Wyoming Game and Fish Department

2020 Bighorn Mountains Enhanced Elk Brucellosis Surveillance Program
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Abstract

In the Bighorn Mountains, the Wyoming Game and Fish Department (WGFD) documented two brucellosis seropositive hunter-harvested elk in 2012. In 2013, surveillance efforts were increased to better understand the extent and distribution of brucellosis seropositive elk within Bighorn Mountain Elk Hunt Areas (HAs) 33-41, 45, 47-49, and 120. Primary objectives of the Bighorn Mountains enhanced elk brucellosis surveillance program were to increase elk hunter contacts resulting in an increase in the number of testable blood and supramammary and/or iliac lymphatic samples, ultimately for informed decision making. From 2013-2016, we documented nine additional seropositive elk, including two adult females associated with captures. Funding, seasonal personnel, hunters contacted, and blood kits deployed began to diminish in 2018 and were lowest in 2020 following four hunting seasons of documenting no seropositive elk. We found that businesses with walk-in coolers (i.e., convenience stores, processors) functioned well as sample drop-off locations, and among drop-off location types (e.g., strategically located coolers, check stations), coolers collected the most blood samples while check stations had the highest average return per day. We found increased number of blood kits deployed and hunters contacted rather than elk harvested was associated with increased return of blood samples. Despite decline of hunters contacted and kits deployed starting in 2017, inception in 2018 of a raffle of prizes donated and valued at $2900, $4200, and $7200 increased the proportion of blood samples returned by an estimate of successful hunters who received kits. Although we collected 2,533 useable blood samples and found no seropositive elk from 2017-2020, we caution stakeholders who use this information to make non-wildlife decisions.

Introduction

Brucellosis, a zoonotic disease caused by the bacteria *Brucella abortus*, is endemic in elk and bison of the Greater Yellowstone Ecosystem (GYE). In elk, the disease typically causes abortion from February to mid-June (peaking from March to mid-May) and is transmitted primarily through contact of animals with infected aborted fetuses, placentas, bodily fluids, or milk and ingestion of the bacteria. In the GYE, spillover transmissions from elk to livestock have increased over the last 20 years causing economic hardship for affected producers and need for accurate data to help make informed management decisions. To understand the prevalence and distribution of brucellosis, particularly in non-feedground elk herds, WGFD annually provides 8,000 to 10,000 blood sampling kits (kits) to limited quota license elk hunters in targeted surveillance elk hunt areas (HAs) around Wyoming. In the Bighorn Mountains, brucellosis seropositive elk were first documented in two hunter-harvested elk in 2012. Beginning in 2013, the WGFD increased surveillance efforts through additional funding and personnel to better understand the seroprevalence, and attempt to document any culture-positive elk. Primary objectives of the Bighorn Mountains enhanced sampling program ("program") were to increase elk hunter contacts resulting in an increase in the number of testable blood and supramammary and iliac lymphatic tissue samples, ultimately to facilitate informed decision-making. As the program continued through time and more data were compiled, a secondary objective included understanding factors potentially affecting the number of samples returned by hunters.

Study Area & Methods

The Bighorn Mountains lie within the north-central portion of Wyoming and are comprised of HAs 33-41, 45, 47-49, and 120 of the North Bighorn, Medicine Lodge, and South Bighorn elk herd units in the Sheridan, Cody, and Casper regions (Figure 1). Generally among years, seasons in August and November to January target antlerless elk; September is any-elk, archery; and October often permits antlerless harvest early in the month, and any-elk in the later half. The number of issued limited quota elk license holders who reported hunting in the Bighorn Mountains rose from 8972 in 2011 to 10,193 in 2020.

The area typically has cold winters and hot summers with precipitation increasing in elevation and occurring as snow or rain depending on season. Elevational precipitation gradient coupled with north-south position of the Bighorn Mountains results in habitat ranging from desert to alpine to grassland from west to east over the range. Public land access is greatest in the northern two-thirds of the range, yet early season snow storms often limit access to tracked vehicles or lower elevations.

