Wyoming Game & Fish Department
Wildlife Health Laboratory

Wyoming Game and Fish Department
2020 Brucellosis Surveillance in Non-Feedground Elk Herds
March 2020

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. Surveillance is generally concentrated in herds that surround the Brucellosis Designated Surveillance Area (DSA) that do not use state or federal feedgrounds (see Figure 1), and in elk herd units (HUs) of the Bighorn Mountains. In addition, around a quarter of the all hunt areas (HAs) located outside of the DSA are surveyed each year, providing coverage of the entire brucellosis non-endemic area every 4-5 years.

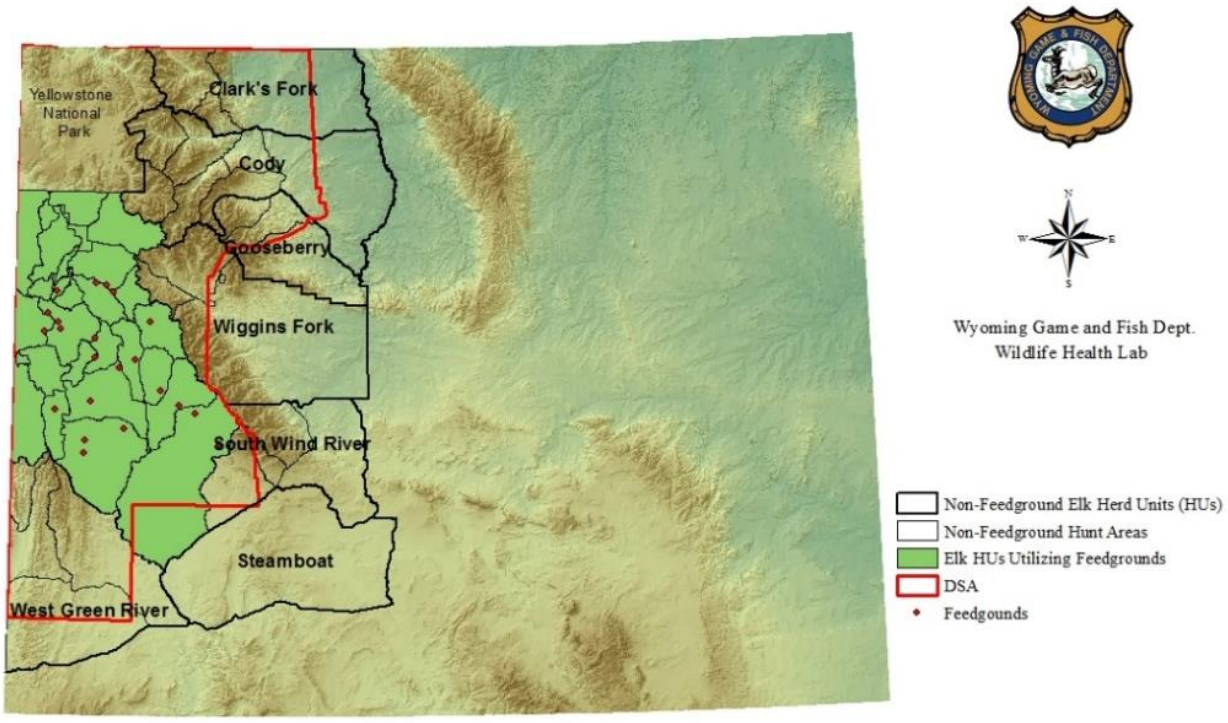

Figure 1. Locations of Wyoming feedgrounds, surrounding non-feedground elk herd units, and the Designated Surveillance Area (DSA)

The brucellosis surveillance program in non-feedground elk began in 1991, and over 19,000 blood samples have been analyzed for brucellosis since its inception. Brucellosis prevalence in the western portion of the state varies between 0-5% in the herd units south of the Greater Yellowstone Area (GYA) (i.e. South Wind River, and West Green River), and between 8-22% in the HUs east of the GYA (i.e. Clark's Fork, Gooseberry, Cody, and Wiggin's 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 has been sporadically detected in several hunt areas along the western slope of the Bighorn Mountains. Due to the lack of effective control measures to mitigate 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 multi-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 a predictive model of how brucellosis may further expand. Understanding the route of spread will enable development of management strategies that could minimize spread to neighboring elk herds and exposure to domestic cattle. Research elk that test seropositive for brucellosis are recaptured, euthanized, and tissues collected for culture and *Brucella* genomics.

