - 10.8%	8 (3.4%)	1.5% - 6.6%

Table 3. 2009-10 serologic test results from adult male elk. Sample sizes, the number and proportion (%) of adult female elk considered to be reactors to brucellosis on standard serology and the number and proportion (%) testing positive for exposure to brucellosis based on western blot assay (WB-seropositive) are reported. The 95% confidence interval was calculated for HD's where reactors or WB-seropositive elk were detected.

HD	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-seropositive 95% CI
104	1	0		0	
300	3	0		0	
301	1	0		0	
310	12	2 (16.7%)	2.1% - 48.4%	0	
311	11	0		0	
312	3	0		0	
313	10	2 (20.0%)	2.5% - 55.6%	1 (10.0%)	0.2% - 44.5%
314	7	0		0	
315	3	0		0	
317	1	0		0	
320	3	0		0	
323	4	2 (50.0%)	6.8% - 98.2%	1 (25.0%)	0.6% - 80.6%
324	3	0		0	
325	1	0		0	
327	1	0		0	
328	1	0		0	
330	3	0		0	
333	2	0		0	
360	9	3 (33.3%)	7.5% - 70.0%	1 (11.1%)	0.3% - 48.2%
393	11	0		0	
520	6	0		0	
560	1	0		0	
Total	97	9 (9.3%)	4.3% - 16.9%	3 (3.1%)	0.6% - 8.6%

2008-09 and 2009-10 Pooled Data

Due to the small samples sizes received, data from the 2008-09 and 2009-10 surveillance seasons were pooled for analysis. During the last two years, eight hundred and twenty-nine (829) blood samples from adult female, 234 blood samples from adult male elk and 137 blood samples from calves were tested for exposure to brucellosis. All of the calves tested were considered to be sero-negative. Analysis of seroprevalence data focused on adult elk with emphasis on females. Based on standard serologic tests, a total of 79 reactors (65 adult females and 14 adult males) were detected over the two years of surveillance. Potential cross-reactions on standard serology, as determined by western blot assay, were detected in 55 of the 79 samples identified as reactors on standard serologic tests (45 adult females and 10 adult males). As a result, 24 (2.2%) of the 1063 samples tested were considered to be WB-seropositive for exposure to brucellosis.

Adult females (yearling and older)

WB-seroprevalence by hunting district varied greatly across the survey area (Table 4, Appendix A). During the last two years of surveillance, WB-seropositive adult female elk were detected in hunting districts 313, 314, 360 and 362 although reactors on standard serology were detected in five additional hunting districts (HD's 317, 323, 324, 326 and 327). Sample sizes were small resulting in large confidence intervals for many hunting districts even though two years of surveillance data were pooled. Observed WB-seroprevalence was highest in hunting districts 313 (8.2%) and 362 (4.3%). Elk exposed to brucellosis have been detected in these hunting districts since the 1980's (MFWP unpublished data). However, seroprevalence generally was less than 2% in both hunting districts prior to the mid 1990's (Figures 3 and 4). When multiple years are pooled, seroprevalence within HD 313 appears to have increased significantly ($p<0.05$) since the early 1990's from 0.9% to 6.1% when the data from the early 1990's and data from 2004-2009 were pooled (MFWP unpublished data). A similar trend was observed in HD 362 with seroprevalence increasing from 1.8% in the early 1990's to 4.7% by the mid and late 2000's, although the increase was not considered statistically significant (MFWP unpublished data). The percentage of reactors on standard serologic tests (western blot results not applied) increased greatly since the early 1990's in both hunting districts (Figures 3 and 4).

