USING TEST AND SLAUGHTER TO REDUCE PREVALENCE OF BRUCELLOSIS IN ELK ATTENDING FEEDGROUNDS IN THE PINEDALE ELK HERD UNIT OF WYOMING; RESULTS OF A 5 YEAR PILOT PROJECT

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Executive Summary: The U.S. Fish and Wildlife Service (USFWS) began the practice of providing supplemental feed to elk in winter during 1912 on the National Elk Refuge (NER) to reduce winter mortality and maintain populations beyond what native winter ranges could support, and to reduce damage to stored crops on private lands. The Wyoming Game and Fish Department (WGFD) began feeding elk in 1929 for the same reasons, and today between 20,000 and 25,000 elk are fed on 22 feedgrounds in Lincoln, Sublette, and Teton counties in western Wyoming. Around 80% of elk within the 7 herd units containing feedgrounds utilize supplemental feed during winter. Brucellosis is a bacterial disease endemic in populations of free-ranging elk (*Cervus elaphus*) and bison (*Bison bison*) in the Greater Yellowstone Ecosystem transmitted by ingestion of the bacteria (*Brucella abortus)* during contact with infected fetuses and placentas from abortion events. Prevalence of brucellosis among elk attending winter feedgrounds is elevated. Although operation of feedgrounds reduces risk of interspecific brucellosis transmission risk by facilitating the separation of elk and domestic cattle (*Bos Taurus*), several *B. abortus* infections have been recently discovered in cattle near feedgrounds, leading to expensive testing requirements and trade sanctions. In an effort to reduce prevalence of brucellosis among elk, the WGFD implemented a pilot project using test

and slaughter on three feedgrounds in the Pinedale elk herd unit from 2006 to 2010. Seroprevalence of antibodies to *B. abortus* of elk captured from the Muddy Creek feedground fell significantly from 37% (n = 158) in 2006 to 5% (n = 141) in 2010 with the slaughter of 107 seropositive animals. Although at least two trapping attempts were conducted every year at Muddy Creek feedground, cumulatively only 646 of 1,321 (49%) adult and yearling female elk available were captured and tested. Slaughter of seropositive elk at Muddy Creek did not appear to prevent brucellosis transmission events. Brucellosis seroprevalence reductions were also observed on the Fall Creek and Scab Creek feedgrounds following removal of 32 and 58 seropositive elk, respectively.

INTRODUCTION

Brucellosis is an infectious zoonotic disease caused by bacteria of the genus *Brucella*. *Brucella abortus* was likely introduced into North America with the importation of European domestic cattle (Meagher and Meyer, 1994; Cheville et al. 1998; Thorne, 2001). The most significant form of brucellosis transmission occurs by ingestion of the bacteria during contact of susceptible animals with infected aborted fetuses, fetal membranes and fluids or uterine discharges (Thorne, 2001). Mohler (1917) first reported incidence of the disease in wildlife with two bison from Yellowstone National Park (YNP) that were positive for antibodies against *B. abortus,* an infection likely originating from commingling of infected cattle with bison or from infected bovine milk fed to captive bison calves (Cheville et al., 1998).

Brucellosis was first detected in wild elk in 1930 from samples collected on the NER (Murie, 1951). The NER, along with 22 additional winter elk feedgrounds currently

managed by the WGFD, annually provide feed to between 20,000 and 25,000 elk in western Wyoming (Fig. 1). Supplemental winter feeding artificially congregates animals from November through April, overlapping the brucellosis transmission period of February through June (Roffe et al., 2004; Cross et al., 2007). Brucellosis seroprevalence of yearling and adult female elk captured from feedgrounds from 1985 to 2009 was 22% (731/3327), whereas none of the 1,930 elk sampled from 1991-2008 in elk herd units in Wyoming distant from the feedgrounds (i.e., elk herds in central and eastern Wyoming not adjacent to elk herd units containing feedgrounds or to YNP) were positive (Scurlock and Edwards, 2010). Although there is evidence suggesting brucellosis is being maintained in elk populations in Wyoming which are not fed during winter, these herds are located either adjacent to feedground elk herds or to YNP and the infection likely originated from the feedgrounds (Cross et al., 2010; Scurlock and Edwards, 2010).

