Wyoming Game and Fish Department
2017 Brucellosis Surveillance in Non-Feedground Elk Herds
March 2018

Overview:
Each year the Wyoming Game and Fish Department (WGFD) monitors the distribution and prevalence of brucellosis within the state's elk populations by utilizing blood samples collected by hunters from their harvested animal. Approximately 10,000 blood collection kits are assembled and mailed to elk hunters successful in acquiring limited quota licenses within target surveillance areas. In general, hunters return between 1,100 and 1,600 blood samples to the laboratory, which equates to a return rate of between 25% and 36% from successful harvests. Surveillance is generally concentrated in elk herd units (HU) of the Bighorn Mountains and herds that surround the Brucellosis Designated Surveillance Area (DSA) that do not use state or federal feedgrounds (see Figure 1). In addition, nearly a quarter of the all hunt areas occurring outside of the DSA are surveyed each year; providing coverage of the entire brucellosis nonendemic area every 4-5 years.

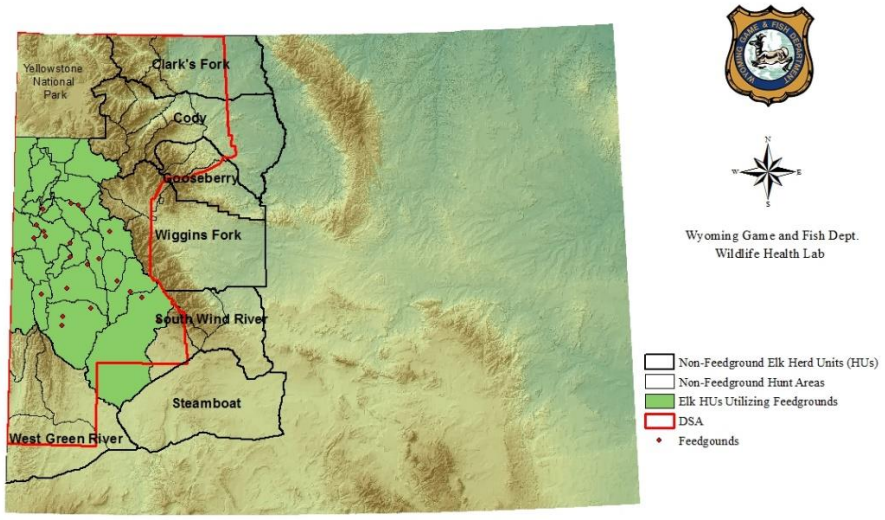

Figure 1: Locations of Wyoming Feedgrounds with Surrounding Non-Feedground Elk Herd Units and the Designated Surveillance Area (DSA)

The brucellosis surveillance program in non-feedground elk began in 1991, and over 15,600 blood samples have been analyzed for brucellosis since its inception. Brucellosis prevalence in the western portion of the state varies between 0-4% in the herd units (HUs) south of the Greater

Yellowstone Area (GYA) (i.e. South Wind River, and West Green River), and between 1-23% in the HUs east of the GYA (i.e. Clarks Fork, Gooseberry, Cody, and Wiggins Fork). In 2012, this disease was documented outside the GYA when it was discovered in elk of the northwestern Bighorn Mountains. Since the initial discovery, this disease continues to be documented at very low levels in several hunt areas along the western slope of the Bighorn Mountains. Due to the lack of effective control measures to stop the spread of this disease, the documentation of seropositive elk outside of the GYA is alarming to both livestock and wildlife managers.

To better understand brucellosis in the Bighorn Mountains, a three-year elk movement study was initiated in early 2016 to determine how this disease may have been introduced as well as to explore management implications should it become established. The study will examine movement and interactions of elk herds in the Bighorn Mountains as well as elk populations in the Bighorn Basin where seropositive animals have been previously documented. In addition, calving areas will be identified, as well as a predictive model on how brucellosis may further expand. Understanding the route of spread will enable development of management strategies that could minimize spread to neighboring elk herds as well as exposure to domestic cattle. Research elk that test seropositive for brucellosis are recaptured, euthanized, and tissues collected for culture and *Brucella* genomics.