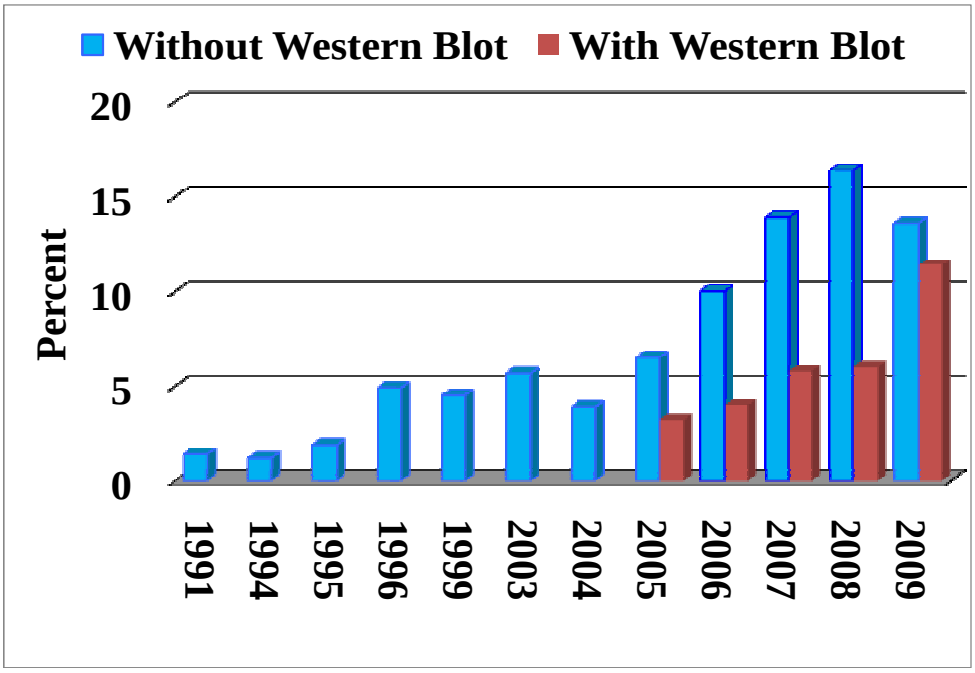

Figure 3. A comparison of seroprevalence of adult female elk from HD 313 based solely on standard serologic tests (reactors) and when the western blot results have been used to detect possible cross - reactions (false positives) on standard serologic tests. The western blot assay has been used as a definitive test for determine seroprevalence in HD 313 elk populations since 2005.

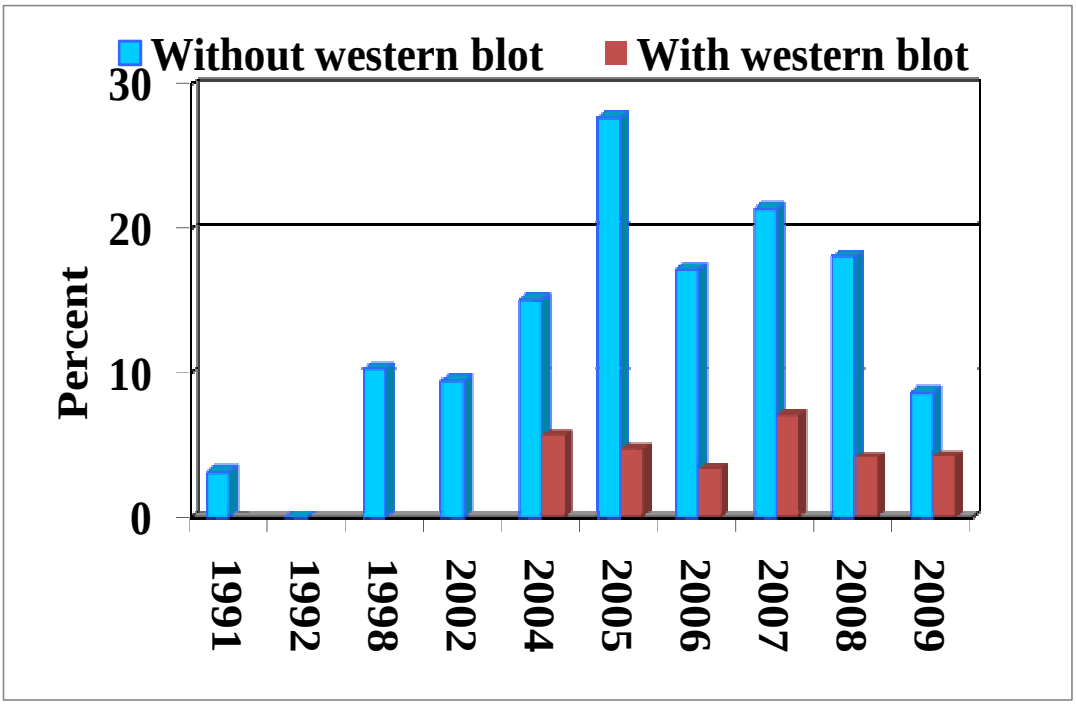

Figure 4. A comparison of seroprevalence of adult female elk from HD 362 based solely on standard serologic tests (reactors) and when the western blot results have been applied to detect possible cross-reactions (false positives) on standard serologic tests. The western blot assay has been used as a definitive test for determine seroprevalence in HD 362 elk populations since 2004.

WB-seroprevalence by herd unit also varied greatly (Table 5, Appendix A), being highest (23.4%) in the Northern Yellowstone-North Area. However, samples sizes were small for most herd units, including the Northern Yellowstone-North Area (n = 17) resulting in large confidence intervals. Reactors on standard serology were detected in 11 herd units within the survey area. Potential cross-reactions to *Yersinia* were detected in western blot assays in six herd units, reducing the number of herd units considered WB-seropositive to five, all of which were within the known distribution of brucellosis in elk herds of the GYA.

Analysis of brucellosis serologic data within Montana focuses on adult female elk. Adult male WB-seroprevalence by hunting district and herd unit is presented in Tables 6 and 7 in Appendix A for comparison. Further analysis of WB-seroprevalence and the percentage of reactors as defined above for adult male elk were not completed for this summary.

