Wyoming Game and Fish Department

Bighorn Mountains Enhanced Elk Brucellosis Surveillance Program

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Summary

In the Bighorn Mountains, we documented nine brucellosis seropositive elk from hunter-harvested elk during 2012-2016. Starting in 2013, Wyoming Game and Fish Department (WGFD) increased surveillance efforts to better understand the extent and distribution of brucellosis seropositive elk within Bighorn Mountain Elk Hunt Areas (HA) 33-41, 45, 47-49, and 120. Primary objectives of the Bighorn Mountains surveillance program are to increase elk hunter contacts resulting in an increase in the number of testable blood and supramammary and iliac lymphatic tissue samples. During the 2016 hunting season in the Bighorn Mountains, the WGFD Wildlife Health Laboratory (WHL), Cody and Sheridan regional personnel contacted at least 2,895 elk hunters, distributed 7,606 blood sample kits (kits), collected 724 blood samples (74.1% useable; 463 mail, 261 non-mail sources) and 64 lymphatic tissue samples. In addition, we documented two seropositive elk (adult female, HA 40; adult male, HA 49). In 2016, WGFD used two At-will Employee Contract(ed) (AWEC) technicians based in Sheridan region to collect samples and a Cody region permanent brucellosis-habitat biologist whose focus was to collect blood samples and monitor brucellosis seroprevalence and distribution in the Bighorn Mountains. Two additional AWEC technicians from other WGFD projects helped collect samples in November. In field trials, we tested disposable hand warmers and freezer-packs to prevent blood from freezing, with no success, yet 100% (7/7) of blood samples that separated prior to freezing were found to be useable for testing. Since 2011, useable blood and tissue samples increased and were likely a function of communication with hunters and key individuals (landowners who allowed elk hunting and outfitters), and improved training of field personnel. To facilitate enhanced brucellosis surveillance, we continue to target sampling in cooperation with landowners and outfitters. We developed a proposal and submitted to the WHL to incentivize brucellosis sample returns by hunters to improve blood sample returns that is under consideration for the 2018 season.

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, debate over appropriate management actions, and need for more seroprevalence data. To understand the prevalence and distribution of brucellosis, particularly in non-feedground elk herds, WGFD annually provides about 10,000 blood sampling kits to limited quota license elk hunters in targeted surveillance HAs around Wyoming. In the Bighorn Mountains, brucellosis seropositive elk were documented in nine hunter-harvested elk from 2012-2016. 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 surveillance program are to increase elk hunter contacts resulting in an increase in the number of testable blood and supramammary and iliac lymphatic tissue samples.

Study Area & Methods

The Bighorn Mountains lie within the north-central portion of Wyoming and are comprised by 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). In 2016, elk hunting seasons ranged from 15 August 2016 to 15 January 2017. Among all HAs, there were at least 10,986 limited quota elk license holders who reported hunting in the Bighorn Mountains in 2016.