2017 Surveillance:
The 2017 surveillance program again concentrated on the Bighorn Mountains; particularly in HAs 39, 40, 41, and 49; but was also was focused on those HAs surrounding the DSA to ensure continued monitoring of the endemic/nonendemic border. The Cody Regional Wildlife Disease Biologist continued to focus on increasing blood sample returns from hunters as well as implementing several measures to preserve blood samples prior to shipment to the laboratory. These efforts have been successful in both the northeastern DSA and the Bighorn Mountains. Surveillance outside of the known brucellosis endemic area occurred in the eastern quadrant of the State (see Figure 2).

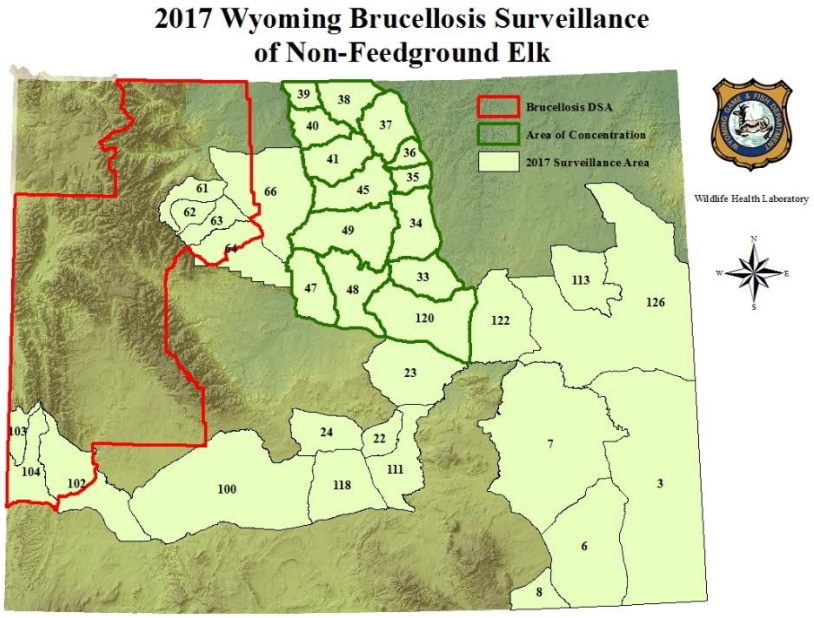

Figure 2: Elk HAs surveyed in 2017 for brucellosis in hunter-killed elk

The total number of HAs surveyed and the total number of blood collection kits to be mailed to hunters was based on the priorities of the WGFD and the Wyoming Livestock Board, while balancing the capacity of the WGFD Wildlife Health Laboratory (WHL). The 2017 surveillance effort was supported by the Department, and by a cooperative agreement with the Animal and Plant Health Inspection Service.

Methods:

In 2017, over 10,500 blood collection kits were mailed or directly handed to elk hunters successful in limited quota elk license drawings in the select (target) HAs. Kits consist of a 15ml sterile polypropylene conical tube, a paper towel, an instruction/data sheet, as well as a prepaid mailing label for return shipping. Samples were also obtained opportunistically in association with various research efforts where animals were captured and bled for disease testing.

All useable serum samples were analyzed at the WHL. Serologic assays for exposure to *B. abortus* were conducted and interpreted using current National Veterinary Services Laboratories (NVSL) protocols for the rapid automated presumptive (RAP) and fluorescence polarization assay (FPA) in microplates and tubes. Serological profiles were categorized using the United States Department of Agriculture's brucellosis eradication uniform methods and rules for Cervidae (US Department of Agriculture-APHIS 91-45-16, 2003), combined with the tristate agreement with Montana and Idaho on brucellosis testing of free-ranging elk. The RAP and FPA plate test were used to screen all samples. Positive reactions on either assay were confirmed with the FPA tube. Reactors originating outside of the known endemic area were submitted to NVSL for confirmation with the complement fixation test. Serologic data (seroprevalence levels) on elk within the known endemic area is based on yearling and adult females, but males and juveniles are included in surveillance data outside of the known endemic area. Including serologic data from males and juveniles offers improved detection of brucellosis in areas where this disease is not known to occur.