Culture Data

A total of 562 tissue samples from hunter-harvested elk (n = 559) and research animals (n=3) were submitted to NVSL for *B. abortus* culture. *B. abortus* biovar 1 was cultured from 18 hunter-harvested animals (3.2%), 5 males and 13 females. *B. abortus* was not cultured from the three research elk removed from the population even though they were identified as being seropositive based on serology and western blot testing. The hunting district of harvest was not provided for four samples, all of which were culture negative. Culture positive elk were detected in 10 hunting districts within the surveillance area (Table 8). Figure 5 illustrates the herd units where reactors, WB-seropositive and culture positive adult female and adult male elk were found.

Table 8. Hunting districts and herd units (Figure 2) where *B. abortus* biovar 1 was cultured from tissues of hunter-harvested elk over the 2008-09 and 2009-10 hunting seasons.

HD	Herd Units(s)	Sample Size	# Culture Positive	% Culture Positive	95% CI
313	N Yellowstone Elk North and South Herd Units	108	6 (5 females, 1 male)	5.5%	2.0% - 11.6%
314	Trail Creek Herd Unit	71	1 (female)	1.4%	0.0% - 7.6%
317	North of Mill Creek Unit	13	1 (female)	7.7%	1.9% - 34.0%
323	Wall Creek Herd Unit	17	2 (males)	11.8%	1.5% - 36.5%
324	Blacktail Herd Unit	40	2 (1 female, 1 male)	5.0%	0.6% - 16.9%
325	Sage Creek Herd Unit	25	1 (male)	4.0%	0.1% - 20.3%
326	Greenhorns West Slope	10	1 (female)	10.0%	0.2% - 44.5%
327	Blacktail Herd Unit	15	1 (female)	6.7%	0.2% - 31.9%
360	East of Ennis Herd Unit	67	1 (female)	1.5%	0.0% - 8.0%
362	Sun Ranch Herd Unit	49	2 (females)	4.1%	0.5% - 14.0%

Serology and culture results were available for 136 elk tested during the 2008-09 and 2009-10 survey periods, seven of which were culture positive. Six of the culture positive elk were considered reactors on standard serologic tests. One culture-positive sample was considered negative on standard serology and not submitted for western blot analysis. Of the six reactors, western blot results were reported as being a cross-reaction with *Yersinia enterocolitica* for four and brucellosis exposure for two (Table 9).

