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 all the elk 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 18,000 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 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. 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 examines 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 are identified, and a predictive model will be developed to determine 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.

**Methods:**

In 2019, around 10,000 blood collection kits were mailed or directly handed out to elk hunters successful in limited quota elk license drawings in the HAs targeted for surveillance. 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 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 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). 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. Over 98% of serum samples
received in 2019 were considered testable for exposure to *B. abortus*. Hemolyzed serum samples were only discarded if the samples were received from inside the endemic area. 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. Research continues on FPA testing of hemolyzed samples and a final report is expected in the coming year.

**2019 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 WHL. These efforts included a chance at a raffle for 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 2019 again concentrated on the Bighorn Mountains as well as those hunt areas that surround the eastern DSA border (see Figure 2). Surveillance within the DSA shifted to the Cody HU. Statewide surveillance normally alternates through the elk hunt areas in the southern and eastern portions of the state, and in 2019 this effort was directed to the southwestern area of Wyoming (HAs 30, 31, 32, 106, 107, and 124).

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 2019 surveillance effort was supported by the WGFD, and by a cooperative agreement with the Animal and Plant Health Inspection Service.

![Figure 2](image)

**Figure 2.** Elk HAs surveyed in 2019 for brucellosis in hunter-killed elk.

**Results and Discussion:**
A total of 1,366 elk blood samples were received by the WHL in 2019. Of those, 1,344 were suitable for testing. 519 useable samples were collected from the Bighorn Mountains; 126 of those
were from yearling or adult cows harvested in hunt areas where seropositive elk had been previously documented (see Figure 3). No seropositive elk were detected in 2019, marking the third year that no new seropositive elk have been identified in the herd units that comprise the Bighorn Mountains.

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

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 HAs 58-59 and 61-63. In the last five years, the combined seroprevalence in these areas has averaged 19.6% (n=373). Many of the subpopulations in these HAs 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. Brucellosis DSA and Endemic Elk Hunt Areas in Wyoming.

Brucellosis surveillance in the combined northern HUs (Clark’s Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA (see Figure 1) reported a slight drop in seroprevalence over the past five years (12.6%; n=886 samples) compared to the previous five-year average of 14.3% (n=1,274) (see Figure 5). Attempts to increase surveillance numbers continued in 2019 along the southeastern border of the DSA in the South Wind River and West Green River HUs (see Figure 1). Brucellosis positive elk have been previously identified in these areas, but they have not been extensively surveyed in recent years. 2018 was the beginning of an effort to sample the area more intensively, and this year a combined 66 samples were received from the southern HUs with one seropositive cow identified in HA 27. The five year prevalence in these HUs remains low compared to the northern HUs (see Figure 5). These HUs will remain a focus of the surveillance effort in 2020.
Figure 5. Seroprevalence through time in cow elk from combined northern and southern elk herd units (HUs) surrounding the DSA.

The five-year average seroprevalence varied considerably between the four northern HUs (see Figure 6). It is important to note that sample sizes from the Wiggins Fork (n=52 in 2019) and Clarks Fork (n=18 in 2019) HUs 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.
Additionally, 120 useable samples were collected from the rotating surveillance program target areas in the southwestern part of the state. All samples tested negative for exposure to *B. abortus* on serological tests. In the past 29 years, 6,535 samples from the non-endemic area have been analyzed. To date, this disease has not been documented outside of western half of the state.

**2020 Surveillance:**
In 2020, the rotating surveillance area will shift to target the Snowy Range and the Sierra Madre HUs in southeastern Wyoming (see Figure 7). This encompasses HAs 8-13, 15, 16, 19, 21, 108, 110, 114, 125, and 130. Surveillance within the DSA will focus on parts of the Cody HU and the Gooseberry HU. Efforts will continue to emphasize surveillance around the eastern and southern borders of the DSA and maintain surveillance along the northeastern border and in the Bighorn Mountains.

The return rate of blood samples from hunters successful in harvesting an elk has remained relatively constant over the past five years with around 1,500 hunters submitting blood samples for analysis every year. On a per hunter basis, approximately 32% of successful hunters that received a blood collection kit voluntarily collected and submitted a sample for surveillance.
Figure 7. Elk hunt areas targeted for brucellosis surveillance in 2020.

Literature cited: