Wyoming Game & Fish Department
Wildlife Health Laboratory

Wyoming Game and Fish Department
2021 Brucellosis Surveillance in Non-Feedground Elk Herds
March 2021

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. Between 8,000 and 9,000 blood collection kits are 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). Additionally, 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.

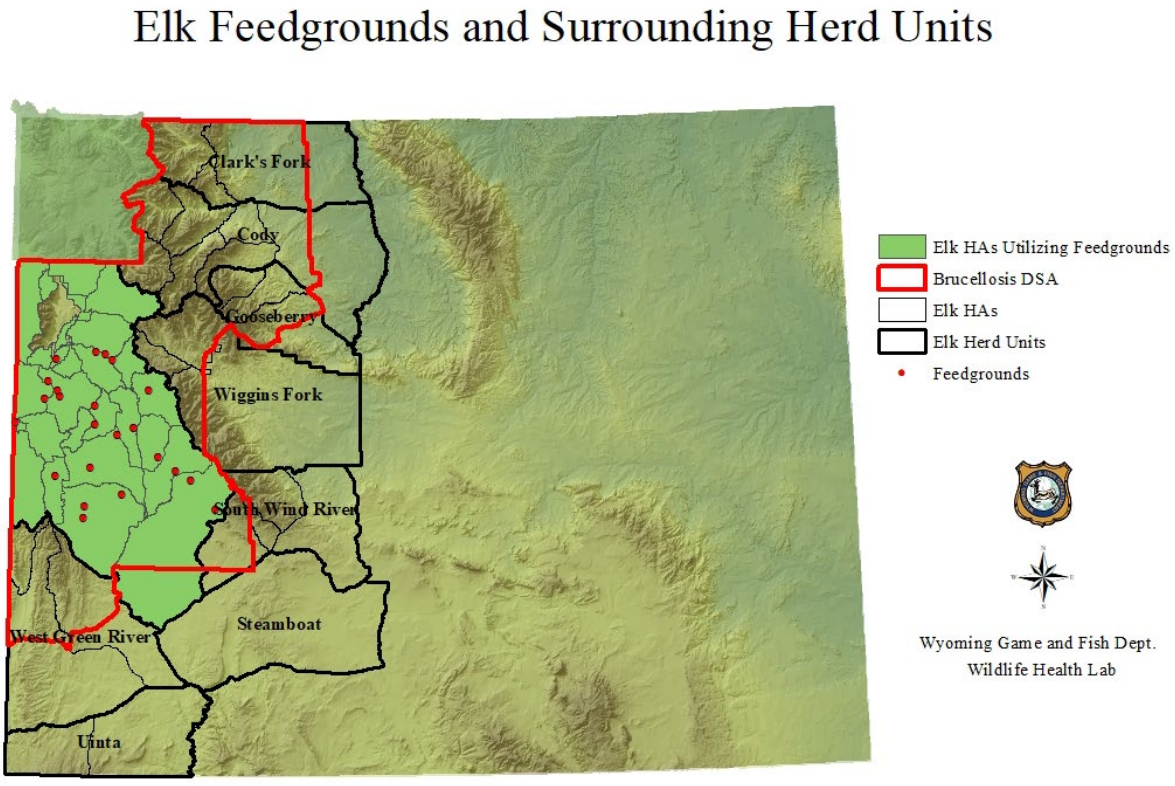

Figure 1. Locations of Wyoming feedgrounds, surrounding non-feedground elk herd units, and the Designated Surveillance Area (DSA)

The brucellosis surveillance program in non-feedground elk began in 1991, and approximately 20,500 blood samples have been analyzed for brucellosis since its inception. Brucellosis prevalence south of the Greater Yellowstone Area (GYA) in elk herd units (HUs) in the southern DSA, varies between 0-6% (i.e. South Wind River and West Green River), and between 8-22% in HUs east of the GYA (i.e. Clark's Fork, Gooseberry, Cody, and Wiggin's Fork). Antibodies to this disease were detected in a hunter-harvested elk on the western slope of the Bighorn Mountains in HA 41 in 2012. Over the course of the next four years, a total of 11 seropositive elk were detected in four nearby hunt areas (see Figure 2), but there have been no detections since 2016, despite focused surveillance efforts. 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.