Nearly all serum samples received in 2017 were tested for exposure to *B. abortus*. This was a departure from previous years, where only the transparent serum samples were retained and tested. As serologic tests have improved and become less subjective, most hemolyzed serum samples are now suitable for testing and can contribute to surveillance data. Research by the WHL found that titers remained detectable even at 100% hemolysis, but those individual titers varied depending on the degree of hemolysis (Jessica Jennings-Gaines, unpublished data). Serum samples were only discarded if FPA delta values varied more than 15 points between duplicate runs on the same assay and could not be confirmed with the RAP, FPA tube or FPA plate. Samples that had less than 15-point variation, but could not be confirmed with RAP, FPA tube or FPA plate were submitted to NVSL for testing and classification.

Results and Discussion:

A total of 1,438 elk blood samples were received by the WHL with 1,337 (93%) of those being suitable for testing. The majority of the samples were collected from the Bighorn Mountains where 708 useable samples were tested; 153 of those were from yearlings or adult cows harvested in hunt areas where seropositive elk had been previously documented. No seropositive elk were documented in the herd units that comprise the Bighorns. Unfortunately, attempts to obtain a *B. abortus* isolate through culture of lymph nodes collected from hunter killed or movement study animals have thus far been unsuccessful.

Table 1 outlines the number of samples analyzed in each of the HAs in the Bighorn Mountains as well as the associated HU. The 95% confidence interval is also listed for each HA and HU in Table 1. This value is calculated from the total samples collected from 2012 to 2017 and provides 95% certainty the prevalence of brucellosis within that HA/HU falls within the specified range (see 95% confidence lower and upper columns), not the given prevalence determined for a particular year. Locations of all seropositive elk identified in the Bighorns are shown in Figure 3.

		2017			Total Samples 2012-2017			95% Confidence (2012-17)	
Elk HuntArea / Herd Unit (HU)	**Age/Sex**	**Samples**	**Positive**	**Prevalence**	**Samples**	**Positive**	**Prevalence**	**Lower**	**Upper**
33	All	31	0	0.0%	106	0	0.0%	0.0%	3.4%
34	All	36	0	0.0%	162	0	0.0%	0.0%	2.3%
47	All	4	0	0.0%	42	0	0.0%	0.0%	8.4%
48	All	56	0	0.0%	142	0	0.0%	0.0%	2.6%
49	All	94	0	0.0%	274	1	0.4%	0.0%	2.0%
	Cows	50	0	0.0%	81	0	0.0%	0.0%	4.5%
120	All	27	0	0.0%	117	0	0.0%	0.0%	3.1%
Total South Bighorn HU	All	248	0	0.6%	843	1	0.1%	0.0%	0.7%
	Cows	50	0	0.0%	81	0	0.0%	0.0%	4.5%
35	All	25	0	0.0%	136	0	0.0%	0.0%	2.7%
36	All	19	0	0.0%	73	0	0.0%	0.0%	4.9%
37	All	41	0	0.0%	134	0	0.0%	0.0%	2.7%
38	All	93	0	0.0%	538	0	0.0%	0.0%	0.7%
39	All	47	0	0.0%	205	1	0.6%	0.0%	2.7%
	Cows	25	0	0.0%	114	0	0.0%	0.0%	3.2%
40	All	76	0	0.0%	365	8	2.2%	1.0%	4.3%
	Cows	31	0	0.0%	203	7	3.4%	1.4%	7.0%
Total North Bighorn HU	All	301	0	0.0%	1451	9	0.6%	0.3%	1.2%
	Cows	56	0	0.0%	317	7	2.2%	0.9%	4.5%
41	All	80	0	0.0%	449	1	0.2%	0.0%	1.2%
	Cows	47	0	0.0%	259	0	0.0%	0.0%	1.4%
45	All	79	0	0.0%	347	0	0.0%	0.0%	1.1%
Total Medicine Lodge HU	All	159	0	0.0%	796	1	0.1%	0.0%	0.7%
	Cows	47	0	0.0%	259	0	0.0%	0.0%	1.4%
Total Bighorns	All	708	0	0.0%	3090	11	0.4%	0.2%	0.6%
	Cows	153	0	0.0%	657	7	1.1%	0.4%	2.2%