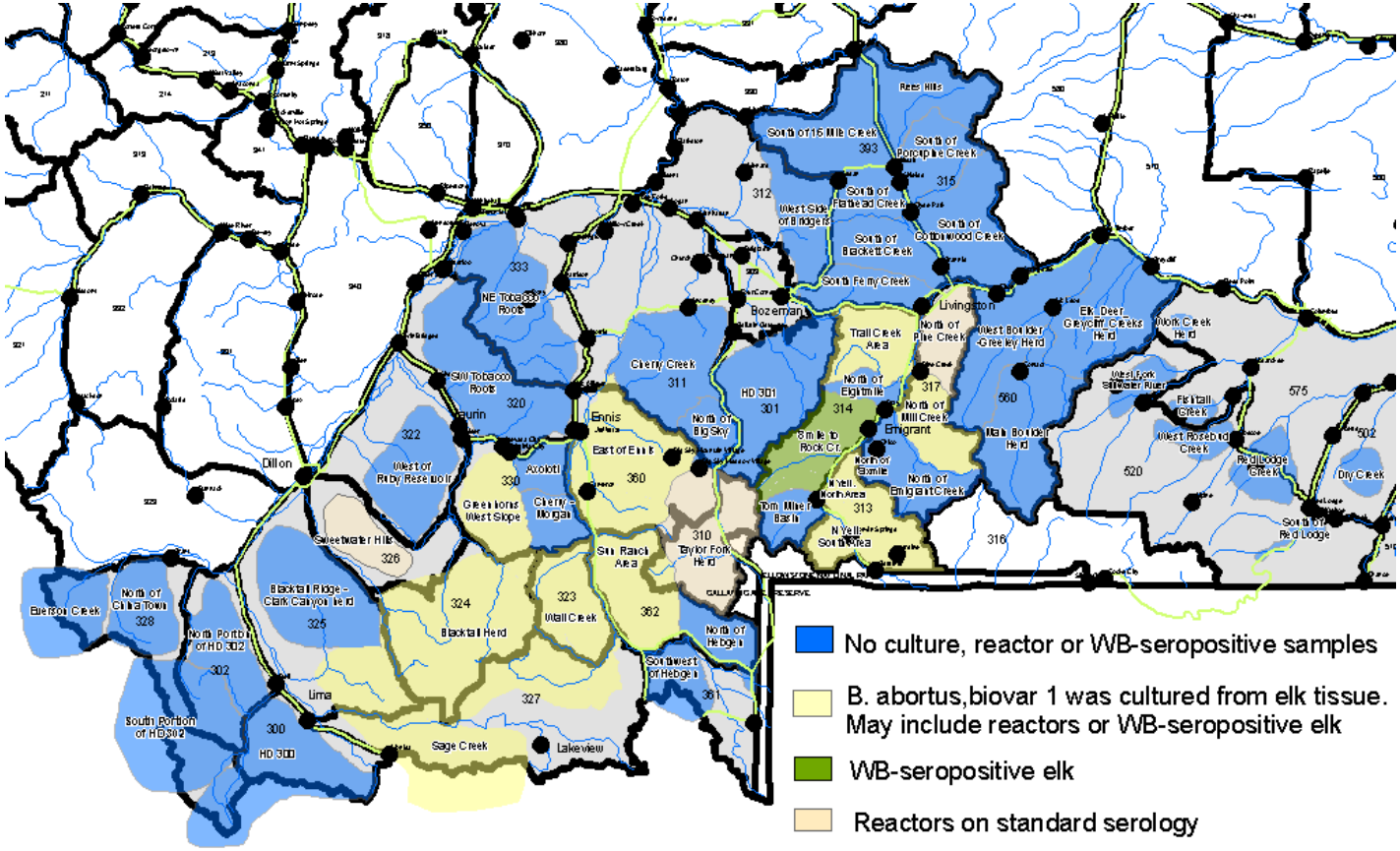

Figure 5. Elk herd units where *B. abortus*, biovar 1 was cultured from elk tissue and reactors and WB-seropositive were detected during the 2008-09 and 2009-10 survey period. Both adult male and adult female reactors, WB-seropositive and culture positive animals are included.

Ileocecal material from 23 hunter-harvested elk was submitted to the Wyoming Game and Fish Diagnostic Laboratory in efforts to culture *Y. enterocolitica*. All samples were culture negative for the bacteria. However, *Y. enterocolitica* is extremely difficult to culture. Negative culture results do not necessarily indicate that the elk was not infected. The bacteria may have been present but not in the sample collected, the bacteria may not have survived the collection and handling process or it may not have been present in the elk.

Table 9. Serology, western blot and culture results for elk where both serology and culture results were available. The western blot assay was only conducted if a sample was considered a reactor on standard serologic tests. Standard serologic tests and the western blot assay are not 100% accurate at detecting exposed or infected animals. A suite of tests are used to improve our ability to determine true brucellosis exposure. Infrequent findings of seronegative but culture positive animals do occur. It is assumed that any culture positive animal has the potential to shed the bacteria through birth fluids and birth tissue.

Standard Serology Results	Western Blot Results	Culture Results	Number of samples	Comments
Negative	Not Tested	B. abortus not detected	118	Highly unlikely to have brucellosis
Negative	Not Tested	B. abortus biovar 1	1	Infrequent cases of seronegative but culture
Reactor	Yersinia exposure only	B. abortus not detected	6	A cross reaction resulting in a potential false-positive result
Reactor	Brucella and/or Yesinia	B. abortus not detected	5	Believed to be true positives indicating exposure to Brucella
Reactor	Yesinia and Brucella exposure	B. abortus biovar 1	2	Confirmed *B. abortus* infected, supported by serology and western blot.
Reactor	Yersinia exposure only	B. abortus biovar 1	4	Confirmed *B. abortus* infected, supported by serology but not western blot.

Discussion

The primary goals for the 2008-09 and 2009-10 surveillance efforts were to determine the geographical distribution and seroprevalence of brucellosis in elk populations in southwestern Montana with a relatively high level of statistical confidence. Currently the number of samples received does not allow us to determine the presence or absence of brucellosis within elk populations outside of the known distribution of brucellosis with a high level of statistical confidence. Hunter-harvested elk have comprised the majority of samples collected for this and historic surveillance efforts. Participation by hunters harvesting elk within the surveillance area was approximately 14.7% in 2008-09 (Anderson et.al. 2009). The participation rate declined to about 8% in 2009-10 despite increased educational efforts. In 2008-09 a drawing for various prizes was offered to hunters submitting blood samples. The drawing was not offered in 2009-10. This may have resulted in decreased willingness to participate in the 2009-10 surveillance efforts, although other factors may have contributed as well.

Adult female elk were the primary focus of surveillance efforts for brucellosis in Montana because brucellosis infected adult females comprise the segment of the population most likely to transmit brucellosis to other elk or livestock through aborted fetuses and birth tissues. Adult female elk may also serve as a better indicator of brucellosis establishment in a population than adult males which tend to move greater distances, especially during rut. Historically, few calves test positive for exposure to brucellosis making them poor sentinels for detecting the disease (Anderson et. al. 2009, MFWP unpublished data).

Due to the small samples sizes achieved in individual hunting districts or elk herd units, data from the 2008-09 and 2009-10 survey seasons were pooled for evaluation. Over the last two years reactors on standard serologic tests have been detected in adult female elk from nine hunting districts (HD's 313, 314, 317, 323, 324, 326, 327, 360 and 362) within southwestern Montana. Western blot was used to determine if a cross-reaction could have resulted in possible false positives on standard serologic tests. WB-seropositive adult male elk were detected in hunting districts 313, 323, 324 and 360 while reactors were detected in two additional hunting districts (HD 310 and HD 317). Reactors on standard serologic tests and elk defined as WB-seropositive for this report were only detected within the known historic distribution of brucellosis or where the disease was expected to be present based on elk movement information.