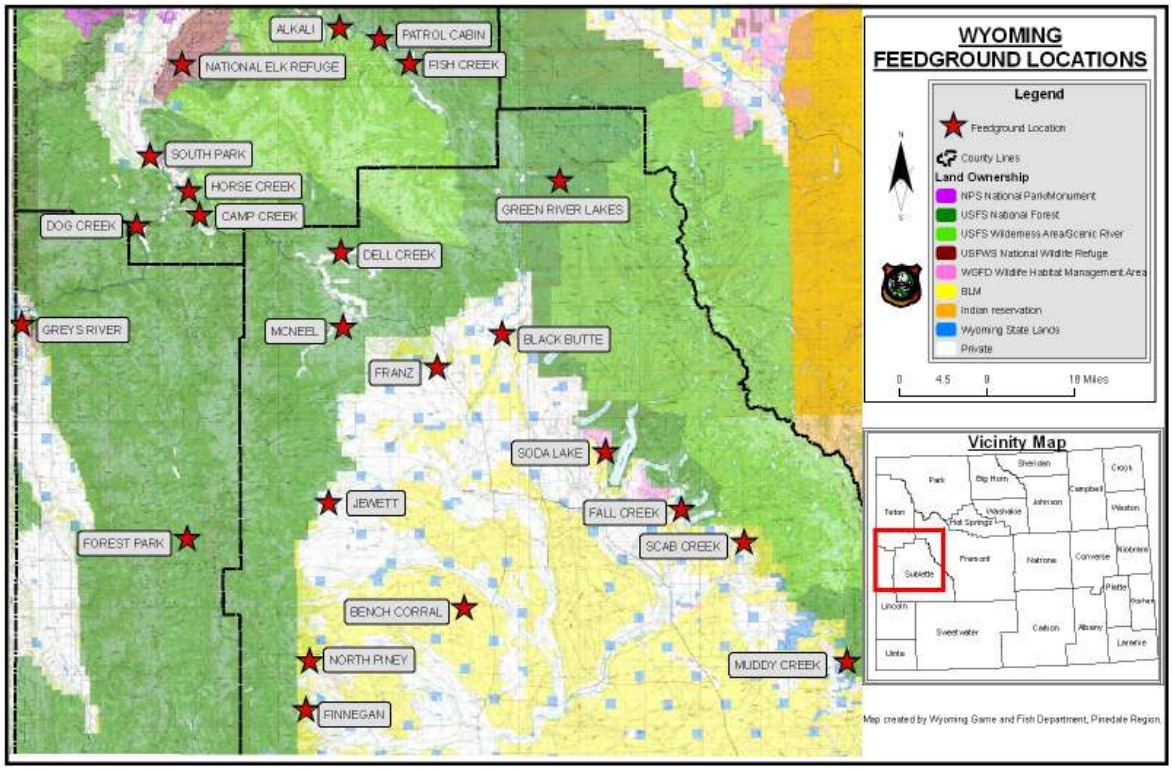

Figure 1. Locations of Feedgrounds in Wyoming

Supplemental feeding of elk in Wyoming began primarily to limit elk impacts on agricultural lands and to maintain larger populations than winter ranges could support. Feedgrounds continue that function today, and also facilitate separation of wildlife and cattle, reducing the potential for interspecific brucellosis transmission (Smith, 2001). However, elk feedgrounds do not provide complete separation of elk and cattle over the duration of the brucellosis transmission period.

Efforts to eradicate brucellosis in U.S. cattle began in 1934 as part of an economic recovery program to reduce the cattle population because of the Great Depression and severe drought conditions. Using primarily a combination of test and slaughter with vaccination, nationwide brucellosis reactor rates in cattle fell from 11.5% in 1934-1935 to 0% in 2000, the first time in history there were no affected cattle herds in the U.S. (Ragan, 2002). However, several outbreaks of brucellosis were detected in cattle herds of the GYA portions of Wyoming, Idaho, and Montana from 2003 to 2008; all are believed to have been of wildlife origin. In Wyoming, four separate *B. abortus* infections in cattle from Teton and Sublette counties from 2004-2009 likely originated from elk (Beja-Pereia et al., 2009).