Table 1. Total useable blood samples tested from elk harvested in the Bighorn Mountains along with the 95% confidence interval of seroprevalence based on total samples 2012 to 2017

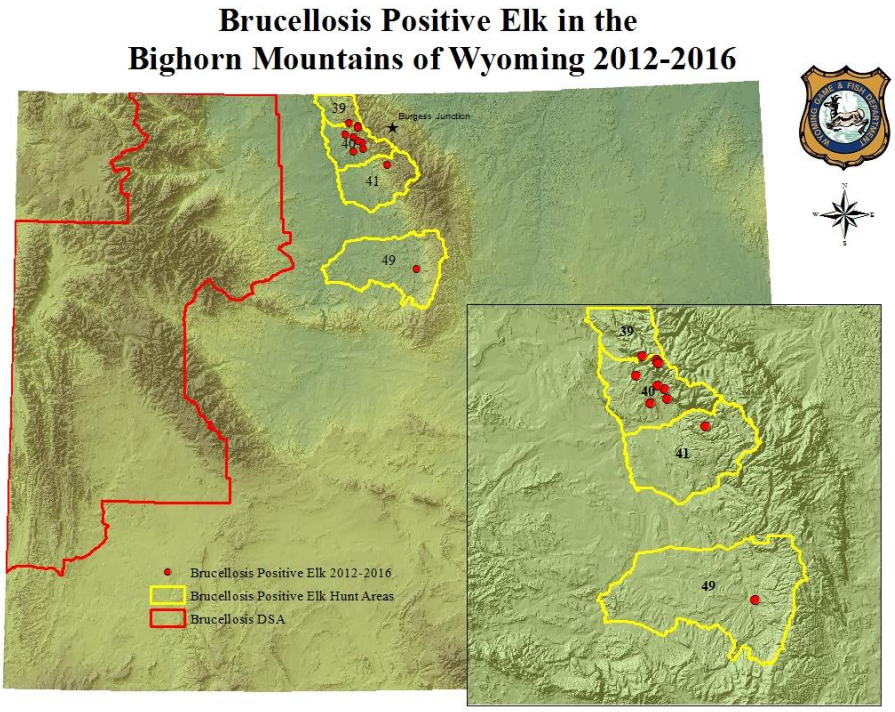

Figure 3: Locations of seropositive elk in the Bighorn Mountains, no new positives were identified in 2017.

Brucellosis surveillance in the combined northern HUs (Clark's Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA, documented an increase in seroprevalence over the past five years (15.9%; n=862 samples) compared to the previous five-year average of 12.6% (n=1,324) (see Figure 4). The five-year average seroprevalence between the four northern HUs varied considerably (see Figure 5). Brucellosis seroprevalence in the Gooseberry and Cody HUs has significantly increased ($p<0.10$) from 11.1% in 2008-2012 (n=569) to 14.7% in 2013-2017 (n=566), and 17.5% in 2008-2012 (n=291) to 22.7% in 2013-2017 (n=225) respectively. Sample sizes obtained from the Wiggins Fork and Clarks Fork HUs in 2003 through 2017 were insufficient to accurately estimate prevalence, and it is important to note that in most hunt areas, the sample sizes achieved through our annual surveillance are insufficient to estimate prevalence with good precision. Therefore, prevalence figures are combined into five-year totals to improve sample size and allow for statistical analysis.