Methods:

In 2020, around 9,000 blood collection kits were mailed or directly handed out to elk hunters that were successful in limited quota elk license drawings, for targeted surveillance hunt areas. Kits consist of a 15 ml 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 sampled for disease testing.

All useable serum samples were analyzed at the WGFD Wildlife Health Laboratory (WHL). Serologic assays for exposure to *B. abortus* were conducted and interpreted using current National Veterinary Services Laboratories (NVSL) protocols for fluorescence polarization assay (FPA) in tubes and assay kit protocols for FPA in plates. The FPA plate test was used to screen all samples, positive reactions on the plate assay were confirmed with the FPA tube test. 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.

As serologic tests have improved and become less subjective, most hemolyzed serum samples are now suitable for testing and can contribute to surveillance data (Jennings-Gaines et al., 2021). Over 96% of serum samples received in 2020 were considered testable for exposure to *B. abortus*. Hemolyzed serum samples were only discarded if the samples were received from inside the endemic area within the DSA. If FPA results varied more than 15 points between duplicate runs

on the same assay, and could not be confirmed upon re-test, the sample was considered not testable. Samples that had less than 15-point variation, but could not be confirmed were submitted to NVSL for testing and classification.

2020 Surveillance:
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 included a chance at a raffle to win valuable hunting equipment for submitting a testable blood sample, and setting up multiple sample drop off points to prevent freezing of blood samples while in the mail. Brucellosis surveillance for 2020 concentrated on the western slope of the Bighorn Mountains as well as the Snowy Range, Sierra Madre and the southern border of the DSA (see Figure 2). Surveillance within the DSA shifted to the Cody, Clarks Fork and Gooseberry HUs (see Figure 5). Statewide surveillance normally alternates through the elk hunt areas in the southern and eastern portions of the state, and in 2020 this effort was directed to the southcentral portion of the State.

The number of HAs surveyed and the number of blood collection kits mailed to hunters was based on the priorities of the WGFD and the Wyoming Livestock Board, while balancing the capacity of the WHL. The 2020 surveillance effort was supported by the Department, and by a cooperative agreement with the Animal and Plant Health Inspection Service.