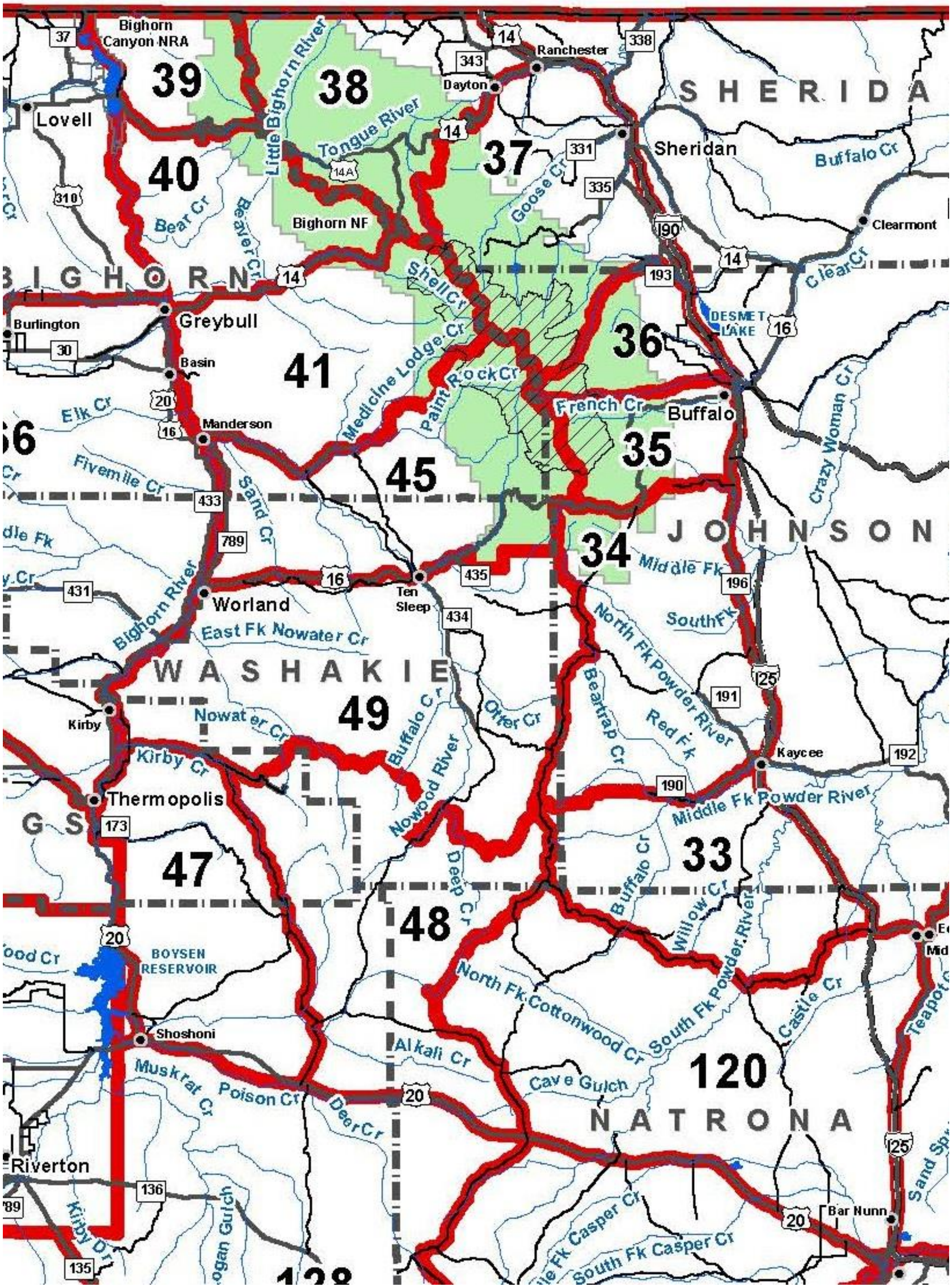

Figure 1. Elk Hunt Areas 33-41, 45, 47-49, and 120, Bighorn Mountains, WY.

From 2013-2020, AWEC technicians in the Sheridan and Cody regions were deployed in the northern and central Bighorn Mountains, operating the US Highway 14 check station almost daily in October to early November 2013-2018. In 2019 and 2020, the sole technician primarily conducted camp and cooler checks in HAs 38-41. From 2013-2018, one AWEC technician at the Wildlife Health Lab (WHL) in Laramie also provided sample testing and database management (Table 1). Additionally, in 2016 a permanent wildlife disease biologist moved to the Cody region to assist and eventually lead sampling efforts. To increase public awareness of brucellosis, field personnel discussed brucellosis ecology and harvest data, both formally and informally, with hunters, landowners, outfitters, merchants, and other publics prior to and throughout the hunting season.

Table 1. Field and lab technician expenses for enhanced elk brucellosis sampling program, 2013-2020.

