Wyoming Game and Fish Department
2016 Brucellosis Surveillance in Non-Feedground Elk Herds
March 2017

Overview:
Each year the Wyoming Game and Fish Department (WGFD) monitors the distribution and prevalence of brucellosis within the state's elk populations by requesting hunters to collect blood samples from their harvested animal. Surveillance is generally concentrated in elk herds 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 brucellosis non-endemic area is surveyed each year, which provides coverage of the entire area every 4-5 years. Approximately 10,000 blood collection kits are assembled and mailed to elk hunters successful in acquiring limited quota elk licenses within target surveillance areas. In general, hunters return between 1,000 and 1,500 blood samples to the laboratory, of which approximately 60% are suitable for testing (samples often freeze in the return mailing, rendering them untestable).

Since 1991 over 14,300 elk blood samples have been analyzed for brucellosis. In the western portion of the state, brucellosis prevalence varies between 0-4% in the herd units (HUs) (South Wind River, West Green River) south of the Greater Yellowstone Area (GYA), and between 1-23% in the HUs (Clarks Fork, Gooseberry, Cody, and Wiggins Fork) east of the GYA. In 2012, this disease was documented outside the GYA when it was discovered in elk of the northwestern Bighorn Mountains. Since the initial discovery through 2015, a combined total of seven elk have been identified from Elk Hunt Areas (HAs) 39, 40, and 41 on the northwestern slope. 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.

An elk movement study was initiated in early 2016 to evaluate movement and interactions of elk herds in the Bighorn Mountains, determine route of brucellosis spread to the Bighorn Mountains, identify calving areas, and model how brucellosis may spread further if it becomes established. Understanding the route of spread will enable development of management strategies that could minimize further spread to neighboring elk herds as well as exposure to domestic cattle. The study plan is to capture and place Global Positioning System (GPS) collars on approximately 150 cow elk over a period of 3 years. Collars are placed in different elk herds and HAs across the Bighorns Mountains as well as HA 66 in the Bighorn Basin; focusing on locations where seropositive animals have been documented. Blood samples are collected from captured animals and tested for brucellosis as described above. Elk testing seropositive are recaptured, euthanized, and tissues collected for culture and *brucella* genomics.

To better manage and monitor brucellosis in the Bighorn Mountains and the Cody Region as a whole, a permanent, full time position was transferred from the Pinedale Region Brucellosis Feedground and Habitat unit to the Cody Region. The core responsibilities of this position are to

increase surveillance efforts in hunter-killed elk, compile and maintain Brucellosis Management Action Plans for elk herd units of the region, and to aid in the development of management strategies that can minimize the incidence and spread of brucellosis in elk.

2016 Surveillance:
The 2016 surveillance program again concentrated on the Bighorn Mountains; particularly in HAs 39, 40, and 41; but was also was focused on those HAs surrounding the DSA to ensure continued monitoring of the endemic/nonendemic border. Surveillance outside of the known brucellosis endemic area occurred in the northeastern corner of the state near Gillette and the Black Hills (see Figure 2).

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 2016 surveillance effort was supported by the WGFD using general funds, and by a cooperative agreement with the Animal and Plant Health Inspection Service. Funding was used for additional WHL personnel for kit assembly, field technicians to maximize sample collection and submission, as well as numerous other costs associated withthis large scale surveillance project.

Methods:
In 2016, 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 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 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 (prevalence levels) on elk within the known endemic area is based on yearling and adult females, but males are included in surveillance data outside of the known endemic area. Including serologic data from males offers improved detection of brucellosis in areas where this disease is not known to occur.

Results and Discussion:
A total of 1,398 elk blood samples were received by the WHL with 1,035 (74%) of those being suitable for testing. The majority of the samples were collected from the Bighorn Mountains where 592 useable samples were tested, and 546 of those were from yearlings or adults.