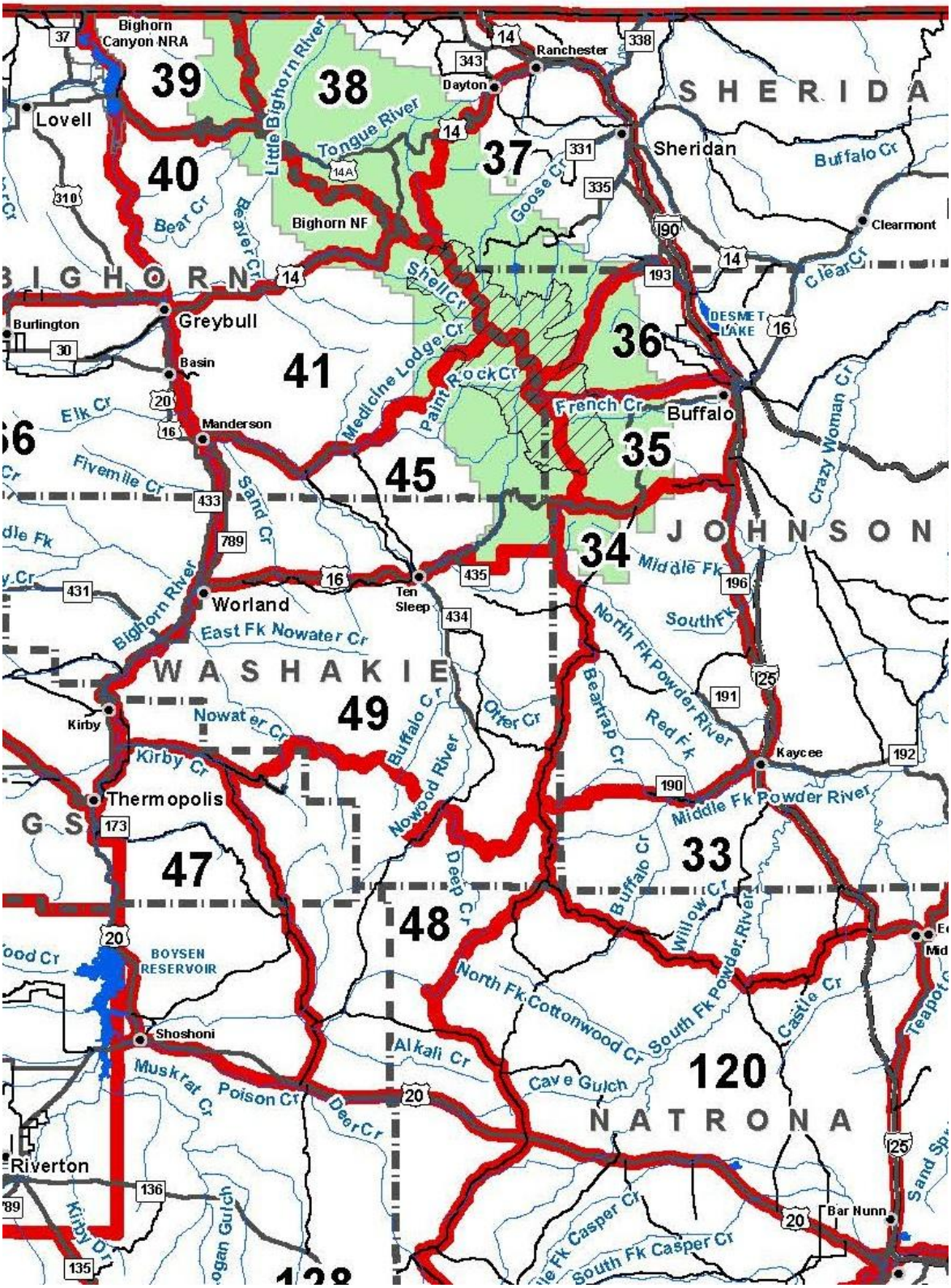

Figure 1. Elk hunt areas, Bighorn Mountains, WY, 2016.

In 2016, two AWEC technicians (Sheridan Region), two AWEC technicians (Cody Region) and a Cody Region permanent, brucellosis-habitat biologist position collected blood samples to monitor brucellosis seroprevalence and distribution (Table 1). Technicians were hired to work from September to December, and the brucellosis-habitat biologist moved from the Pinedale Region to the Cody Region on 1 October 2016 and assumed duties specific to brucellosis and habitat management. To increase public awareness of brucellosis, Cody and Sheridan Wildlife Division personnel discussed brucellosis ecology and harvest data, both formally and informally, with hunters, landowners, outfitters and other publics throughout the hunting season. About 7,606 kits were assembled and distributed to hunters (7,256 by mail and 350 handed out in field) by WHL and Cody and Sheridan personnel (Table 2).

Table 1. Summary expenses for brucellosis technicians, 2016-2017.