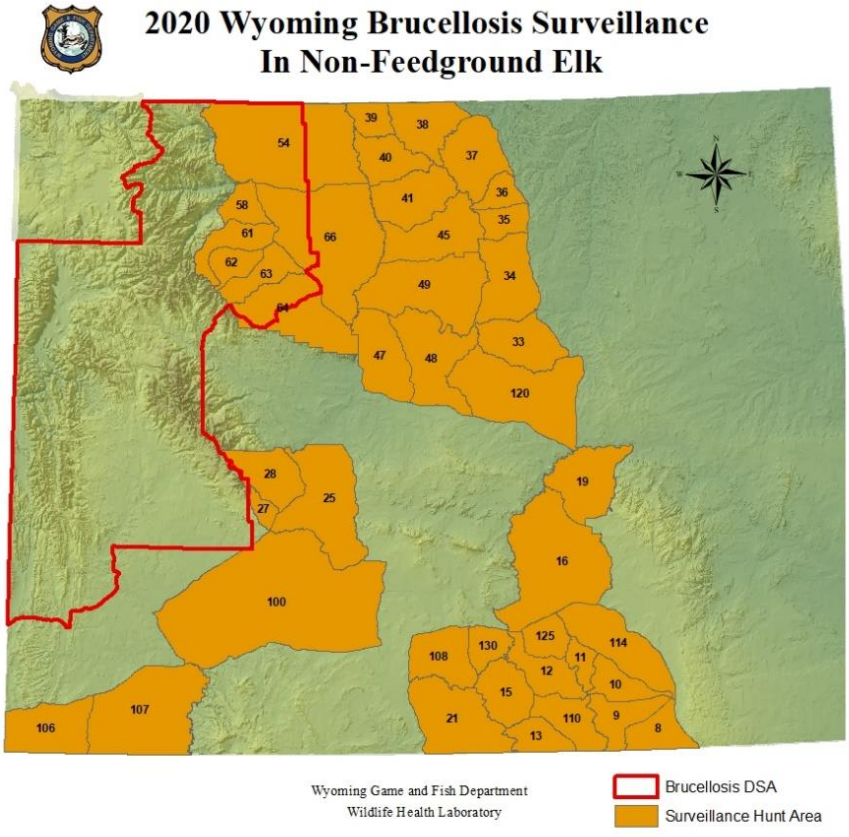

Figure 2. Elk HAs surveyed in 2020 for brucellosis in hunter-killed elk.

Results and Discussion:

A total of 1,130 elk blood samples were received by the WHL in 2020. Of those, 1,093 were suitable for testing. 444 useable samples were collected from the Bighorn Mountains; 105 of those were from adult cows harvested in hunt areas where seropositive elk had been previously documented (see Figure 3). No seropositive elk were detected in 2020, marking the fourth year that no new seropositive elk have been identified in the herd units that comprise the Bighorns.