Hunt Areas Where Seropositive Elk Have Been Identified

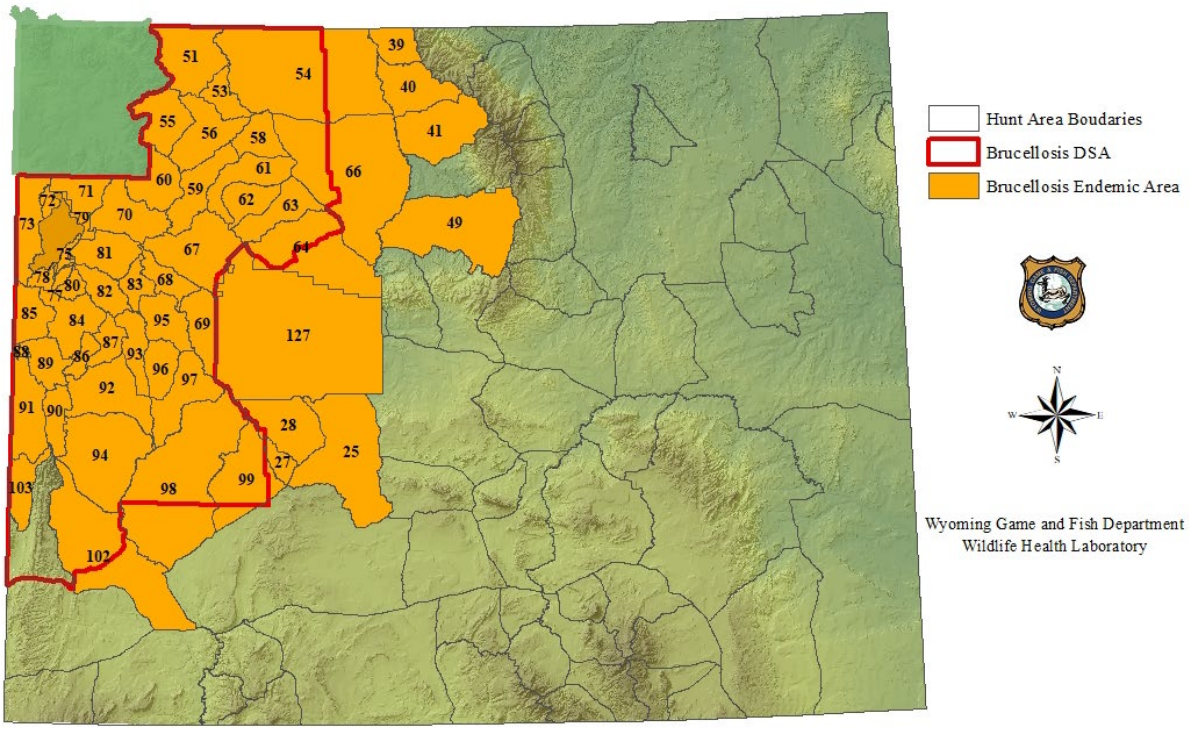

Figure 2. Hunt Areas with known seropositive elk and Brucellosis DSA.

Methods:

Blood collection kits were mailed or directly handed out to elk hunters in targeted hunt areas. Kits consisted of a 15 ml sterile polypropylene conical tube, a paper towel, an instruction/data sheet, and 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 samples were analyzed at the WGFD Wildlife Health Laboratory (WHL). Serologic assays for exposure to *Brucella abortus* were conducted and interpreted using current assay kit protocols for FPA plate tests and National Veterinary Services Laboratories (NVSL) protocols for fluorescence polarization assay (FPA) tube tests. The FPA plate test was used to screen all samples, and all positive reactions on the plate assay were confirmed with the FPA tube test. Serologic data (seroprevalence levels) on elk within the known endemic area are 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 increased sample size in those areas outside the known endemic area (Jennings-Gaines et al., 2021). Hemolyzed serum samples received from within the DSA are discarded.