		Labor		Vehicles		
Technician Positions (N)	Period Worked	Hrs.	Cost ($)	Miles	Cost/ mi	Cost ($)
Cody (2)[a]	Nov-Dec 2016	209	3,097.38	7,805	$0.30	2,341.50
Sheridan (2)	Oct-Dec 2016	811	12,019.02	9,003	$0.30	2,700.90
Wildlife Health Lab (1)	Jun 2016-Mar 2017	N/A	11,561.00[b]	N/A	N/A	N/A
Total		1,020	26,677.40	16,808		5,042.40

[a] Two technicians from another project helped collect samples later in the hunting season

[b] Cost for WHL Technician is amount budgeted

Kits consisted of a 15-ml sterile polypropylene conical tube, paper towel, instruction/data sheet, a prepaid mailing label for return shipping. Kits were collected opportunistically during hunter field checks; at game check stations; regional WGFD offices; and at strategic drop-off locations (i.e., road junctions, convenience stores, grocery stores, processors, check stations) around the Cody and Sheridan Regions. Kit drop-off locations (N=42) consisted of a cooler (typically exposed to ambient outdoor weather), a 12”x18” instructional sign, and were checked daily throughout the hunting season, especially when night-time low temperatures were predicted to be below freezing. Uncontaminated and unfrozen blood samples were separated into red blood cells and serum using a centrifuge. Serum was frozen for shipment to the WHL, usually once a week. We collected supramammary and iliac lymphatic tissues during field checks and at check stations,

when possible. To potentially increase the percentage of useable blood samples, we tested several types of heat sources placed in coolers to prevent blood from freezing during cold day-time, or over-night temperatures. We measured cooler internal temperatures using temperature loggers with either: no heat source (control), small hand warmers, large hand warmers, and unfrozen freezer packs (e.g., "Blue Ice®") exchanged daily. We hoped that blood samples would remain above freezing during below-freezing nights to increase usable samples.

To potentially increase the number of lymphatic tissue samples, we coordinated with one key landowner and seven associated hunters in HA 41 in mid-October. The goal of this coordination was to collect samples, describe brucellosis ecology, our need for additional tissue samples, and help hunters and landowners learn the locations and collect supramammary and iliac lymph nodes when they harvested an elk when an employee could not be there to take a tissue sample. We provided the seven hunters with Whirlpacks® (plastic bag) to place their tissue samples if they harvested an elk. Prior to submission to WHL, tissue samples were checked to ensure quality and accuracy. We also contacted three game processors in the Cody Region (mid-Nov to mid-Dec) to ask permission to collect elk blood samples from their customers. Game processors specified that Mondays were often a good day to collect blood samples from hunters, and one processor called after confirming that hunters had blood samples.

Results

In 2016, we contacted and provided brucellosis information to at least 2,895 hunters and 40 key individuals during face-to-face informal and formal conversations, including stock growers in HA 49 following harvest of a seropositive adult male elk. Of the 724 kits returned with blood, 74% were useable, with 463 received by mail and 261 samples received during field contacts or at a WGFD regional office. Most of the unuseable samples were contaminated (strong urine and/or rumen odor). Seropositive elk harvested from HA 40 (adult female) and HA 49 (adult male) did not have lymphatic tissue samples collected as we never contacted the hunters with the carcass, or were able to go to the kill site and inspect the gut pile or carcass for tissues. Since 2011, we increased kits distributed; blood and tissue samples collected, and number/proportion of useable samples. Since 2013, percentage of kits returned relative to kits distributed and elk harvested was stable but relatively low (Table 2).

Table 2. Brucellosis hunter-harvest sampling summary for Elk Hunt Areas 33-41, 45, 47-49 and 120 in the Bighorn Mountains, WY, 2011-2016 hunting seasons.