It appears that seroprevalence has increased since the mid 1990's in the two hunting districts where brucellosis testing has occurred with the greatest frequency (HD 313 and HD 362). In HD 313, elk populations have declined significantly, being less than half of what they were in the mid 1990's yet brucellosis seroprevalence has increased. Elk populations in HD 362 have not declined to the same extent, but seroprevalence appears to have increased in this elk population as well, although that increase is not statistically significant ($p > 0.05$). If results from the western blot assay were not used, seroprevalence in both HD 313 and HD 362 would be much higher and similar to that reported in areas of Wyoming where seroprevalence is increasing in elk populations not directly associated with feedgrounds (Wyoming Game and Fish, 2010). Increasing seroprevalence within elk populations of HD

313 and HD 362, which demonstrated different trends in population levels, suggests that seroprevalence may not be a function of overall population density. The reason for increasing seroprevalence is not known but could be a function of larger winter elk aggregations (Cross et.al. 2010) as well as a combination of many other potential variables. In 1995 the state of Montana passed a law making it illegal for private citizens to feed ungulates. As a result feedgrounds in the West Yellowstone were eliminated. Those elk have since dispersed utilizing area winter range. It was unknown what the seroprevalence of that population was prior to elimination of the feedgrounds, but if seroprevalence was similar to what can be found on Wyoming feedgrounds, the dispersal of elk into adjacent herds in the Madison Valley and Gardiner area could contribute to the observed increases in the late 1990's. Other factors such and increased predation risk and changes in land management strategies may have resulted in shifts in distribution, changes emigration and immigration, shifts in calving areas and artificially high concentrations of elk in certain area. Wintering elk normally form groups which can, at times, be quite large. Simply having large groups of elk on the landscape may not have contributed to the observed increase in seroprevalence as much as the length of time those elk were in the large groups. Changes in behavior that result in large groups persisting on the landscape for extended periods of time during the third trimester of pregnancy could increase the likelihood of exposure should a brucellosis related abortion occur. As seroprevalence increases, concern that brucellosis may be maintained in Montana's free-ranging elk populations without augmentation from outside sources also increases. Should that happen, areas where brucellosis is maintained and possibly increasing in prevalence may serve as a source of infection to adjacent herds, enlarging the geographic area affected by brucellosis.

B. abortus was cultured from elk for the first time in HD's 317, 324, 325, 326 and 327 during the last two years of surveillance. Additional cultures were obtained in HD's 313, 314, 323, 360 and 362. *B. abortus* was cultured from female elk in HD's 313, 314, 317, 324, 326, 327, 360 and 362 and adult male elk in HD's 313, 323, 324 and 325. The distribution of culture positive elk is consistent with the distribution of reactors detected during serologic surveys during the last two years, and includes four hunting districts where WB-seropositive elk were not detected. The detection of seropositive and culture positive adult female elk suggests that the disease is established in the population, but in many areas the prevalence of the disease is not well understood.

Cross-reactions on standard brucellosis serologic tests due to exposure to bacteria with similar biochemical makeup to *B. abortus* is well documented (Bundle et.al. 1984, Chukwu 1987, Nielsen 1990, Kittelberger et.al. 1995, Schoerner et.al. 1990). Currently the western blot assay is the only test available to MFWP to detect potential cross-reactions. Both Montana and Idaho use the western blot assay to assess whether cross-reactions on standard serology may have resulted in false positives for exposure to brucellosis on standard serology in elk. MFWP has used the western blot assay in its testing protocol since 2004. However, the western blot is considered a research tool and has not been validated in elk. In an effort to better understand the utility of the western blot assay, MFWP has been opportunistically collecting blood and tissue samples from elk harvested within the survey area. Over the last two years tissue samples were collected for *B. abortus* culture and a blood sample was

submitted for serologic testing from 136 individual elk. Of those 136 paired tissue and blood samples, seven were culture positive for *B. abortus* biovar 1. Of the seven culture positives, one was considered negative and six were reactors based on standard serology. The western blot assay was conducted on the six reactors to determine if a cross-reaction had resulted in a false positive on serology.

Results from the western blot assay suggested that four of the six reactors on standard serology were the result of a cross-reaction to *Yersinia* and the remaining two were due to true brucellosis exposure, even though all were culture positive for *B. abortus*. There are three possible explanations for the failure of western blot to detect evidence of brucellosis exposure on four culture positive samples: 1) western blot did not accurately differentiate between *Yersinia* and *Brucella* antibodies, 2) the level of *B. abortus* infection was not sufficient to maintain a humoral immune response resulting in loss of antibody production for brucellosis, but the elk was also exposed to and produced antibodies against *Yersinia* or 3) the elk was infected with *B. abortus* and failed to produce antibodies against it, but was also exposed to and did generated antibodies against *Yersinia*. Should the first scenario be true, western blot failed to accurately identify exposure to brucellosis in four of six (67%) culture positive samples. This could have a drastic affect on reported seroprevalence rates in portions of Montana's elk populations. However, the sample size used to evaluate the western blot assay is extremely small and was not conducted in a controlled experimental setting, making interpretation of test results difficult. Regardless of the reason why western blot did not identify 4 of the 6 *B. abortus* culture positives, a reevaluation of the testing protocols used on free-ranging elk in Montana is warranted.