2021 Surveillance:
In 2021, 9,000 test kits were mailed to or directly handed out to hunters with licenses within targeted surveillance areas. Surveillance included the western slope of the Bighorn Mountains, the eastern border of the DSA, and down to the southwestern corner of the state (see Figure 3). Surveillance within the DSA included Cody, Clarks Fork, Gooseberry, and Wiggins Fork HUs. Statewide surveillance alternates through elk areas outside of the DSA and in 2021 this effort was directed to the eastern side of the state.

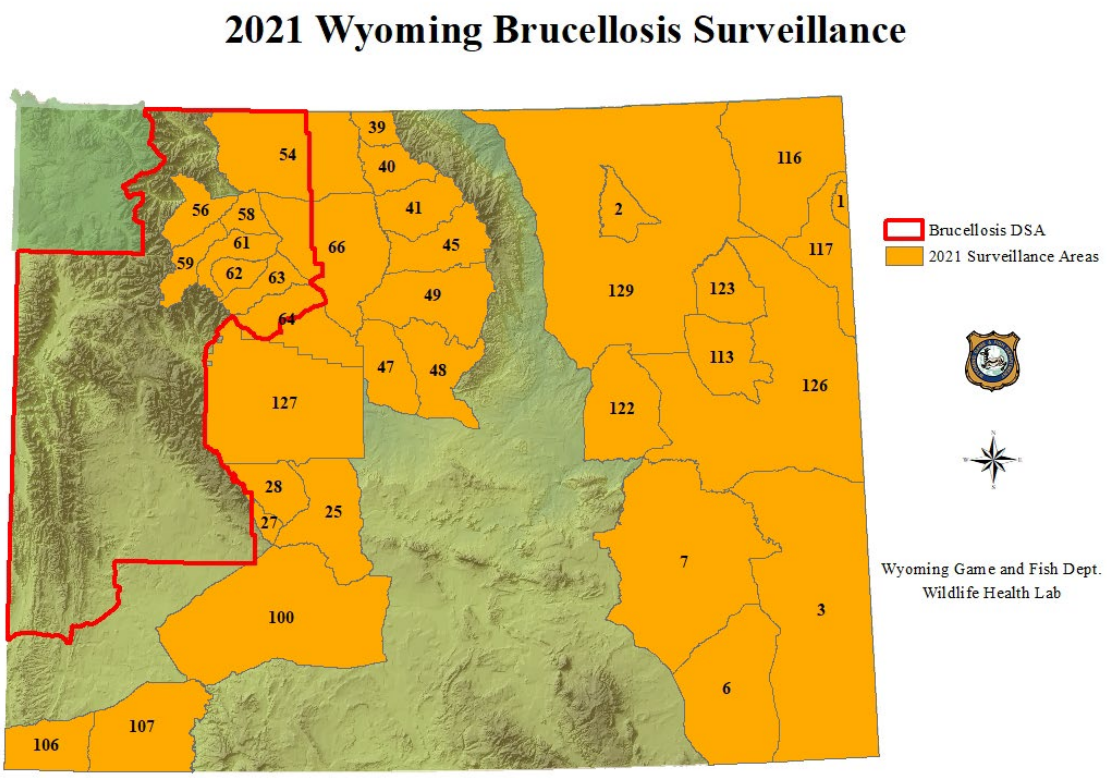

Figure 3. Elk Hunt Areas surveyed in 2021 for brucellosis in hunter-killed elk.

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 2021 surveillance effort was supported by the Department, and by a cooperative agreement with the Animal and Plant Health Inspection Service.

Results and Discussion:
In 2021, 875 elk blood samples were received by the WHL. Of those, 815 were suitable for testing. These sample numbers are lower than returns seen in previous years. In 2020, the WHL received 1,130 samples (1,093 testable) and in 2019, 1,369 samples were received (1,344 testable). This year, approximately 3,500 kits were not shipped out as scheduled, and as a result, only 5,000 kits reached hunters in time for season openings. This delay in shipping likely contributed to lower sample returns.