		Cost ($)[a]			
Year	**Personnel (N)**	**Personnel**	**Vehicles**	**Total**	**Funding Source**
2013	5	29,030	5,416	34,446	WY Governor's Brucellosis Surveillance
2014	5	30,347	5,903	36,250	WY Governor's Brucellosis Surveillance
2015	4	30,578	5,134	35,712	WGFD, USDA-APHIS
2016	5	28,188	5,042	33,230	WGFD, USDA-APHIS
2017	5	36,724	3,188	39,930	WGFD, USDA-APHIS
2018	2	12,819	2,510	15,329	WGFD, USDA-APHIS
2019	1	10,324	3,000	13,324	USDA-APHIS
2020	1	10,863	2,880	13,743	USDA-APHIS
Total	**27**	**188,873**	**6,073**	**221,946**	

[a] Does not include supplies, permanent field personnel, or housing.

Kits were primarily assembled by and distributed from the WHL. From 2018 to 2020, volunteers with the Cody Chapter of the Rocky Mountain Elk Foundation assembled 1,500 kits for field distribution. Kits consisted of a 15-ml sterile polypropylene conical tube, paper towel, instruction/data sheet, a prepaid mailing label for return shipping. Kits with blood samples (samples) were collected opportunistically at hunter field checks, game check stations, regional WGFD offices, and strategic drop-off locations (i.e., road junctions, convenience stores, processors) around the Cody and Sheridan regions. Blood sample drop-off locations consisted of a 12"x18" instructional sign displayed conspicuously, and a cooler. Coolers exposed to ambient weather conditions, kept indoors, or indoors within refrigerated units were typically checked daily, twice per week, or once per week, respectively.

Uncontaminated and unfrozen blood samples were separated into red blood cells and serum using a centrifuge operated at 2500 revolutions per minute for 10-20 minutes. To facilitate WHL sample testing and research, field personnel categorized blood samples that would not separate based on smell (rumen, urine, and/or putrid), and texture or color/transparency (frozen, or frozen/thawed). Samples were transferred to cryovials, frozen, and shipped weekly to WHL. When available, we collected supramammary and/or iliac lymphatic tissues. Lymphatic tissue samples were placed in a Whirlpack® (plastic bag), labeled with specific sample information, and frozen.

To thank hunters and potentially increase return of blood samples, we implemented a raffle for hunters who submitted useable samples from 2018 to 2020. The raffle was open to any hunter in a HA targeted for brucellosis sampling throughout Wyoming, and hunters could submit up to three blood samples, the maximum number of elk licenses allowed per hunter per year. Raffle sponsors agreed to provide prizes in return for WGFD advertising in all forms of raffle marketing (Table 2). Marketing throughout each hunting season included press releases, social media posts, targeted emails to hunters who were mailed kits, and flyers that were distributed through target HAs. The raffle prize drawing was conducted in mid-March following the season. Winners were contacted and had mailing address confirmed in late March, with prizes shipped in early April. Several winners provided pictures used in follow-up media efforts.

Table 2. Prizes, value, and sponsors of the enhanced elk brucellosis sample kit return raffle, 2018-2020.

Year	Prizes	Total Value ($)	Sponsors[a]
2018	Rifle/Scope, Spotting Scope, Binoculars, Hooded Sweatshirts	2,900	RMEF/Vortex, Vortex Maven & WYTWS WGFD
2019	Rifle/Scope, Spotting Scope, Binoculars, Range Finders Hooded Sweatshirts	4,200	RMEF/Vortex, Leupold Maven & WYTWS, WSG Gillette WGFD
2020	Rifle/Scope & Spotting Scope, Rifle, Rifle, Binoculars, Hooded Sweatshirts	7,200	WSG Gillette/Vortex & Sig Sauer WSG Gillette, RMEF, WGFD Maven & WYTWS

[a] RMEF, Rocky Mountain Elk Foundation; WYTWS, Wyoming Chapter of The Wildlife Society; WSG, Wyoming Sportsman's Group; WGFD, Wyoming Game and Fish Department

We compiled number of kits deployed, elk harvested, blood and lymphatic tissue samples submitted, proportion of useable samples, and number of seropositive elk documented among HAs 33-41, 45, 47-49, and 120 in the Bighorn Mountains from 2011 to 2020. To provide adequate sample size for statistical confidence, we used data from 2016-2020 to calculate average seroprevalence (±95% CI). To understand efficiency of kit returns at non-mail sources, we categorized type and number of drop-off sites, including WGFD AWEC and permanent field personnel, WGFD office, check station, cooler, ranch, outfitter, and processor. We calculated total per season and average return of kits per drop-off site type per day assuming each site type was available seven days per week. We calculated proportion of emails opened relative to all emails sent to hunters who were mailed kits. To understand the potential impact of harvest, hunter contacts, and number of kits deployed on total return of blood kits, we performed leased-squares linear regression on data from 2011 to 2020. Prior to analyses on hunter contacts, we excluded years 2011-2013 and 2015 as data were incomplete.