	Total				% Return[b]		% Samples Useable (N)[c]	Seropositive Elk	
Year	Kits[a]	Harvest	Samples		Kits	Harvest		N	Hunt Area(s)[d]
			Blood	Tissue					
2011	2829	3057	241	UK	8.5	7.9	55.6 (134)	0	N/A
2012	2885	3785	244	UK	8.5	6.4	43.4 (106)	2	40
2013	7626	3364	736	48	9.7	21.9	67.0 (493)	2	40, Unk[e]
2014	7350	3880	812	27	11.0	20.1	71.2 (578)	3	39,40,41
2015	6,640	4053	700	34	10.5	17.3	68.9 (482)	0	N/A
2016	7,606	4247	724	64	9.5	17.0	74.2 (537)	2	40, 49

[a] Total mailed plus estimated total distributed to hunters in field

[b]Percentage of blood samples returned relative to total kits distributed to hunters or elk harvested

[c]Number of useable blood samples (N) divided by number of blood samples returned

[d]Hunt area(s) where samples from seropositive elk were harvested

[e]Sample did not have a location or HA information

We found that hand warmers did not prevent the inside of coolers from dropping below zero, and unfrozen freezer packs did not prevent freezing of hunter-harvested blood samples. However, 100% (7/7 blood samples) of frozen sera that had separated from red blood cells prior to freezing and was scraped from the sample tube was later found testable. Of the hunters who attempted to collect tissue samples, 100% (7/7) successfully harvested iliac and/or supramammary tissue samples along with corresponding useable blood samples in HA 41. Lymphatic tissue samples were obtained in HA 45 from three hunter-harvested elk at a processor.

Discussion

The relative increase in useable blood and tissue samples since 2011, particularly on and after 2013, was likely facilitated by committed funding; educating hunters on the importance of blood sample collection, and increased training for WGFD field personnel. This also includes training for brucellosis-specific field and lab technicians, and maintaining contact with hunters and key individuals. To ensure sustained sample numbers in future years, we think it is beneficial to maintain seasonal field technicians, in addition to one WHL technician, and one permanent brucellosis-habitat biologist.

Repeated detection of seropositive females in HA 40 suggests this area is a source for brucellosis, and subsequent detection in surrounding HAs suggests the disease is expanding. In the event that additional seropositive elk from the Bighorn Mountains is detected, we will continue to try to collect tissue samples to determine if there are culture positive elk in the Bighorns. Cultured *Brucella* from a hunter-killed elk may provide information on the origin of the bacteria by DNA analysis.

In our field trial, hand-warmers failed to prevent blood samples from freezing and was likely due to the warmers must be constantly "massaged" to prevent solidification of granular contents and permit air flow for chemical reaction and heat generation. Freezer packs failed likely because thermal conductivity was too low to be sustained in below-zero temperatures. From our frozen serum samples that were successfully tested, we estimate that blood must sit for >12 hours to separate prior to freezing and still be usable. We will continue to experiment with various blood kit drop-off coolers and samples to determine optimal designs and thresholds.

Given the willingness of landowners and relative success of their hunters at collecting samples, we think there is potential in certain situations to allow hunters to collect tissue samples. We will continue to work with key landowners during peak hunter numbers days in the rifle hunting seasons (i.e., Saturday-Sunday, opening or closing season dates, holiday weekends) and maybe later expand to include tissue collection by specific outfitters and guides. Low numbers of tissue (and blood) samples from processors was probably due to soliciting these individuals in November, well

past their peak operations. We will continue to work with processors, particularly during and after peak hunting days in October (e.g., Monday-Tuesday).

State-wide, approximately 10% to 15% of kits mailed to hunters are returned, and of those returned kits approximately 60% contained useable samples. Similarly from the Bighorn Mountains, sustained low levels of returned samples relative to kits deployed or elk harvested are likely functions of hunter education and incentive. We drafted a proposal to simplify the blood kit information card and incentivize hunters submitting useable samples via raffle. These changes will be further discussed with the WHL for possible adoption in 2018.

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