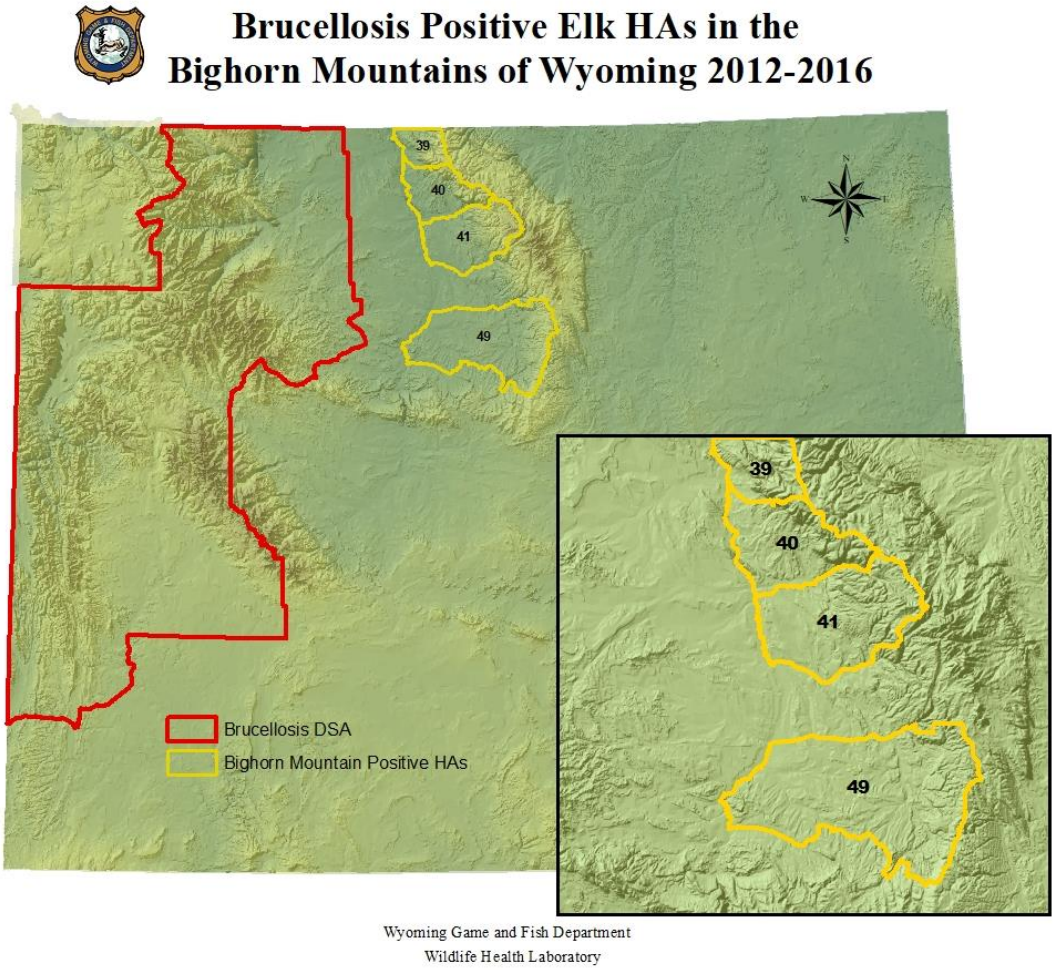

Figure 3. HAs of seropositive elk in the Bighorn Mountains; no new positives have been identified since 2016.

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 2016 to 2020 and provides 95% certainty that 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.

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 2016 to 2020.

		2020			Total Samples 2016-2020			95% Confidence (2016-2020)	
Elk Hunt Area / Herd Unit (HU)	**Age/Sex**	**Samples**	**Positive**	**Prevalence**	**Samples**	**Positive**	**Prevalence**	**Lower**	**Upper**
33	All	6	0	0.0	84	0	0.0%	0.0%	4.3%
34	All	23	0	0.0	181	0	0.0%	0.0%	2.0%
47	All	14	0	0.0	48	0	0.0%	0.0%	7.4%
48	All	25	0	0.0	190	0	0.0%	0.0%	1.9%
49	All	42	0	0.0	343	1	0.3%	0.0%	1.6%
	Cows	19	0	0.0	182	0	0.0%	0.0%	2.0%
120	All	17	0	0.0	123	0	0.0%	0.0%	3.0%
Total South Bighorn HU	All	127	0	0.0	969	1	0.1%	0.0%	0.6%
	Cows	59	0	0.0	434	0	0.0%	0%	0.8%
35	All	9	0	0	116	0	0.0%	0.0%	3.1%
36	All	9	0	0	75	0	0.0%	0.0%	4.8%
37	All	12	0	0	159	0	0.0%	0.0%	2.3%
38	All	71	0	0	482	0	0.0%	0.0%	0.8%
39	All	61	0	0	237	0	0.0%	0.0%	1.5%
	Cows	36	0	0	123	0	0.0%	0.0%	3.0%
40	All	47	0	0	352	3	0.9%	0.2%	2.5%
	Cows	18	0	0	154	3	1.9%	0.4%	5.6%
Total North Bighorn HU	All	209	0	0.0	1421	3	0.2%	0.0%	0.6%
	Cows	129	0	0.0	824	3	0.4%	0.1%	1.1%
41	All	63	0	0.0	423	0	0.0%	0.0%	0.9%
	Cows	32	0	0.0	249	0	0.0%	0.0%	1.5%
45	All	45	0	0.0	352	0	0.0%	0.0%	1.0%
Total Medicine Lodge HU	All	108	0	0.0	775	0	0.0%	0.0%	0.5%
	Cows	60	0	0.0	458	0	0.0%	0.0%	0.8%
Total Bighorns	All	444	0	0.0	3165	4	0.1%	0.0%	0.3%
	Cows	248	0	0.0	1716	3	0.2%	0.0%	0.5%

Brucellosis seroprevalence is monitored within individual elk hunt areas of the DSA (see Figure 4). Over the past twenty-five years, seroprevalence has gradually increased in hunt areas 58-59 and 61-63. In the last five years, the combined seroprevalence in these areas has averaged 21.2% (*n*=405). 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. 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., 2014). 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. 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.