To understand the possible influence of the raffle on the return of kits, we first calculated the proportion of samples returned by an estimate of successful hunters who received a kit in HAs 33-41, 45, 47-49, and 120 from 2011 to 2020, where the proportion of successful hunters was provided by the annual harvest survey (Model 1). We utilized this metric because data specifying if hunters with active licenses had received and utilized kits if successful at harvesting an elk were not available. We performed least squares linear regression on proportion of samples returned by an estimate of successful hunters who received kits from 2011 to 2020.

Model 1. Proportion of samples returned by an estimate of successful hunters who received a blood sample kit, HAs 33-41, 45, 47-49, and 120 in the Bighorn Mountains, WY, 2011-2020. Pooled percent harvest success is calculated from the annual harvest survey.

$$Proportion\ of\ Samples\ Returned = \frac{samples\ returned}{(kits\ deployed * pooled\ \%\ harvest\ success)}$$

Results

After inception of the program, the number of kits deployed, hunters contacted in person, blood samples submitted, percent return of kits deployed per total elk harvested, and useable blood samples were higher than before inception (Table 3). Throughout the program, the proportion of useable blood samples and harvest of elk increased, yet starting in 2018, number of kits deployed, hunters contacted, and proportion of blood samples returned per elk harvested declined. Most seropositive elk were detected in and around HA 40, and were last detected in 2016. Average seroprevalence estimate for the Bighorn Mountains from 2016-2020 was 0.1% (95% CI, 0.0%, 0.3%). Number of lymphatic tissue samples collected was lowest (27) and highest (64) in 2014 and 2016, respectively, declining to 33 in 2020. Lymphatic tissue samples were not collected from any hunter-harvested seropositive elk, and we were unable to culture *B. abortus* from two elk confirmed seropositive elk euthanized following capture in HA 40 in 2016.

Table 3. Brucellosis hunter-harvest sampling summary for Elk Hunt Areas 33-41, 45, 47-49, and 120 in the Bighorn Mountains, WY, 2011-2020 hunting seasons.

	Total				**% Return[c]**		**% Samples Useable (N)[d]**	**Seropositive Elk**	
Year	**Kits[a]**	**Harvest**	**Hunter Contacts[b]**	**Blood Samples**	**Kits**	**Harvest**		**N**	**Hunt Area(s)[f]**
2011	2829	3057	Unk	241	8.5	7.9	56 (134)	0	N/A
2012	2885	3785	Unk	244	8.5	6.4	43 (106)	2	40
2013	7626	3364	231	785	10.3	23.3	67 (545)	2	40, Unk[g]
2014	7675	3880	2906	773	11.0	20.1	84 (646)	3	39,40,41
2015	6285	4053	513	700	11.1	17.3	68 (482)	0	N/A
2016	7606	4247	2895	724	9.5	17.0	74 (537)	4[e]	40, 49
2017	6373	3577	2032	719	11.3	20.1	92 (661)	0	N/A
2018	6366	4515	2171	792	12.4	17.5	99 (784)	0	N/A
2019	5898	4132	439	562	10.0	14.3	99 (556)	0	N/A
2020	4003	4193	551	449	11.2	10.7	99 (444)	0	N/A

[a] Total mailed plus minimum total distributed to hunters in field
[b] Hunters contacted specifically to discuss brucellosis and sampling; data incomplete 2013 and 2015
[c] Percentage of blood samples returned relative to total kits distributed to hunters or elk harvested
[d] Number of useable blood samples (N) divided by number of blood samples returned
[e] Includes two adult females identified and lethally removed following capture
[f] Hunt area(s) where samples from seropositive elk were harvested
[g] Unk = Unknown; sample did not have Hunt Area information

From 2017-2020, proportion of kits returned at non-mail sites relative to total kits returned ranged from 45% to 60%. Coolers produced most total kits returned, while on average per drop-off site per day, kits were collected most frequently at check stations (Table 4).