From the 815 blood samples tested for *B. abortus* specific antibodies, 43 were classified as positive. All positive elk were harvested within the DSA.

Northern DSA Surveillance:
Brucellosis surveillance in the combined northern HUs (Clark's Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA (see Figure 1) reported a slight increase in seroprevalence over the past five years (15.3%, n=927) compared to the 2016-2020 five-year average of 14.4% (n=967).

The five-year average seroprevalence varied between the four northern HUs (see Figure 4). It is important to note that sample sizes are generally low and affect the accuracy of prevalence estimates for the individual HUs. Therefore, prevalence figures are combined into five-year totals to improve sample size and allow for statistical analysis

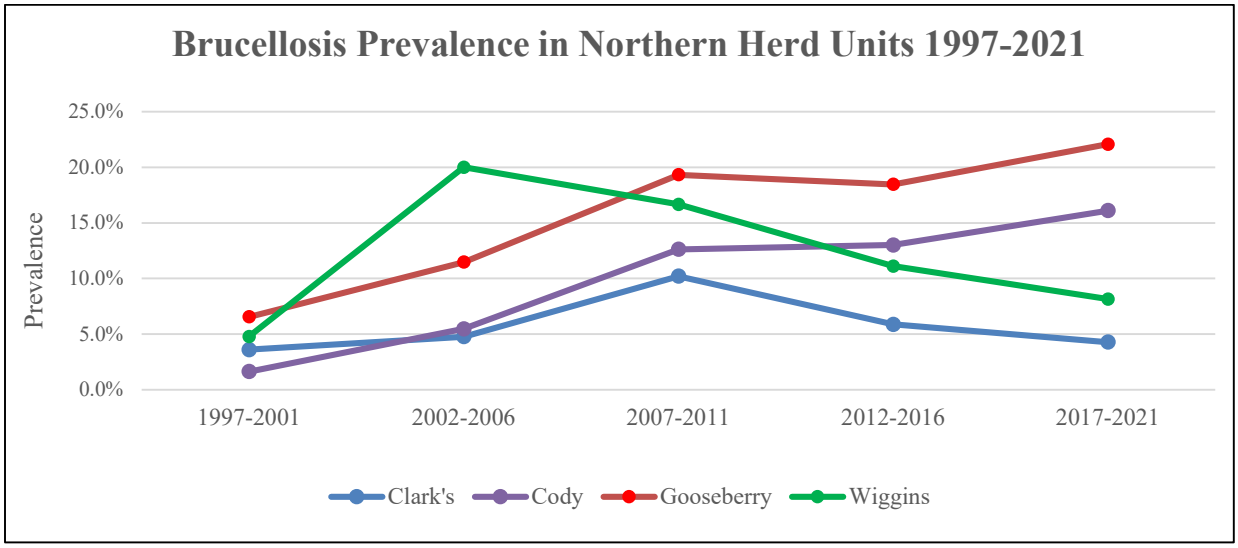

Figure 4. Seroprevalance over time in cow elk from the Northern HUs.

Many of the subpopulations in the northern HUs 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). 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 HUs 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.

Southern DSA Surveillance:
In 2018, enhanced surveillance efforts were initiated in southern HUs bordering the DSA. A combined 62 samples were tested from the South Wind River and the West Green River in 2021 with no seropositive elk identified. This brings the 5-year seroprevelence in the southern HUs to 0.4% (n=247).

Rotating Statewide Surveillance:
From the rotating surveillance program target areas (1 ,2, 3, 6, 7, 113,116,117, 122, 123, 126, and 129), 178 useable samples were collected. All samples tested negative for exposure to *B. abortus* on serological tests. In the past 30 years, 7,086 samples from non-endemic areas statewide have been tested. To date, this disease has not been documented outside of western half of the state.