One of the goals of surveillance since 2008 has been to determine the geographic distribution of brucellosis in free-ranging elk populations on southwestern Montana. These efforts were hampered by small sample sizes, limited hunter participation, potential cross-reactions resulting in false positives on standard serologic tests, and difficulties in interpreting test results knowing that cross-reactions are possible. In order to better elucidate the current geographic distribution of brucellosis and the potential of brucellosis infected elk herds to pass the infection on to adjacent uninfected herds, better surveillance and additional information on elk movement patterns is required. Previous and current surveillance has relied heavily on hunter participation in the form of blood samples from harvested animals to ascertain seroprevalence in elk populations. Hunter-harvested samples, although convenient, are not collected in a random manner and when used alone may not be effective at producing accurate disease prevalence estimations or detecting disease presence or absence (Nusser et. al., 2008; Walsh and Miller, 2010). Likewise, disease distribution is rarely random or evenly distributed throughout a population. A sampling design that incorporates biological information about the host as well as potential disease distribution within a population would likely be more effective at detecting the disease at low prevalence. Surveillance strategies that combine convenience samples such as hunter-harvested blood collections and probability sampling that accounts for potential differences in disease distribution are more advantageous methods for disease detection and provide statistically quantifiable estimates of disease prevalence (Nusser et. al, 2008.). In order to enhance the probability of detecting brucellosis should it exist in an elk population, additional information on factors that may result in increased brucellosis seroprevalence and transmission among elk is needed. Based on current

knowledge and published literature of the disease, this could be in areas where large numbers or groups of elk congregate on winter range (Cross et.al. 2010).

In many elk populations the amount of mixing that occurs between the herds is not well understood and may be highly variable. Within the GYA winter range movement or exchange rates ranging from 3 - 17% have been reported for adult female elk (Smith and Anderson, 2001; Hamlin and Ross, 2002; Hamlin and Cunnigham, 2008; Gower et. al., 2009). Recent changes in land management practices and increased pressure from predators may also influence elk distribution or dispersal. Understanding the amount of intermixing and dispersal that occurs among populations is important in understanding potential disease transmission, should a disease of concern become established within a population. Increasing brucellosis seroprevalence in some elk herds within the GYA is a concern, not only where brucellosis exists, but in areas containing herds that may exchange elk with an infected population. A better understanding of elk movement and dispersal patterns is needed to determine the potential movement of brucellosis, and other diseases such as chronic wasting disease should it be detected in the GYA.

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Appendix A.

Table 4. Brucellosis surveillance results for adult female elk during the 2008-09 and 2009-10 survey periods by hunting district. The 95% confidence interval (CI) provides a range of where we would expect seroprevalence to be 95 out of 100 times if we conducted surveillance in a similar manner with the same sample sizes.

HD	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-seropositive 95% CI
300	32	0	0% - 11%	0	0% - 11%
301	2	0	0% - 66%	0	0% - 66%
302	12	0	0% - 24%	0	0% - 24%
309	1	0	0% - 95%	0	0% - 95%
310	1	0	0% - 95%	0	0% - 95%
311	15	0	0% - 20%	0	0% - 20%
312	5	0	0% - 43%	0	0% - 43%
313	110	17 (15.4%)	10% - 23%	9 (8.2%)	4% - 15%
314	192	10 (5.2%)	3% - 9%	4 (2.1%)	1% - 5%
315	26	0	0% - 13%	0	0% - 13%
317	8	2 (25.0%)	7% - 59%	0	7% - 59%
319	1	0	0% - 95%	0	0% - 95%
320	13	0	0% - 23%	0	0% - 23%
321	4	0	0% - 49%	0	0% - 49%
322	12	0	0% - 24%	0	0% - 24%
323	12	3 (25.0%)	9% - 53%	0	9% - 53%
324	26	3 (11.5%)	4% - 29%	0	4% - 29%
325	9	0	0% - 30%	0	0% - 30%
326	6	2 (33.3%)	10% - 70%	0	10% - 70%
327	20	1 (5.0%)	0% - 24%	0	0% - 24%
328	8	0	0% - 32%	0	0% - 32%
329	2	0	0% - 66%	0	0% - 66%
330	16	0	0% - 19%	0	0% - 19%
332	1	0	0% - 95%	0	0% - 95%
333	12	0	0% - 24%	0	0% - 24%
340	2	0	0% - 66%	0	0% - 66%
360	89	8 (9.0%)	5% - 17%	2 (2.2%)	1% - 8%
361	6	0	0% - 39%	0	0% - 39%
362	117	19 (16.2%)	9% - 21%	5 (4.3%)	2% - 10%
370	1	0	0% - 95%	0	0% - 95%
380	2	0	0% - 66%	0	0% - 66%
393	57	0	0% - 6%	0	0% - 6%
520	9	0	0% - 30%	0	0% - 30%
560	11	0	0% - 26%	0	0% - 26%
570	1	0	0% - 95%	0	0% - 95%
575	4	0	0% - 49%	0	0% - 49%
Total	829	65 (7.8%)		20 (2.4%)	