Table 4. Percent blood kits returned at non-mail (i.e., drop-off sites) relative to total returned, and average number of kits returned per drop-off site type per day (total) for Elk Hunt Areas 33-41, 45, 47-49, and 120, Bighorn Mountains, WY, 2017-2020 hunting seasons.

Year	% Non-Mail	Average Kits per Day (N)						
		Check Station	**Cooler**	**Outfitter**	**Ranch**	**WGFD Office**	**Field Staff**	**Processor**
2017	49	0.53 (65)	0.11 (161)	0.09 (28)	0.05 (12)	0.04 (11)	0.03 (65)	0.03 (7)
2018	48	0.98 (27)	0.15 (210)	0.15 (36)	0.04 (5)	0.06 (19)	0.02 (54)	0.10 (26)
2019	45	0.35 (6)	0.10 (158)	0.01 (3)	0.01 (1)	0.02 (3)	0.05 (87)	0.03 (9)
2020	60	1.07 (15)	0.13 (129)	0.05 (8)	0 (0)	0.08 (19)	0.04 (81)	0.06 (16)

From two years prior to seven years following inception of the program, we found that number of elk harvested increased (R^2=0.53, P=0.02), yet number of elk harvested had no effect on total blood samples returned (R^2=0.14, P=0.29). We found that number of kits deployed (R^2=0.92, P<0.001) and hunters contacted (R^2=0.77, P=0.02) increased total blood samples returned per year (Figure 2).

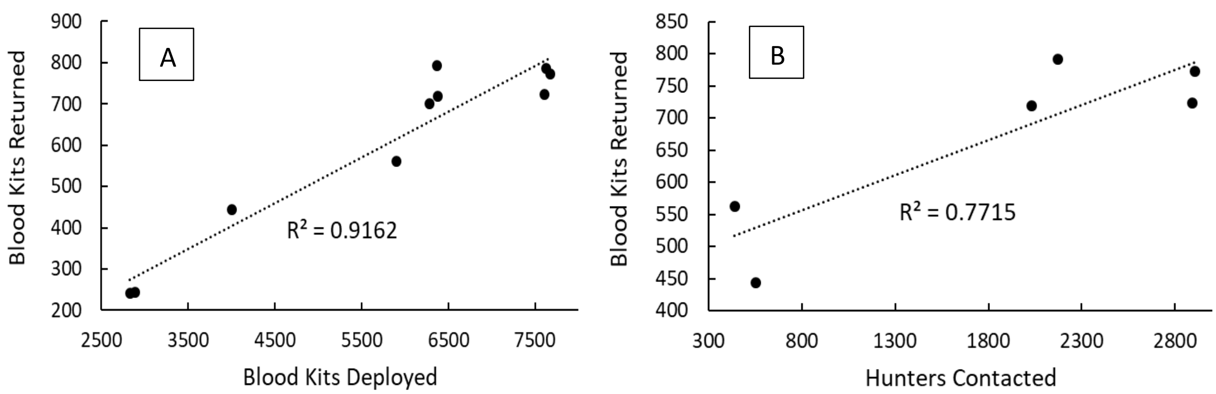

Figure 2. Total kits returned relative to blood kits deployed 2011-2020 (A) and hunters contacted 2014, 2016-2020 (B) Elk Hunt Areas 33-41, 45, 47-49, and 120, Bighorn Mountains, WY.

We found the proportion of blood samples returned by an estimate of successful hunters who received a kit increased from 2011-2020 (R^2=0.46, P=0.03; Figure 3). In 2019 and 2020, the proportion of emails opened relative to all emails sent to hunters was 44% in both years.