Three seropositive cow elk were identified from HA 40 in 2016; two animals were identified through trapping operations in February, and one was harvested during the fall hunting season. Since 2012, seven cows and one bull have been identified with antibody titers to *B. abortus* from HA 40. Brucellosis was also found in one new HA in the Bighorn Mountains when a

seropositive bull was harvested from HA 49 (see figure 3) in mid-October. In an effort to collect additional samples from HA 49, 890 letters and 456 blood collection kits were sent to most HA 49 license holders encouraging them to collect a blood sample from their harvested animal. Additional blood sample drop-off collection coolers were also distributed throughout the HA and field personnel were available to collect samples whenever possible. Unfortunately, attempts to obtain a *B. abortus* isolate through culture of lymph nodes collected from hunter killed animals or collected from seropositive elk movement study animals (n=2), have 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 2016 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.

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 2016

		2016			Total Samples 2012-2016			95% Confidence(2012-15)	
Elk HuntArea / Herd Unit (HU)	**Age/Sex**	**Samples**	**Positive**	**Prevalence**	**Samples**	**Positive**	**Prevalence**	**Lower**	**Upper**
33	All	11	0	0.0%	75	0	0.0%	0.0%	4.8%
34	All	41	0	0.0%	126	0	0.0%	0.0%	2.9%
47	All	6	0	0.0%	38	0	0.0%	0.0%	9.3%
48	All	22	0	0.0%	86	0	0.0%	0.0%	4.2%
49	All	56	1	1.8%	180	1	0.4%	0.0%	3.1%
	Cows	31	0	0.0%	31	0	0.0%	0.0%	11.2%
120	All	28	0	0.0%	90	0	0.0%	0.0%	4.0%
Total South Bighorn HU	All	164	1	0.6%	595	1	0.0%	0.0%	0.9%
	Cows	31	0	0.0%	31	0	0.0%	0.0%	11.2%
35	All	18	0	0.0%	111	0	0.0%	0.0%	3.3%
36	All	14	0	0.0%	54	0	0.0%	0.0%	6.6%
37	All	27	0	0.0%	93	0	0.0%	0.0%	3.9%
38	All	113	0	0.0%	445	0	0.0%	0.0%	0.8%
39	All	39	0	0.0%	158	1	0.6%	0.0%	3.5%
	Cows	22	0	0.0%	89	0	0.0%	0.0%	4.1%
40	All	64	3	4.7%	289	8	2.3%	1.2%	5.4%
	Cows	35	3	8.6%	172	7	3.5%	1.7%	8.2%
Total North Bighorn HU	All	275	3	1.1%	1150	9	0.7%	0.4%	1.5%
	Cows	57	3	5.3%	261	7	2.4%	1.1%	5.4%
41	All	97	0	0.0%	369	1	0.2%	0.0%	1.5%
	Cows	58	0	0.0%	212	0	0.0%	0.0%	1.7%
45	All	56	0	0.0%	268	0	0.0%	0.0%	1.4%
Total Medicine Lodge HU	All	153	0	0.0%	637	1	0.1%	0.0%	0.9%
	Cows	58	0	0.0%	212	0	0.0%	0.0%	1.7%
Total Bighorns	All	592	4	0.7%	2382	11	0.4%	0.2%	0.8%
	Cows	146	3	2.1%	504	7	1.3%	0.6%	2.8%

Brucellosis surveillance in the combined northern HUs (Clark's Fork, Cody, Gooseberry, and Wiggins Fork) of the DSA, documented an increase in seroprevalence from 9.2% (n=76 samples) in 2015 to 15% (n=206) in 2016 (see Figure 4). Seroprevalence in cows of the Cody HU (HAs 55, 56, 58-61, 66) was 12.4% (n=153) which is very similar to the 10 year average of 12.9% (n=1,112). Elk HA 58 had the highest seroprevalence in the HU with 20% (n=25), which is slightly above the 10 year average of 18% (n=89). The Clark's Fork HU (HAs 51, 53, 54) on the other hand observed a decrease in prevalence over the 10 year average of 9.9% (n=573) to 8.7% (n=23) in 2016.