Table 5. Brucellosis surveillance results for adult female elk during the 2008-09 and 2009-10 survey periods by herd unit. The 95% confidence interval (CI) provides a range of where we would expect seroprevalence to be 95 out of 100 times if we conducted surveillance in a similar manner with the same sample sizes.

Herd Unit (Primary HD)	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-seropositive 95% CI
8 mile to Rock Cr. (HD 314)	24	3 (12.5%)	4%-31%	2 (8.3%)	2%-26%
Axolotl (HD 330)	3	0	0%-56%	0	0%-56%
Blacktail (HD 324)	31	2 (6.4%)	2%-21%	0	0%-11%
Blacktail Ridge-Clark Cyn	7	0	0%-35%	0	0%-35%
Cherry Cr.	14	0	0%-22%	0	0%-22%
Cherry-Morgan	3	0	0%-56%	0	0%-56%
East of Ennis	78	7 (9.0%)	4%-17%	1 (1.3%)	0%-7%
Everson Cr.	1	0	0%-95%	0	0%-95%
Fishtail Cr.	4	0	0%-49%	0	0%-49%
Greenhorns-West Slope	10	1 (10.0%)	1%-40%	0	0%-28%
HD 300	32	0	0%-11%	0	0%-11%
HD 301	3	0	0%-56%	0	0%-56%
N. Yellowstone-North Area	17	8 (47.0%)	26%-69%	4 (23.5%)	10%-47%
N. Yellowstone-South Area	94	9 (9.6%)	5%-17%	5 (5.3%)	2%-12%
NE Tobacco Roots	5	0	0%-24%	0	0%-24%
North of Big Sky	1	0	0%-95%	0	0%-95%
North of China Town	7	0	0%-35%	0	0%-35%
North of 8 Mile Cr.	3	0	0%-56%	0	0%-56%
North of Hebgen	1	0	0%-95%	0	0%-95%
North of Mill Cr.	4	2 (50.0%)	15%-85%	0	0%-49%
North of Pine Cr.	1	0	0%-95%	0	0%-95%
North portion of 302	12	0	0%-24%	0	0%-24%
Rees Hills	10	0	0%-28%	0	0%-28%
Sage Cr.	15	2 (13.3%)	4%-38%	0	5%-20%
South of Ferry Cr.	6	0	0%-39%	0	0%-39%
South of 16 Mile Cr.	10	0	0%-28%	0	0%-28%
South of Brackett Cr.	12	0	0%-24%	0	0%-24%
South of Cottonwood Cr.	3	0	0%-56%	0	0%-56%
South of Flathead Cr.	23	0	0%-14%	0	0%-14%
South of Porcupine Cr.	7	0	0%-35%	0	0%-35%
South of Red Lodge	4	0	0%-49%	0	0%-49%
South Portion of 302	1	0	0%-95%	0	0%-95%
Southwest of Hebgen	4	0	0%-49%	0	0%-49%
Sun Ranch Area	126	20 (15.9%)	9%-21%	6 (4.8%)	2%-10%
SW Tobacco Roots	14	0	0%-22%	0	0%-22%
Sweetwater Hills	2	1 (50.0%)	3%-97%	0	0%-66%
Taylor Fork	2	0	0%-66%	0	0%-66%
Tom Miner Basin	7	0	0%-35%	0	0%-35%
Trail Cr.	67	2 (3.0%)	0% -10%	0	0%-5%
Wall Cr.	18	3 (16.7%)	6%-39%	0	0%-18%
West Boulder-Greeley	8	0	0%-32%	0	0%-32%
West of Ruby Reservoir	12	0	0%-24%	0	0%-24%
West Rosebud Cr.	3	0	0%-56%	0	0%-56%
West Side Bridgers	4	0	0%-49%	0	0%-49%
Work Creek	3	0	0%-56%	0	0%-56%

Table 6. Brucellosis surveillance results for adult male elk during the 2008-09 and 2009-10 survey periods by hunting district. The 95% confidence interval (CI) provides a range of where we would expect seroprevalence to be 95 out of 100 times if we conducted surveillance in a similar manner with the same sample sizes.