Brucellosis seroprevalence is also monitored within individual elk hunt areas of the DSA. Over the past 24 years, seroprevalence has gradually increased in hunt areas 58-59 and 61-63 (see Figure 6). Overall, the combined seroprevalence in these areas has averaged 19.5% (n=600) over the last five years; a significant increase over the previous five-year average of 14.3% (n=735). Many of the subpopulations in these hunt areas have been examined to determine if the increase in seroprevalence can be attributed to increasing elk density. The research found that the rates of increase were positively related to both large and small groups at high density, as well as larger groups at low densities (Brennan et al., 2017). In addition, these authors note that disease management strategies aimed at reducing population density or group sizes are unlikely to reduce transmission of the disease. The steady increase in prevalence in these populations is alarming and may soon mirror prevalence's found on many of the State's feedgrounds. Continued monitoring of all HAs along the southeastern slope of the Absaroka Range is

warranted, as well as exploration of management actions that affect the prevalence of brucellosis in these populations.

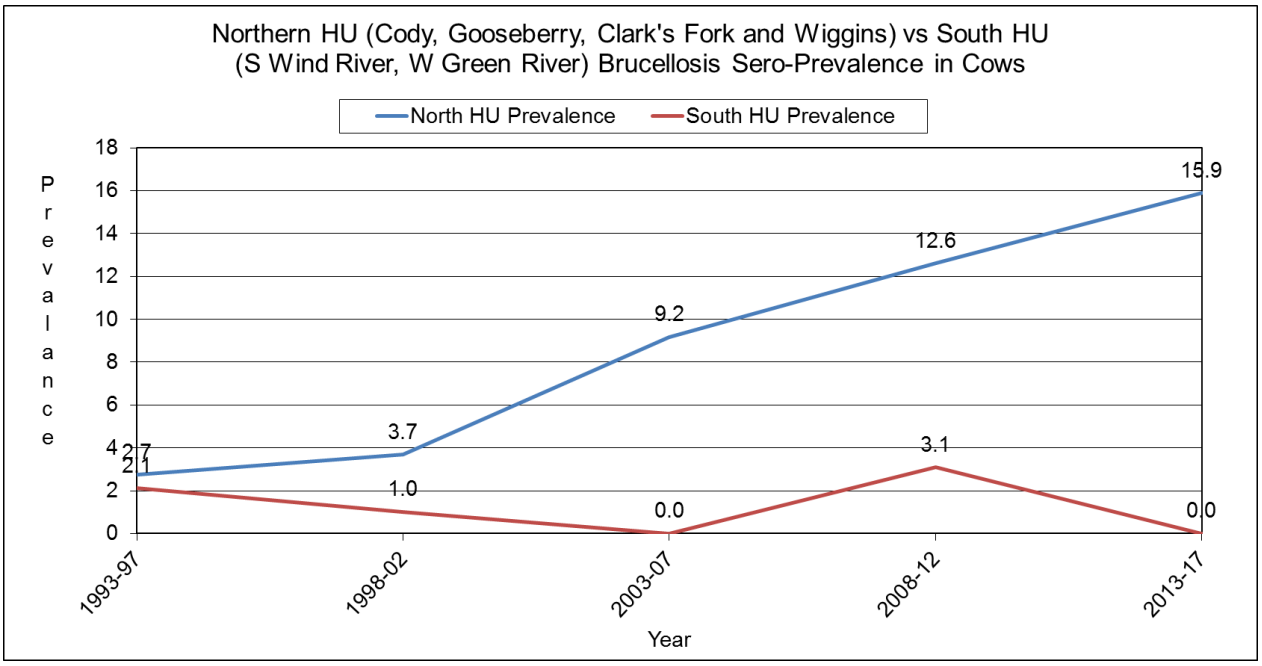

Figure 4: Seroprevalence through time in northern and southern elk herd units (HU) surrounding the DSA