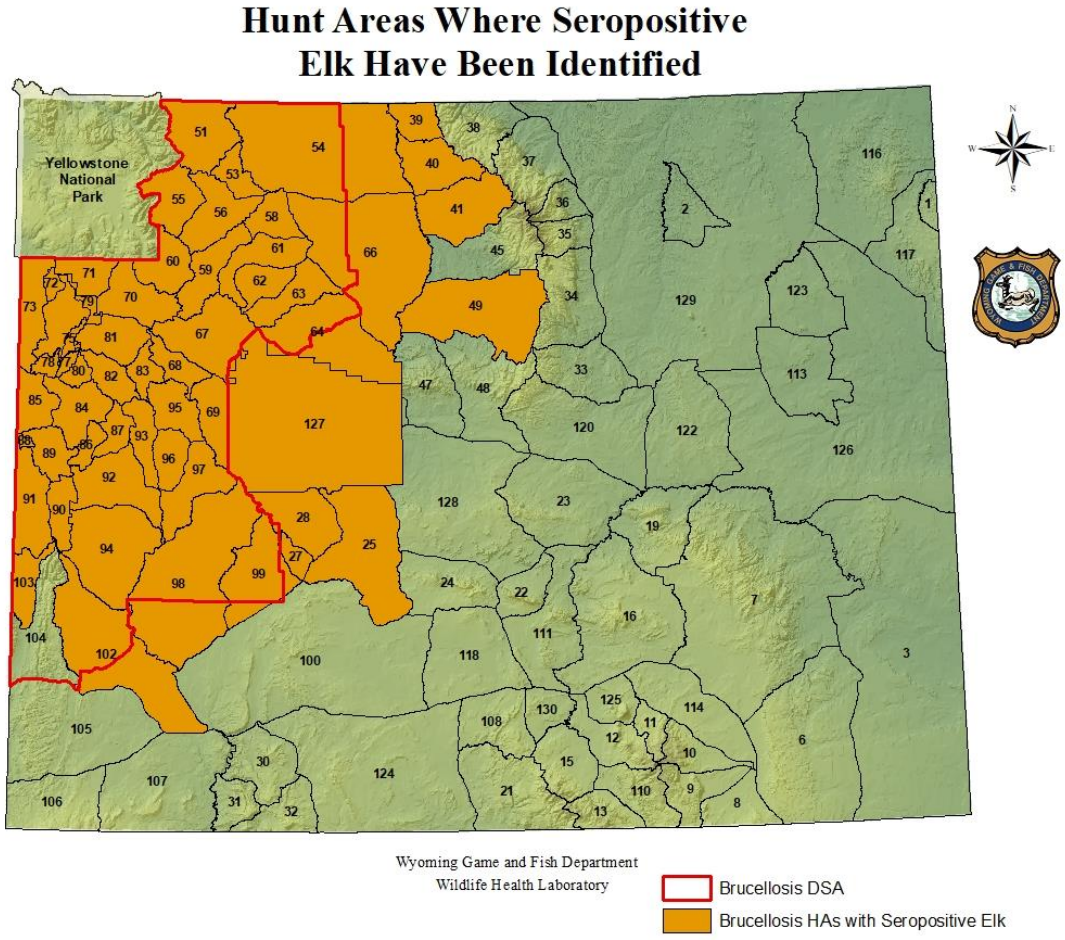

Figure 4. Hunt Areas with Seropositive Elk and Brucellosis DSA.

Brucellosis surveillance in the combined northern HUs (Clark's Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA (see Figure 5) reported a slight increase in seroprevalence over the past five years (14.4%; n=967) compared to the previous five-year average of 12.6% (n=886). Seroprevalence, however, has been relatively stable when compared to the last ten year average (13.9%; n=1,972).