Bighorn Mountains Brucellosis Surveillance Summary (Submitted by Eric Maichak, Cody Region Wildlife Disease Biologist)**:**
Brucella abortus specific antibodies have not been detected in elk of the Bighorn Mountains since 2016, with over 2,700 samples tested from hunter-harvested and elk captured for research. In light of these negative findings, the surveillance effort in the Bighorn Mountains has been scaled back. In 2021, surveillance focused only on HA 39-41, 45, 47-49 along the west side of the Bighorn Mountains. A statewide raffle of quality outdoor recreation oriented prizes was initiated in 2018. The raffle continued this year with $8000 in prizes from sponsors including Benelli, Badlands, Maven, Rocky Mountain Elk Foundation, Vortex, and WGFD.

Targeted hunt areas were mailed a total of 2,654 kits. Because of the previously mentioned mailing delay, 1,354 kits were mailed at least one week late. However, at least 1,403 kits (mailed and handed out) were available for hunters in time for their season opening.

In 2021, 202 total samples (183 hunter-harvested, 19 captured) were tested. Average seroprevalence estimate for the Bighorn Mountains from 2017-2021 was 0% (95% CI, 0.0%, 0.0%). For hunter-harvested samples, 50% (n=92) were returned at drop-off coolers, field personnel, or processors. Relative to previous years, total kits deployed, samples returned, and hunters contacted (n=150) declined. From 2011 to 2021, number of kits returned were positively affected by total kits deployed (R^2=0.94, P<001), elk harvested (R^2=0.67, P=0.002), temporary personnel employed (R^2=0.75, P=0.001), and hunters contacted (R^2=0.74, P=0.02). Estimates of the proportion of kits returned by successful hunters who received kits increased from 2011 to 2021 (R^2=0.57, P=0.01; Figure 5), particularly after inception of the raffle, with eight sample kits (4%, n=183) returned by hunters from areas not targeted for sampling in 2021.

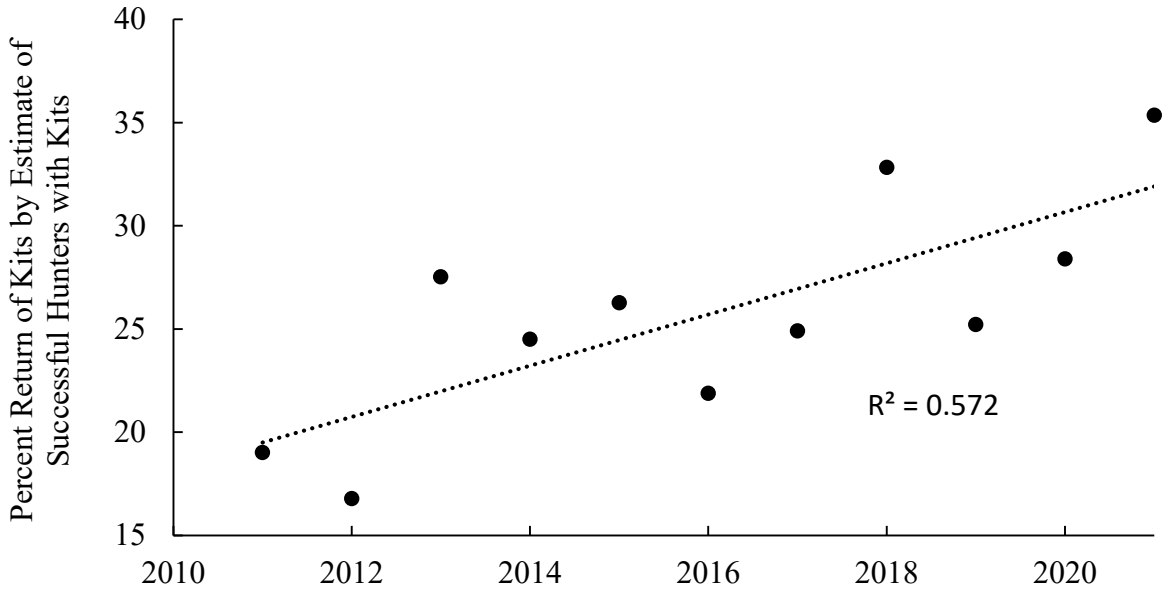

Figure 5. Proportion of blood samples returned by estimate of successful hunters who recieved kits, Elk Hunt Areas 33-41, 45, 47-49, and 120, Bighorn Mountains, WY, 2011- 2021.