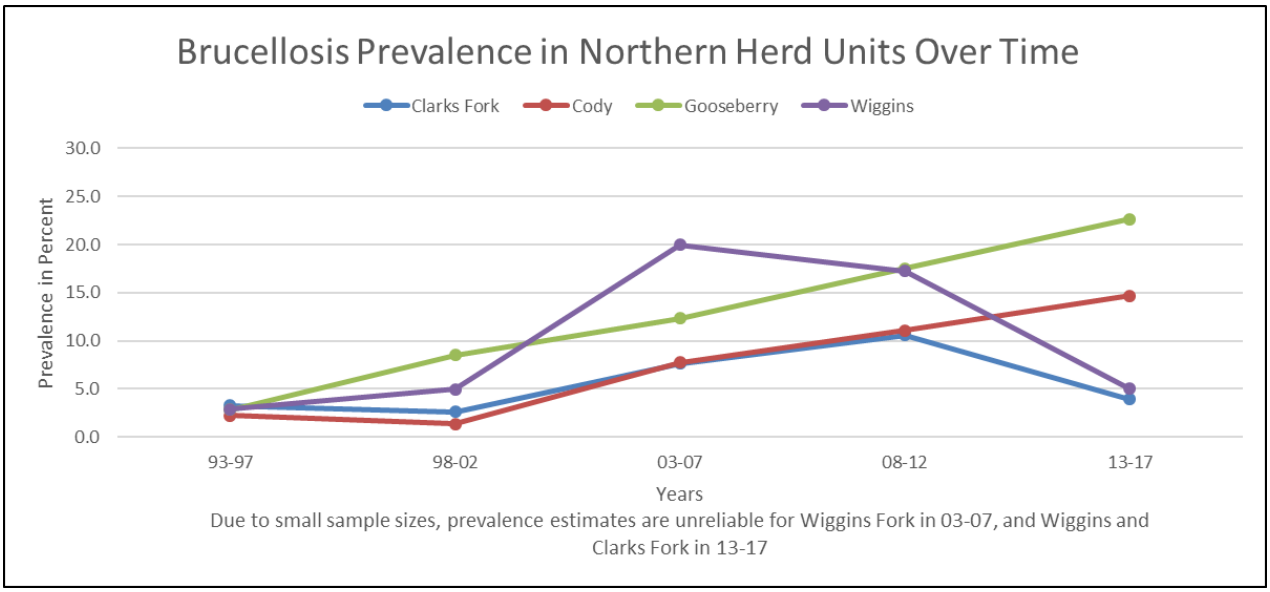

Figure 5: Brucellosis prevalence in the Clarks Fork, Cody, Gooseberry and Wiggins elk herd units over time