In 2018, enhanced surveillance efforts were initiated in Southern HUs bordering the DSA. These efforts continued in 2020 in the South Wind River, Steamboat and Uinta HUs (see Figure 5). A combined twenty-nine samples were received from the southern HUs with no seropositive elk identified. Seroprevalence of the Southern HUs remains lower than the Northern HUs (see Figure 6).

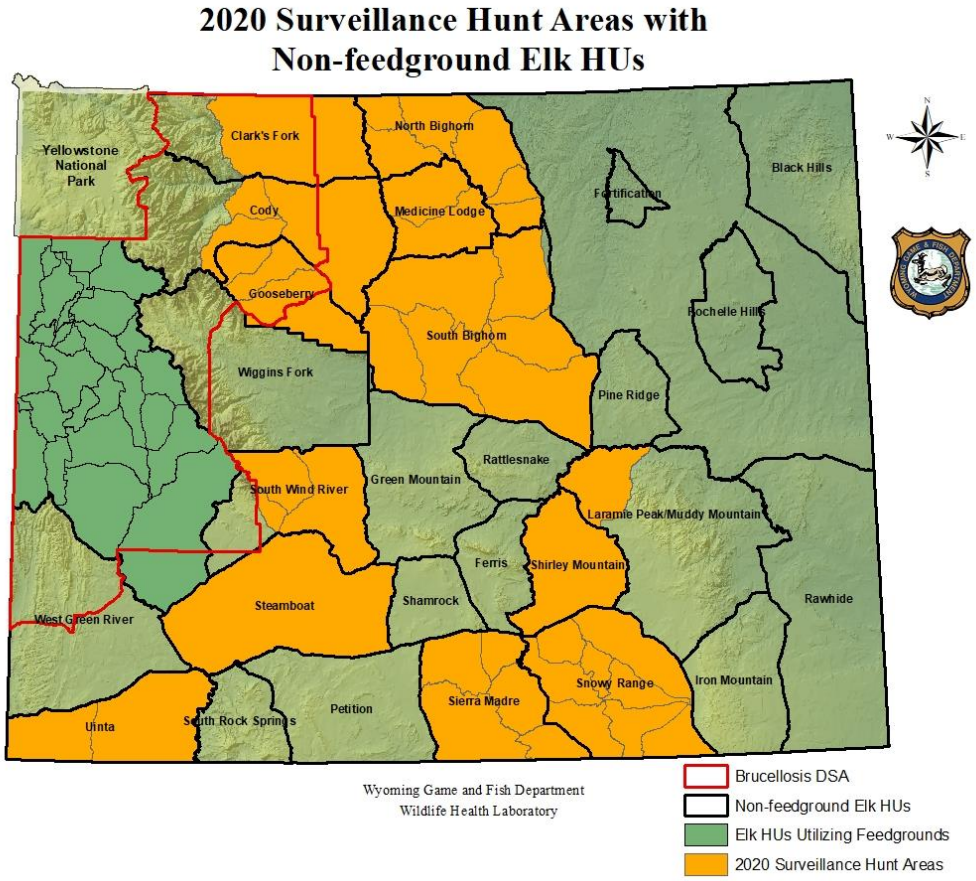

Figure 5. Elk HUs utilizing feedgrounds, non-feedground elk herd units, and the Designated Surveillance Area (DSA).