Our cursory analysis suggests that, despite decreased effort and investment of resources into brucellosis surveillance in the Bighorn Mountains, increased returns suggest a positive effect of the raffle and associated marketing. Although cash incentives (especially when provided in advance) typically provide greater response rates, our results are supported by previous studies showing that prizes can increase survey response rates relative to when no incentive is offered (Singer et al. 1999, Gneezy et al. 2011). The raffle is anticipated to continue a fifth season in 2022 with sponsors committed.

The failure to detect brucellosis in over 2,000 samples over the last five years (2017-2021) throughout the Bighorn Mountains is encouraging. However, we urge caution for individuals or entities using this knowledge to make non-wildlife management decisions. Our 2021 data suggest that in HAs 39-41 and 49 where brucellosis was previously detected (see Figure 6), approximately 1 in 10 elk harvested are tested, leaving relative uncertainty in the serostatus of untested harvested elk. Although the seroprevalence estimate we present from the Bighorn Mountains was much lower than estimates from elk populations within the GYE (e.g., >20%; Brennan et al. 2017), it has also been suggested that population seroprevalence is best modeled with the previous eight years of data (Cross et al. 2007). Thus, utilizing all data collected from 2014-2021 in the Bighorns would suggest higher prevalence than what we have presented, and perhaps, a greater likelihood of finding future seropositive elk and possible spillover risk to livestock. Additionally, it has been predicted that reductions of elk density up to 90% will have no measureable effect on host seroprevalence levels that are <1% (Proffitt et al. 2015). Although we do not present results on elk density, WGFD has made no attempt to reduce elk density below established population objectives in HAs 39-41 and 49, and therefore, lack of finding seropositive elk the previous four hunting seasons potentially contradict this prediction. The Bighorn Mountains will be surveyed in

2022 however, surveillance efforts in this area will move from annual surveillance into the four year rotating surveillance schedule (see figures 7 and 8).