The 'Brucellosis Class Free' status of Wyoming's cattle was revoked in 2004 as a result of 37 cattle from two different herds in Sublette County testing positive for antibodies to *B. abortus*. Management of the index herd included calving on private lands in close proximity to the Muddy Creek elk feedground, and the outbreak most likely originated from infected elk associated with this feedground (Bricker and Ewalt, 2005). In response, Wyoming's Governor appointed ranchers, outfitters, sportspersons, conservationists, state and federal land managers, and domestic and wildlife health

managers to a Brucellosis Coordination Team (BCT). The goals of this team were to address: 1) reclaiming Class-Free brucellosis status for cattle, surveillance, and transmission between species; 2) developing an Action Plan of what to do in the event of a new case in cattle; 3) addressing human health concerns; and, 4) reducing, and eventually eliminating brucellosis in wildlife, specifically addressing winter elk feed grounds. While addressing topic 4, the BCT recommended not to terminate any winter elk feedground in the foreseeable future, but to "conduct a limited 5-year pilot project that institutes a seroprevalence reduction program within the Pinedale Elk Herd unit" (Galey, 2005).

In this report, we describe data collected during the pilot Test and Slaughter project conducted by the WGFD on the Fall, Scab and Muddy Creek feedgrounds from 2005 to 2010 as recommended by the BCT. These three feedgrounds are located within the Pinedale elk herd unit, which has a total population objective of 1900 elk wintering both on and off feedgrounds. An average of around 1700 elk wintered on the three feedgrounds during the pilot project. The pilot project was initiated on the Muddy Creek feedground during winter 2005-2006, and continued every winter on this site through 2009-2010. The project was expanded during winters 2007-2008 and 2008-2009 to the Fall and Scab Creek feedgrounds, respectively, continuing through 2009-2010.

MATERIALS AND METHODS

Portable elk traps

Capturing a large proportion of the total female elk attending each feedground every year was determined imperative for the success of the pilot test and slaughter project.

Although permanent corral traps were located on the Muddy and Fall Creek feedgrounds, they lacked the capacity and the technology to hold and efficiently process large numbers of elk; therefore, new trap facilities had to be considered. All new structures had to be portable in design to satisfy federal requirements of the agencies who manage the lands occupied by the Muddy Creek feedground (U.S. Forest Service) and Fall and Scab Creek feedgrounds (Bureau of Land Management).

Wyoming Game and Fish personnel began investigating various portable trap designs and manufacturers during spring 2005. TJ Welding, Inc. (Blackfoot, ID) had experience constructing and operating portable elk traps for the Idaho Fish and Game Department on the Rainy Creek elk feedground. Following a competitive bid process, the WGFD contracted TJ Welding and worked with their professionals as well as Grandin Livestock Handling Systems, Inc. (Fort Collins, CO) to design, and eventually construct 3 large portable elk traps (Figure 2). The traps were erected on Muddy Creek feedground during October 3-6, 2005; on Fall Creek feedground during October 26-29, 2006; and on Scab Creek feedground during August 4-8, 2008.