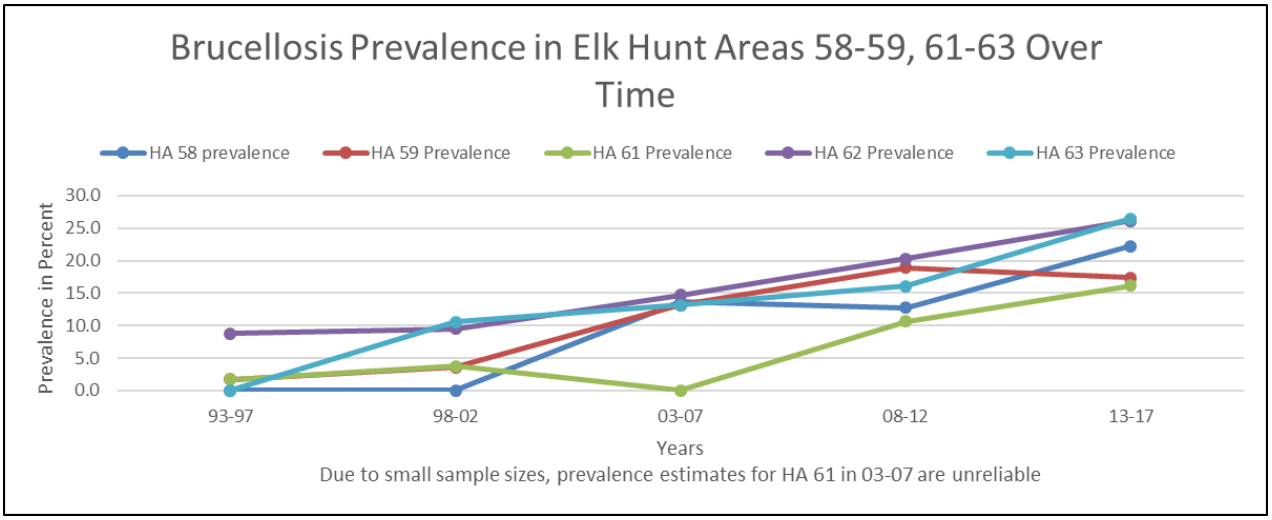

Figure 6. Brucellosis prevalence in elk HAs 58-59 and 61-63 over time; sample sizes in HA 61 in 03-07 are insufficient and unreliable.

In 2017, ten suitable samples were received from cows harvested from either the South Wind River or the West Green River elk HUs of the southern DSA. Over the past five years, a total of only 46 samples have been analyzed from these HU, with no seropositive animals identified (see figure 4).

A total of 112 useable samples were collected in 2017 in the eastern portion of the state (HAs 3, 6-8, 113, 122, and 126). All samples tested negative for exposure to *B. abortus* on serological tests. In the past 27 years, 5,103 samples from the non-endemic area have been analyzed. To date, this disease has not been documented outside of western half of the state (see Figure 7).

The return rate of blood samples from hunters successful in harvesting an elk has remained surprising constant over the past five years. From 2012 to 2016, the average return rate was 1,404 samples with 970 (69%) of those being suitable for testing. On a per hunter basis, approximately 32% of successful hunters that had received a blood collection kit, voluntarily collected and submitted a sample for surveillance (harvest success of elk in Wyoming averages 44%).

The newfound ability to utilize hemolyzed blood samples has greatly increased the number of samples that can now be included in surveillance data. This year, 93% of blood samples received were tested, a significant increase over the past five-year average of 69%. Further research is planned to determine if filtering or other manipulations can increase the number of useable samples.

Brucellosis surveillance for 2018 will again concentrate on the Bighorn mountains as well as those hunt areas that surround the eastern DSA border (see Figure 8). Surveillance within the DSA will shift to the Wiggins Fork HU, which hasn't been surveyed since 2010. Although statewide surveillance normally alternates through the elk hunt areas in the southern and eastern portions of the state, in 2018, this effort will be directed to the south-central area of the State (elk hunt areas 16, 19, 22-25, 27, 28, 98, 99, 111, 118, and 128).