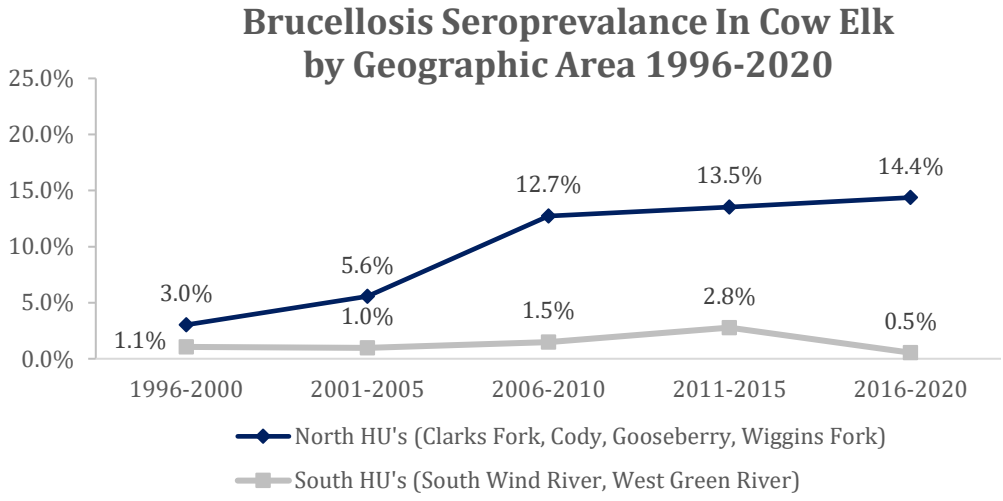

Figure 6. Comparison of brucellosis seroprevalence in cow elk of the Northern and Southern herd units from 1996-2020. Southern HUs sample size are low for years 2013-2017; in years 2001, 2003, 2004, 2008, 2016 $n \leq 1$. Northern HUs sample size are adequate most years; 2002, 2005 $n = 0$.

The five-year average seroprevalence varied between the four northern HUs (see Figure 7). It is important to note that sample sizes from 2001-2005 are low (n ≤ 49) in all northern HUs. Zero samples were collected for each HU 2001-2005, with the exception of Cody (2004; n =36), Gooseberry (2003; n=41) and Wiggins (2001; n=49). Sample sizes are generally low and affect the accuracy of prevalence estimates for the individual HUs. In most areas, 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.

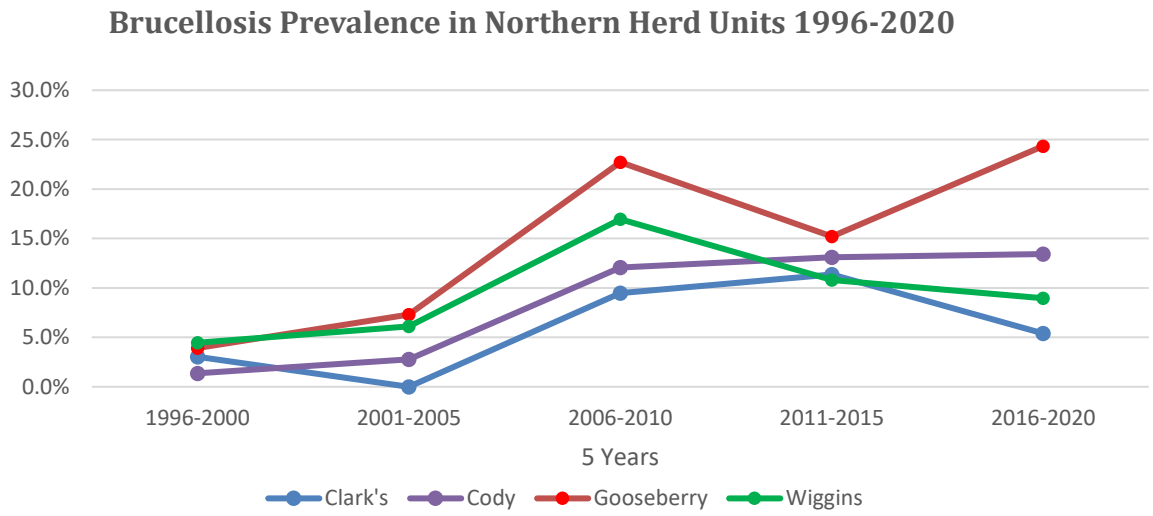

Figure 7. Seroprevalance over time in cow elk from the Northern HUs

From the rotating surveillance program target areas (8,9,10,11,12,13,15,16,19,21,108,110, 114,125,130), 192 useable samples were collected. All samples tested negative for exposure to *B. abortus* on serological tests. In the past 29 years, 6,727 samples from the non-endemic area have been analyzed. To date, this disease has not been documented outside of western half of the state.