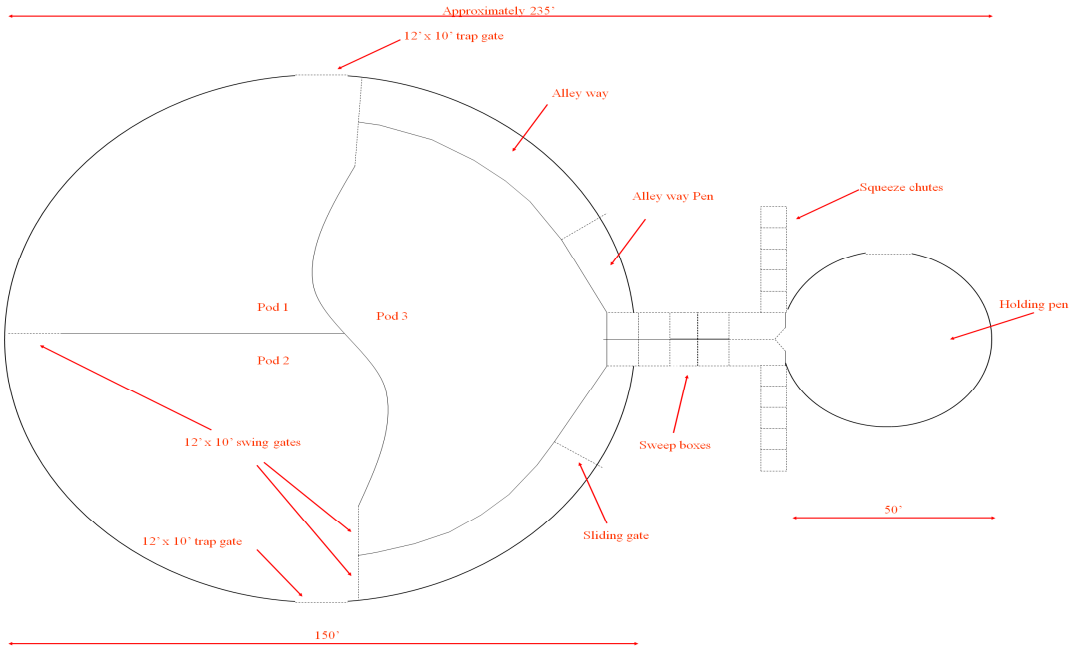

Figure 2. Diagram of portable elk trap

Snow and Ice Removal

Snow accumulation in the main trap corral reduces the height of the walls allowing elk to escape and ice accumulation in all areas of the trap reduces traction and increases risk of injury to elk and personnel. Thus, WGFD personnel removed snow and ice from elk traps after each significant snowfall each year during trapping periods, typically one week in late January and one week in early February. Additionally, nearly 22 miles of road into the three feedgrounds were plowed several times each trapping period to ensure personnel could access the trap and allow for removal of selected elk via stock trailer.

Trap Acclimatization, Capture, Processing, and Serologic Testing

Five to ten days prior to trapping attempts, efforts were made to acclimatize elk to a routine similar to when trapping would be attempted. Time of feeding, distribution of hay on the feedground (including within the trap), and number of humans present when feeding were repeated as consistently as possible. Bull excluders (17" wide x 68" tall metal guards placed over gate openings to deter branch-antlered bulls) were also placed into position 2-7 days before the initial trapping attempt. Generally, elk were more tolerant of trapping-related activities during periods of cold temperatures and deep snow (i.e., more dependent upon supplemental hay).

One to two days prior to capture attempts, hay rations were decreased to encourage elk to enter the trap. During capture attempts, hay was dispersed from the feed sled into each of the 3 main pods of the main corral, a limited amount fed outside the trap, and a line of hay was led from the traps to the main feeding area. WGFD personnel monitored the number of elk entering the trap from nearby blinds, remotely closing the door (Ace

Capture Equipment, Invercargill, New Zealand) when the number of yearling and adult female elk inside the trap peaked.

Once elk were captured, branch antlered bulls, if present, were chemically immobilized and removed from the trap to prevent goring injuries. Personnel then divided animals equally among the three pods of the main trap corral. Elk were then moved from pods 1 and 2 into the long narrow alleyways (Fig 2). A sliding gate in the alleyway allowed elk to be further divided, advancing several animals at a time into the sweep boxes. Elk in sweep boxes were moved towards the squeeze chutes and gradually divided into groups of five, which were then advanced into the squeeze chutes from a corner sweep box. Individual animals were isolated with sliding gates into each squeeze chute, where a pivoting door with multiple drop-down access panels pinned the elk to the adjacent closed sliding gate. Elk in pod 3 were advanced to either pod 1 or 2 when emptied, then moved through the system as previously described.

Elk in squeeze chutes were sexed and aged via incisor wear. Male elk were not targeted due to their insignificance in brucellosis transmission (Cheville et al., 1998), and antibody prevalence of juvenile elk is very low (Scurlock and Edwards, 2010). Thus, all juveniles and males received permanent aluminum ear tags (style #42, Hasco Tag Company, Dayton, KY), and were then released through a pivoting door on the far side of the squeeze chute.

Blood from yearling and adult females was collected into 15 ml sterile polypropylene conical tubes (Falcon Blue Max Jr., Becton Dickinson Labware, Franklin Lakes, NJ), via jugular venipuncture. Bled elk also received ear tags and a polyvinyl visibility collar with unique letter-number combination, and were then moved back through the squeeze

chutes and the corner sweep box into a holding pen. After all elk had been processed, animals in the holding pen were advanced into pod 3, where they were held overnight while serologic testing was conducted.