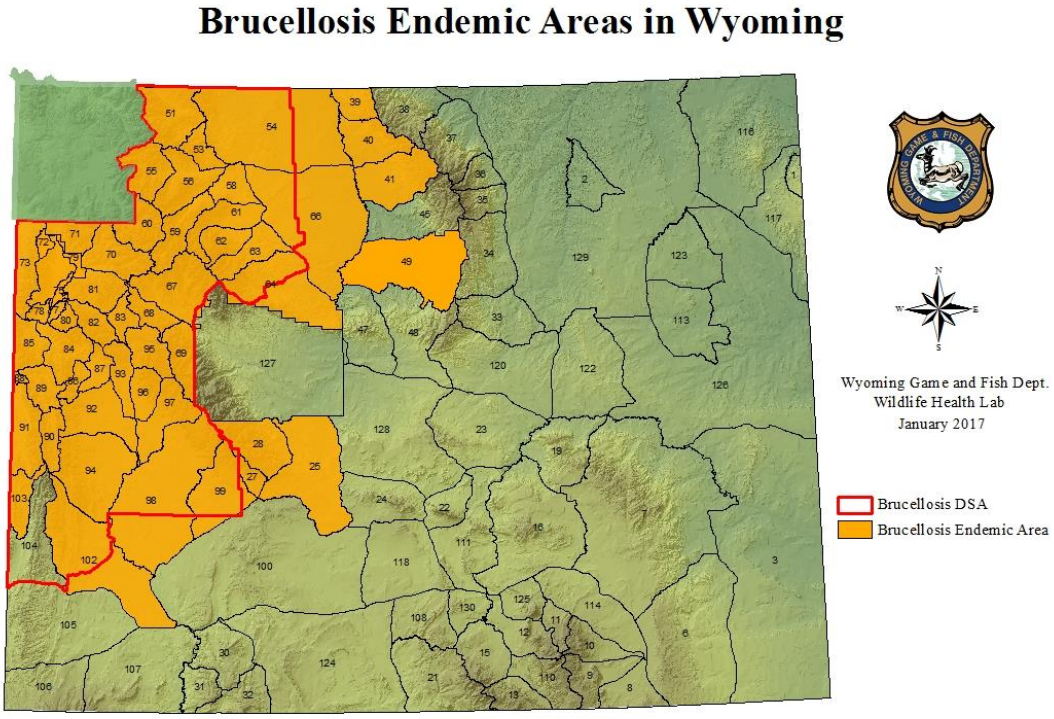

Figure 7: Brucellosis Endemic Elk Hunt Areas in Wyoming

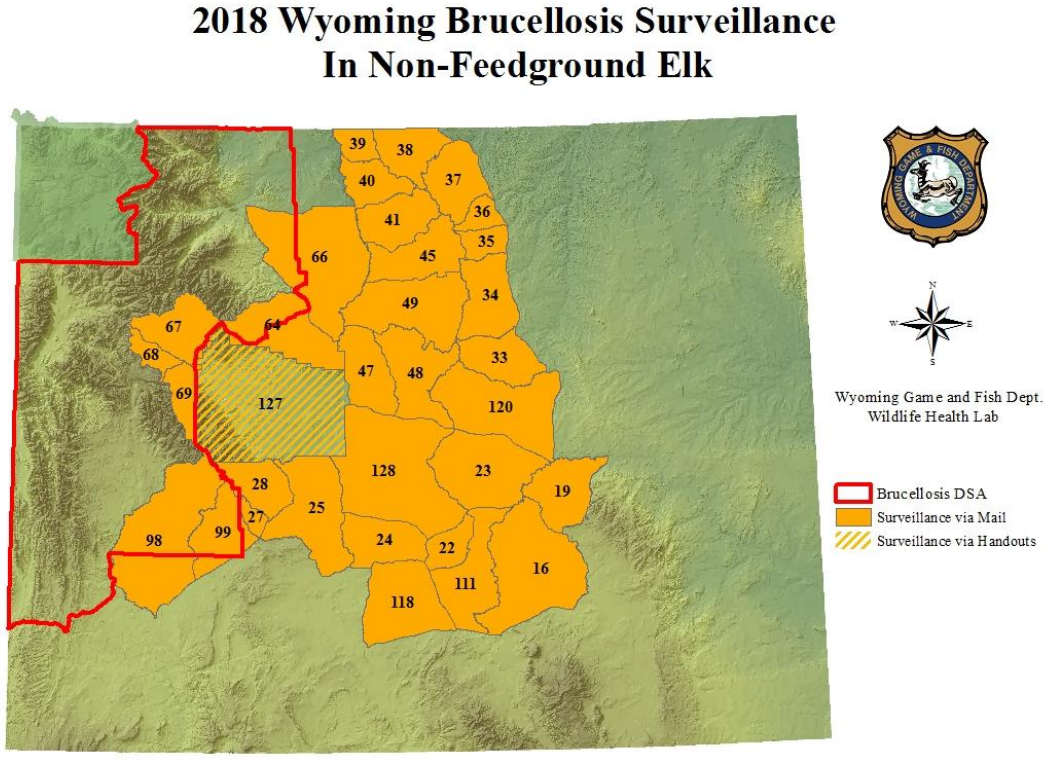

Figure 8: Elk hunt areas targeted for brucellosis surveillance in 2018

Literature cited

Brennan, A., P. C. Cross, M. D. Higgs, W. H. Edwards, B. M. Scurlock, and S. Creel. 2014. A multi-scale assessment of animal aggregation patterns to understand increasing pathogen seroprevalence. Ecosphere 5(10):138. http://dx.doi.org/10.1890/ES14-00181.1