2021 Surveillance:

In 2021, the rotating surveillance area will shift to target the eastern edges of Wyoming (see Figure 8). This encompasses elk hunt areas 1,2,3, 6,7,113,116,117,122,123,126 and 129. Efforts to survey around the DSA border and the western edge of the Bighorn Mountains will continue as well as areas within the DSA (Northern HUs).

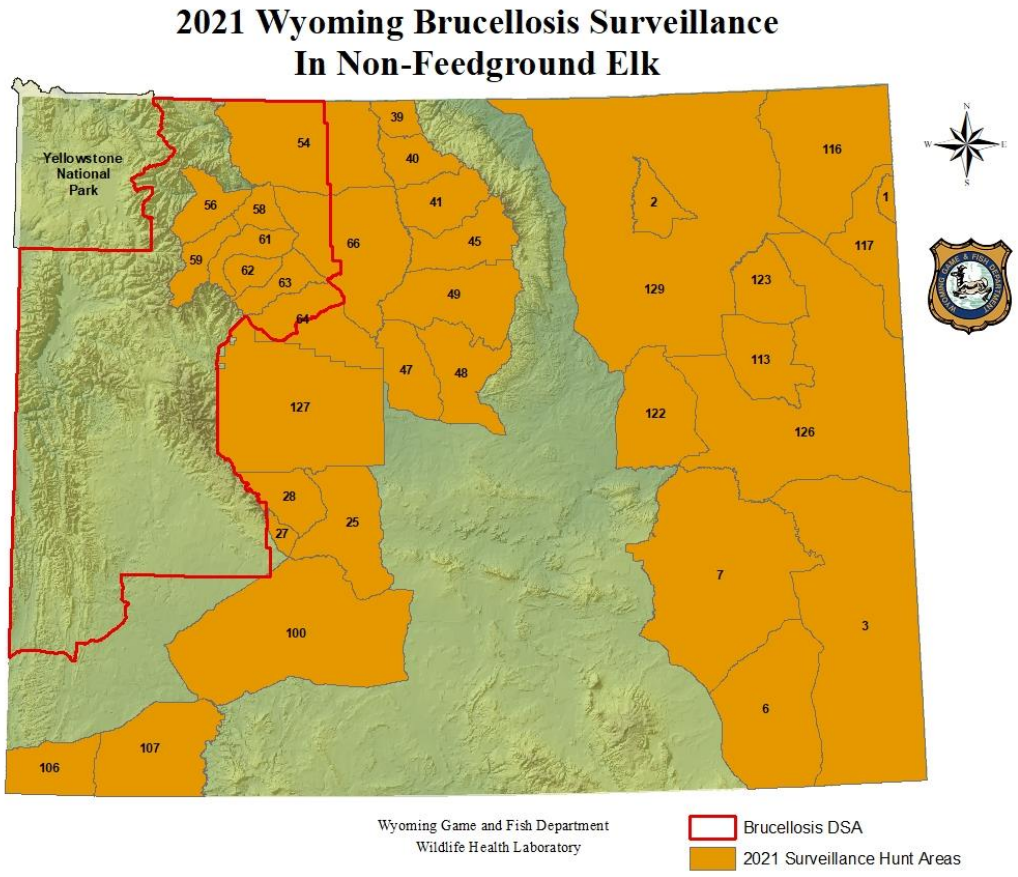

Figure 8. Proposed elk hunt areas to target for brucellosis surveillance in 2021.

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

Jennings-Gaines, J. E., W. H. Edwards, and T. J. Robinson. 2021. Determining Antibody Retention in Hemolyzed, Bacterially Contaminated, and Nobuto Filter Paper-Derived Serum Utilizing Two Brucella abortus Fluorescence Polarization Assays. Journal of Wildlife Diseases 57(2).