HD	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-seropositive 95% CI
104	1	0	0%-95%	0	0%-95%
300	8	0	0%-32%	0	0%-32%
301	2	0	0%-66%	0	0%-66%
302	1	0	0%-95%	0	0%-95%
309	1	0	0%-95%	0	0%-95%
310	13	2 (15.4%)	4%-42%	0	0%-23%
311	12	0	0%-24%	0	0%-24%
312	4	0	0%-49%	0	0%-49%
313	31	3 (9.7%)	3%-25%	1 (3.2%)	0%-16%
314	31	0	0%-11%	0	0%-11%
315	7	0	0%-35%	0	0%-35%
317	4	1 (25.0%)	1%-70%	0	0%-49%
320	4	0	0%-49%	0	0%-49%
323	7	2 (28.6%)	8%-64%	1 (14.3%)	1%-51%
324	17	1 (5.9%)	0%-27%	1 (5.9%)	0%-27%
325	2	0	0%-66%	0	0%-66%
327	5	0	0%-43%	0	0%-43%
328	3	0	0%-56%	0	0%-56%
329	3	0	0%-56%	0	0%-56%
330	4	0	0%-49%	0	0%-49%
333	2	0	0%-66%	0	0%-66%
340	2	0	0%-66%	0	0%-66%
360	13	3 (23.1%)	8%-50%	1 (7.7%)	0%-33%
361	1	0	0%-95%	0	0%-95%
362	12	2 (16.7%)	5%-45%	0 (0%)	0%-24%
393	27	0	0%-12%	0	0%-12%
417	1	0	0%-95%	0	0%-95%
520	9	0	0%-30%	0	0%-30%
560	6	0	0%-39%	0	0%-39%
580	1	0	0%-95%	0	0%-95%
Total	234	14 (6.0%)		4 (1.7%)	

Table 7. Brucellosis surveillance results for adult male elk during the 2008-09 and 2009-10 survey periods by herd unit. The 95% confidence interval (CI) provides a range of where we would expect seroprevalence to be 95 out of 100 times if we conducted surveillance in a similar manner with the same sample sizes.

Herd Unit	# Samples	Reactors	Reactor 95% CI	WB-seropositive	WB-seropositive 95% CI
8 mile to Rock Cr.	7	0	0%-35%	0	0%-35%
Blacktail	19	1 (5.3%)	0%-25%	1 (5.3%)	0%-25%
Blacktail Ridge-Clark Canyon	1	0	0%-95%	0	0%-95%
Cherry Cr.	11	0	0%-26%	0	0%-26%
Cherry-Morgan	1	0	0%-95%	0	0%-95%
Elk-Deer-Greycliff Cr.	1	0	0%-95%	0	0%-95%
East of Ennis	9	2 (22.2%)	6%-55%	1 (11.1%)	1%-43%
Everson Cr.	1	0	0%-95%	0	0%-95%
Fishtail Cr.	1	0	0%-95%	0	0%-95%
Greenhorns-West Slope	3	0	0%-56%	0	0%-56%
HD 300	8	0	0%-32%	0	0%-32%
HD 301	3	0	0%-56%	0	0%-56%
N. Yellowstone-North Area	5	0	0%-43%	0	0%-43%
N. Yellowstone-South Area	26	3 (11.5%)	4%-29%	1 (3.8%)	0%-19%
NE Tobacco Roots	2	0	0%-66%	0	0%-66%
North of Big Sky	2	0	0%-66%	0	0%-66%
North of China Town	5	0	0%-43%	0	0%-43%
North of 8 Mile Cr.	1	0	0%-95%	0	0%-95%
North of Hebgen	1	0	0%-95%	0	0%-95%
North of Mill Cr.	3	0	0%-56%	0	0%-56%
North of Pine Cr.	1	1 (100%)	5%-100%	0	0%-95%
North portion of 302	1	0	0%-95%	0	0%-95%
Rees Hills	6	0	0%-39%	0	0%-39%
Sage Cr.	1	0	0%-95%	0	0%-95%
South of Ferry Cr.	4	0	0%-49%	0	0%-49%
South of 16 Mile Cr.	10	0	0%-28%	0	0%-28%
South of Brackett Cr.	8	0	0%-32%	0	0%-32%
South of Cottonwood Cr.	2	0	0%-66%	0	0%-66%
South of Flathead Cr.	6	0	0%-39%	0	0%-39%
South of Porcupine Cr.	1	0	0%-95%	0	0%-95%
South of Red Lodge	2	0	0%-66%	0	0%-66%
Sun Ranch Area	14	3 (21.4%)	8%-48%	0	0%-22%
SW Tobacco Roots	4	0	0%-49%	0	0%-49%
Taylor Fork	14	2 (14.3%)	4%-40%	0	0%-22%
Tom Miner Basin	1	0	0%-95%	0	0%-95%
Trail Cr.	21	0	0%-15%	0	0%-15%
Wall Cr.	9	2 (22.2%)	6%-55%	1 (11.1%)	1%-43%
West Boulder-Greeley	5	0	0%-43%	0	0%-43%
West Fork Stillwater River	2	0	0%-66%	0	0%-66%
West Rosebud Cr.	4	0	0%-49%	0	0%-49%
West Side Bridgers	3	0	0%-56%	0	0%-56%