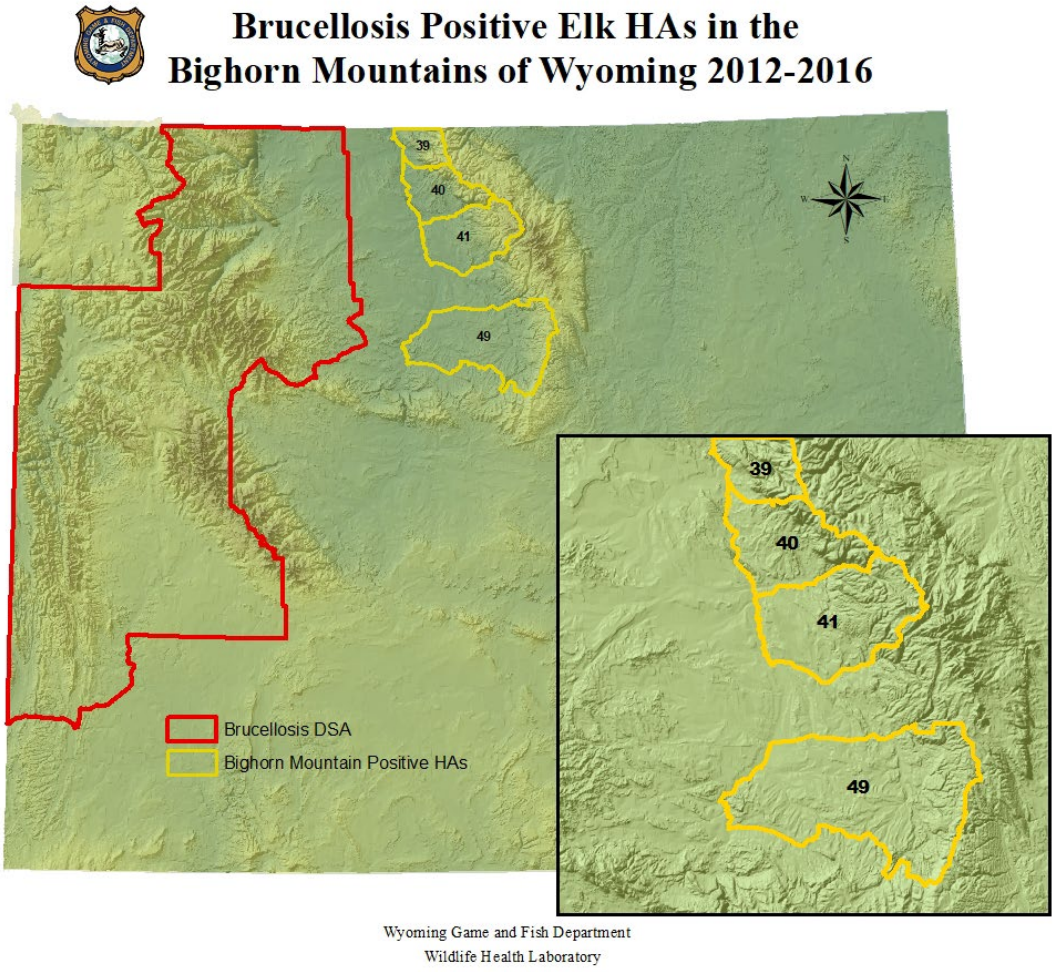

Figure 6. HAs of seropositive elk in the Bighorn Mountains; no new positives have been identified since 2016.

2022 Surveillance:

In 2022, the rotating surveillance area will include the central northern areas of Wyoming, including the Bighorn Mountains (see Figure 7). This encompasses elk hunt areas 33, 34, 35, 36, 37, 38, 39, 40, 41, 45, 47, 48, 49, and 120. Efforts to survey within the DSA (northern HUs) and around the DSA southern border will continue (see Figure 8).

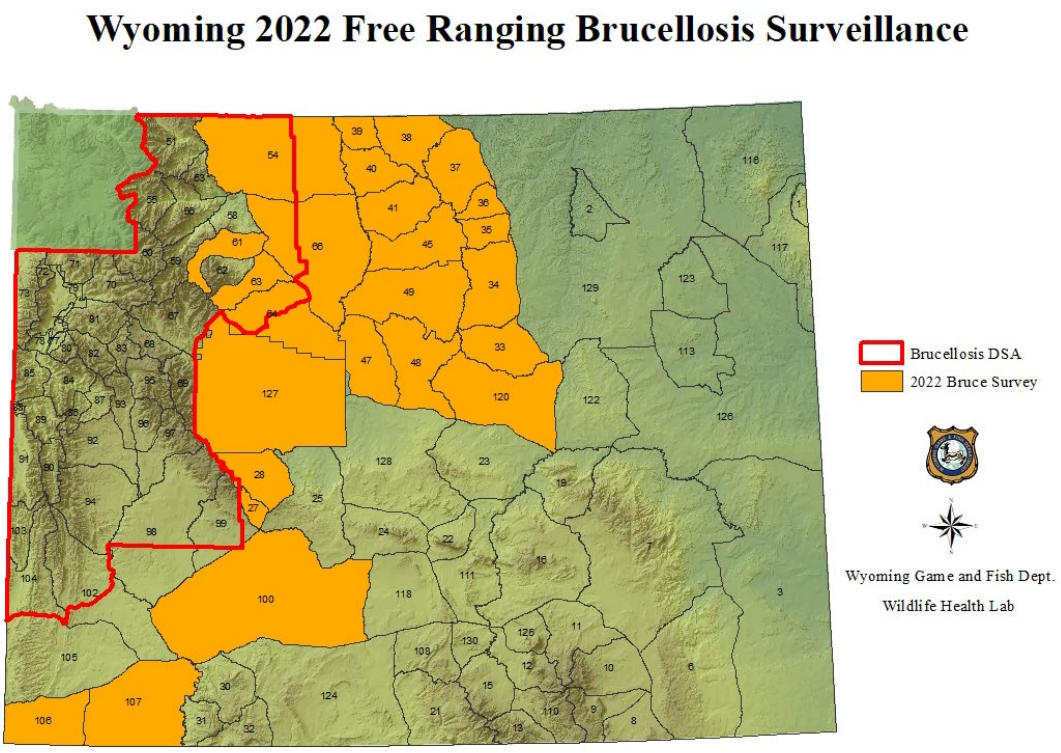

Figure 7. Proposed elk hunt areas to target for brucellosis surveillance in 2022.