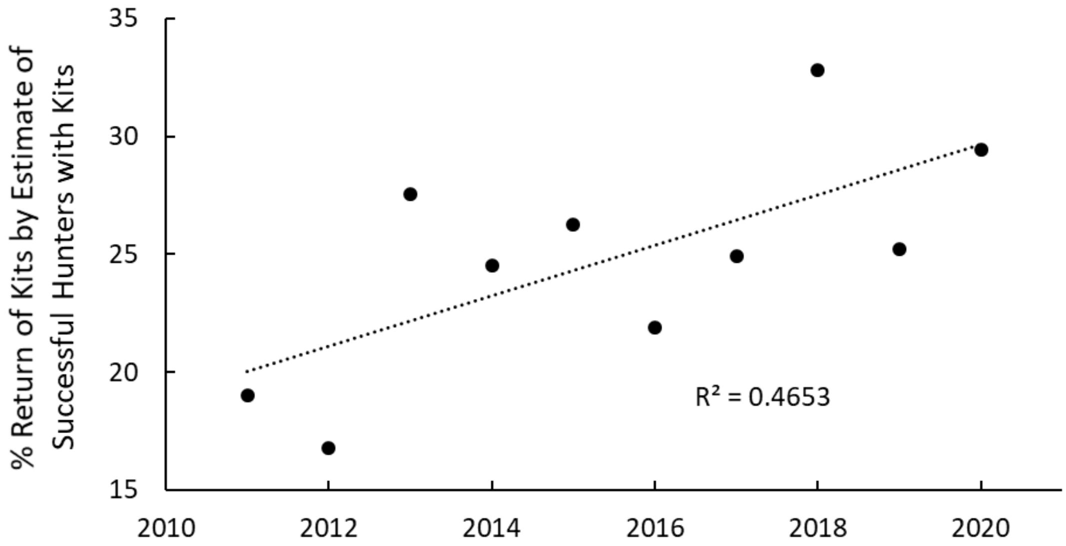

Figure 3. Proportion of blood samples returned by estimate of successful hunters who recieved kits, Elk Hunt Areas 33-41, 45, 47-49, and 120, Bighorn Mountains, WY, 2011- 2020.

Discussion

The finding of brucellosis in the Bighorn Mountains in 2012 initiated a major investment of resources and funding dedicated to contacting hunters and key stakeholders, primarily to procure useable samples to detect the disease and provide data for informed decision-making. From 2013 to 2017, during the years immediately following the initial and last known seropositive elk, total funding and number of seasonal personnel invested were stable, yet declined starting in 2018 as efforts failed to detect the disease in both hunter-harvested elk and elk captured for an associated GPS movement study. The continued years of failed detection of seropositive elk, in addition to initiating a major statewide monitoring program on chronic wasting disease (CWD) in 2018, shifted program funding from state to federal sources. Funding a seasonal technician has been reallocated to assist sampling efforts around Lander during the 2021 hunting season.

Among years of the program, kits deployed, contacts with hunters, and blood samples returned have similar trends as seen for personnel and funding. The increased number of useable blood samples was primarily attributed to WHL ability to accurately test frozen and/or contaminated samples, and assisted by willingness of land and business owners to keep coolers indoors or in refrigerated units, thus protecting blood samples from freezing. From 2017-2020, overall return of blood samples at non-mail sources reflect efforts exerted toward land and business owners to collect and store blood samples. The relatively high average return of blood samples at check stations was likely a function of strategic timing and/or location, and particularly for total return at coolers, persistent availability. Low return of blood samples at processors likely resulted from low submission of elk for processing (estimated <5% of those harvested based on processor check-in sheets), whereas low return for field personnel likely resulted from other job duties and/or field check availability due to when/where harvest occurred.

Our cursory analysis suggests that although harvest has no effect on the return of blood samples, number of kits deployed and hunters contacted does. Furthermore, despite decreased number of kits deployed and hunters contacted in 2018, we observed an increased blood sample return by an estimate of successful hunters who received a kit, suggesting a positive effect of the raffle and associated marketing. Although cash incentives (especially when provided in advance) typically provide greater response rates than prizes via mail, phone, or in-person surveys, 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). For the 2021 hunting season, we have secured one prize, and intend to pursue additional sponsors to secure prizes exceeding total value of those offered in 2020. We will again re-emphasize the need for all regional wildlife personnel to promote collection of blood samples and the raffle to elk hunters and key landowners within program HAs.

The failure to detect brucellosis in over 2,000 useable samples the last four hunting seasons (2017-2020) throughout the Bighorn Mountains is encouraging. However, we urge caution for individuals or entities using this knowledge to make non-wildlife management decisions. Loss of detectable titer occurs in animals older than 10 years of age (Benavides et al. 2017) and may reduce the likelihood of detecting seropositive elk. However, our 2020 data suggest that in HAs 39-41 and 49 where brucellosis was previously detected, approximately 1 in 5 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 seroprevalence 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 2013-2020 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. With failure to detect brucellosis the last four hunting seasons, WGFD will reduce targeted sampling of elk for brucellosis to the western side of the Bighorn Mountains in HAs 39-41, 45, and 47-49 in the 2021 hunting season.

Acknowledgements

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