Seroprevalence in the targeted HAs selected for long-term monitoring (HAs 61, 62, and 63) increased in 2016. Last year's levels were 14.3% in cows (n=35), but increased to 17.9% in 2016 (n=67). Seroprevalence in these three HAs has averaged 15.8% (n=1,088) over the past 10 years, whereas the Gooseberry HU (HAs 62, 63, 64) has averaged 19% (n=480) over the past 10 years, but has increased to 32.1% (n=28) prevalence in cows in 2016. Unfortunately, sample sizes for these HAs and HUs are insufficient for meaningful data analysis or conclusions, but warrant continued monitoring of all HAs along the southeastern slope of the Absaroka Range (HAs 58, 61-64) where the prevalence of brucellosis mirrors those found on many of the State's feedgrounds.

In the southern HUs of the DSA, only seven suitable samples were received from cows harvested from either the South Wind River or the West Green River HUs. Although the sample size is very small, no seropositive animals were identified from these herd units.

A total of 1,073 useable samples were collected over the past four years of surveillance in the northeastern corner of the state (totals include samples collected from HAs on the eastern slope of the Bighorn Mountains). All samples tested negative for exposure to *B. abortus* on serological tests. In the past 25 years, 4,335 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 5).

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

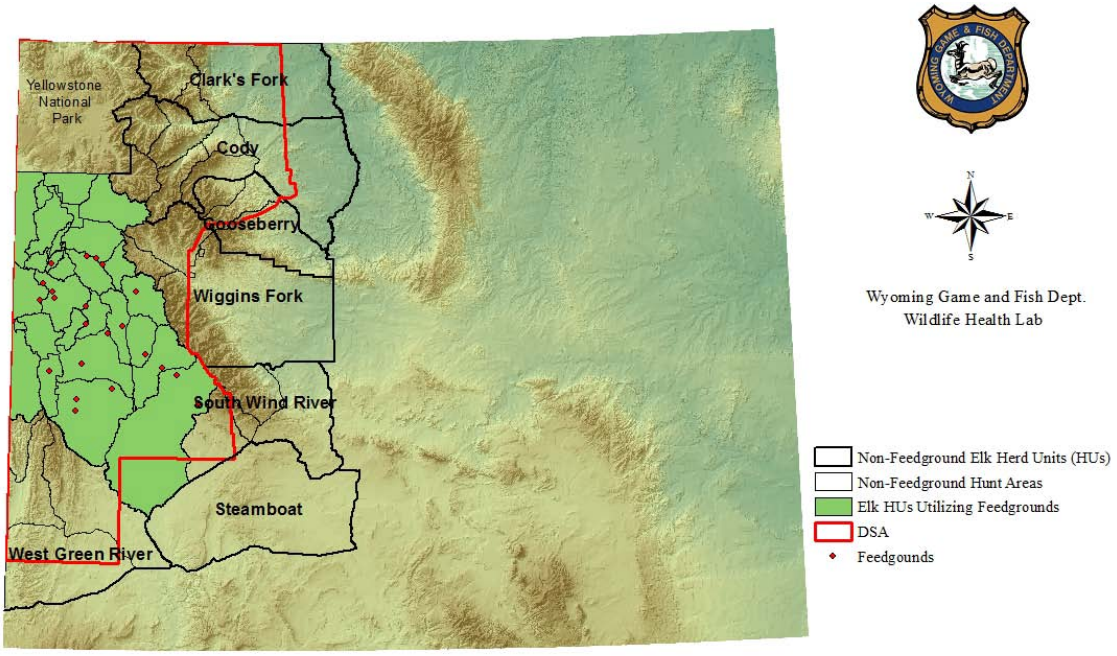

Figure 2: Elk Hunt Areas Surveyed in 2016 for Brucellosis in Hunter-Killed Elk

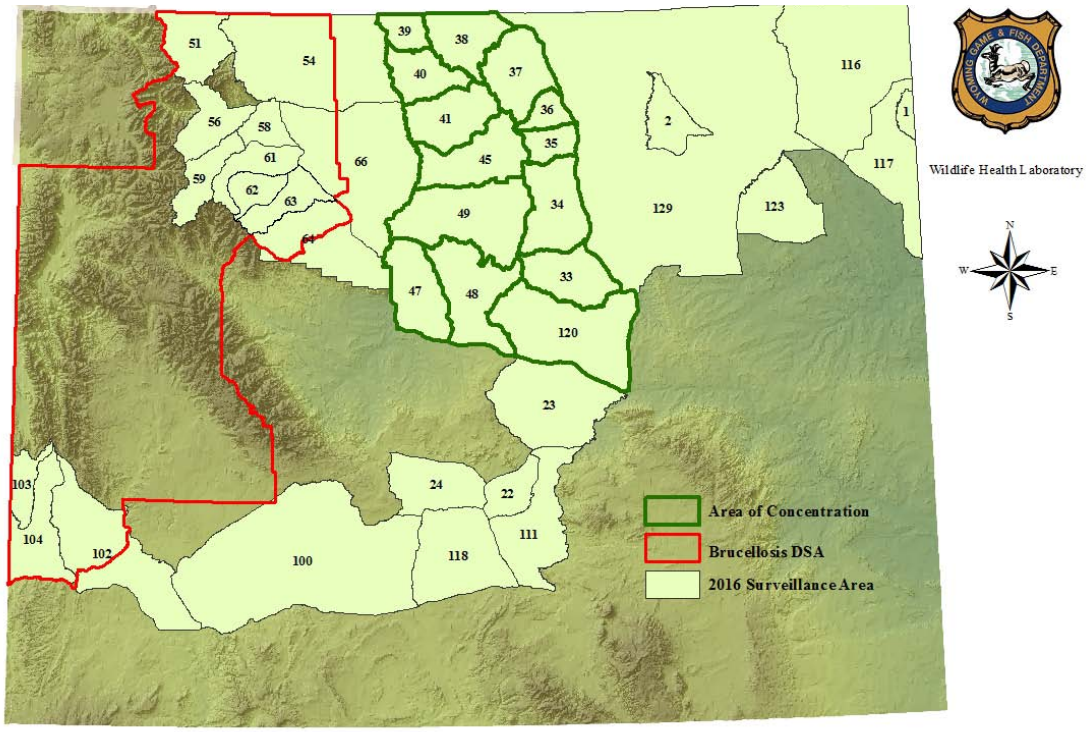

Figure 3: Locations of Seropositive Elk in the Bighorn Mountains 2012-2016

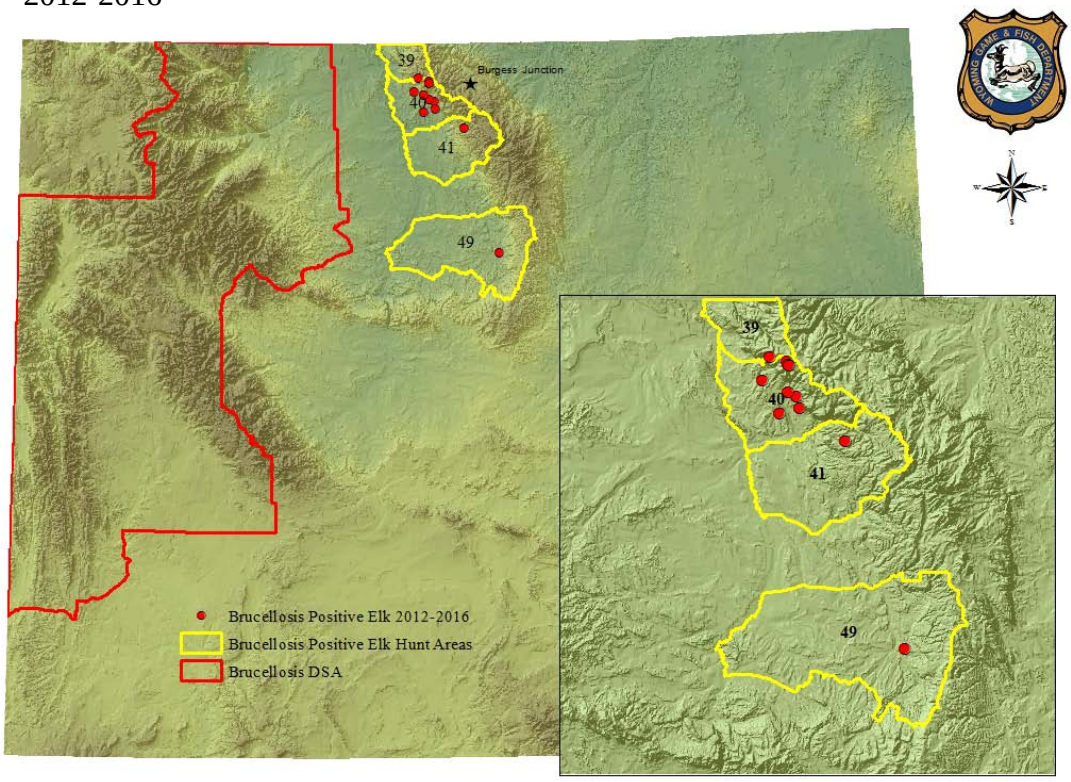

Figure 4: Seroprevalence Through Time in Northern and Southern Elk Herd Units Surrounding the DSA

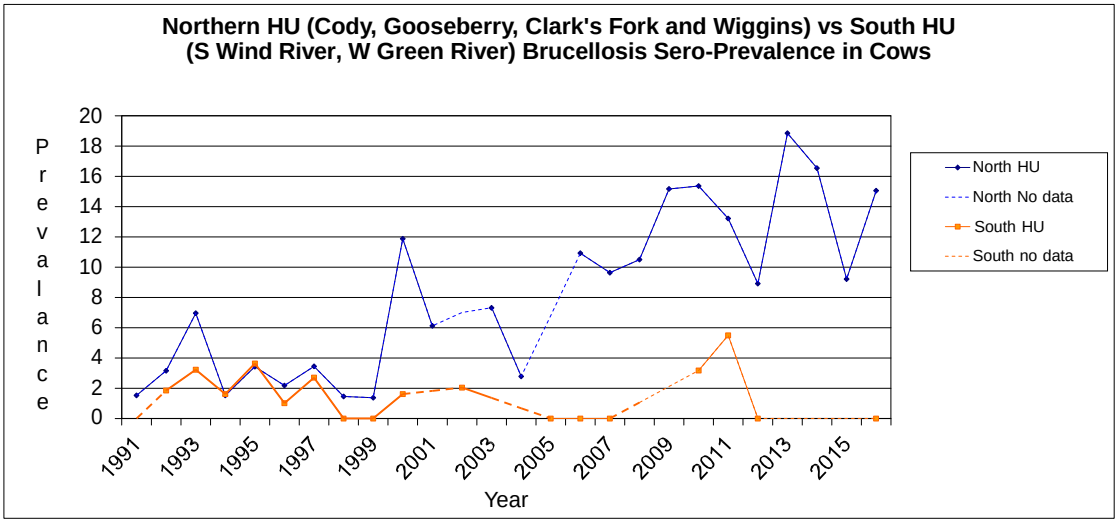

Figure 5: Brucellosis Endemic Elk Hunt Areas in Wyoming