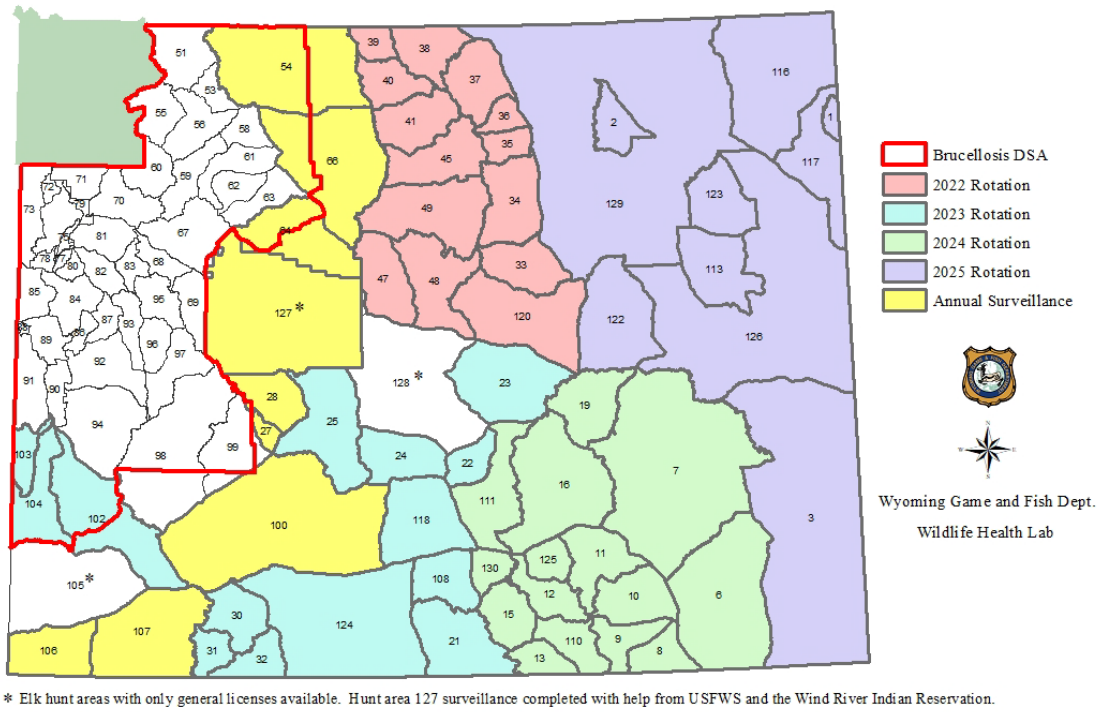

Figure 8. Proposed 4-year rotation schedule of elk hunt areas to target for brucellosis surveillance.

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

Cross P.C., W.H. Edwards, B.M. Scurlock, E.J. Maichak, and J.D. Rogerson. 2007. Effects of management and climate on elk brucellosis in the Greater Yellowstone Ecosystem. Ecological Applications 17:957–964.

Gneezy, U., S. Meier, and P. Rey-Biel. 2011. When and why incentives (don't) work to modify behavior. Journal of Economic Perspectives 25:191-210.

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).

Proffitt K.M., N. Anderson, P. Lukacs, M.M. Riordan, J.A. Gude, and J. Shamhart. 2015. Effects of elk density on elk aggregation patterns and exposure to brucellosis. Journal of Wildlife Management 79: 373–383.

Singer, E., J. Van Hoewyk, N. Gerbler, T. Raghunathan, andK. McGonagle. 1999. The effect of incentives on response rates in interviewer-mediated surveys. Journal of Official Statistics 15:217-230.