Blood samples were transported to the Wyoming Game and Fish Wildlife Disease Laboratory in Laramie, WY, where tubes were centrifuged, serum decanted and placed into sterile 5 ml cryovials (Nalgene Cryogenic Vial, Nalge Nunc International, Rochester, NY). All serological assays were conducted and interpreted using current National Veterinary Services Laboratories protocols for the card test, plate agglutination (SPT), rivanol precipitation – plate agglutination (RIV), fluorescence polarization assay using tubes (FPA), and complement fixation (CF). A competitive ELISA (cELISA) was used to discriminate *B. abortus* strain 19 vaccine from field strain titers (Van Houten et al., 2003). Serologic profiles were categorized using the United States Department of Agriculture's brucellosis eradication uniform methods and rules for *Cervidae* (US Department of Agriculture, 2003), with one variation. A positive classification was based on incomplete or positive agglutination on any two of the following tests: Card, SPT (≥1:100), and RIV (≥1:25). Positive reactions were confirmed with the CF, FPA and cELISA.

Serologic tests were completed in less than 12 hours and results were relayed to field personnel, who then reprocessed and sorted the elk; those determined positive for antibodies to *B. abortus* were moved into the holding pen and those determined negative were released. Seropositive elk were then moved from the holding pen, through the squeeze chutes and into an awaiting stock trailer, for transport to a USDA-approved

slaughterhouse in Rigby, ID. Elk were slaughtered, and the processed meat subsequently donated to food banks throughout Wyoming.

Tissues most likely to yield *B. abortus* culture were collected from each carcass following slaughter. Target lymph nodes included the internal iliac, external iliac, medial retropharyngeal, and supramammary. Reproductive tissues (uterus) were also collected along with the fetus, if present. Two incisor teeth were collected for age determination using cementum annuli analysis. Reproductive tissues and fetuses were transported to the Wyoming State Veterinary Laboratory for necropsies and further culture sample collection from amniotic fluid, lung, abomasal fluid, and cervix. All tissues were frozen within 48 hours of collection at -70^{o}C or -20^{o} C.

Culture

Tissues were frozen at –20^{o}C for at least 24 hours, and then thawed at 22^{o}C for 2 hours or 18-24 hours at 5^{o}C. Tissues were removed from collection dishes and placed into a sterile 15 X 100 mm plastic Petri dish where fat and connective tissue was removed with a sterile #60 scalpel blade and forceps. Tissue was then minced into small (~1 cm^2 pieces) and placed into a 10.2cm X 15.2 cm sample bag (Nalge Nunc International, Rochester, NY) with an equal amount of tryptose broth. The tissue and broth were then homogenized (Metrohm USA, Riverview, FL) for 2 minutes at high speed, then used to inoculate four 15 X 100 mm Petri dishes containing TSAEV semi-solid media (5% donor calf serum, Brucella agar (Difco Laboratories, Detroit, MI) with the addition of 7,500 I.U. bacitracin, 1,800 I.U. polymixin B, 30-mg cyclohexamide, and 0.000125% crystal violet per liter). Plates were inverted and incubated at 37^{o}C under 10% CO_2, for a minimum of seven days. Suspect bacterial colonies were removed and streaked for

isolation on TSAEV media. Isolates were used to inoculate several types of selective media types, including 1:50,000 thionin, 1:50,000 basic fuchsin, urease, erythritol, penicillin and an O_2 incubation on tryptose agar with serum, antibiotics, and ethyl violet. All suspect isolates were tested for dominant antigen and confirmed with BASS PCR (Ewalt and Bricker, 2003).

RESULTS

Capture Data

A summary of capture results is provided in Table 1. A total of 1,416 elk were captured at Muddy Creek feedground during all years of the project. Captures totaled 657 elk at Fall Creek feedground, where trapping occurred during 2008 and 2009. Trapping was attempted at Fall Creek feedground in 2010, but the majority of elk that typically winter on the site did not attend the feedground and trapping was not successful. A total of 551 elk were captured at Scab Creek feedground during the last two years of the project. Overall, trapping was successful during 18 of 52 attempts (35%).

Table 1. Number of female and male elk captured from three feedground in the Pinedale Elk Herd unit, total captured, total bled, number seropositive, and percent antibody prevalence

Feedground	Year	Females				Males				Total Captured	Total Bled	Positive	Prevalence (%)
		Adults	Yrlng	Juv	Total	Adults	Yrlng	Juv	Total				
Muddy Creek	2006	148	10	74	232	3	9	69	81	313	158	58	36.7
	2007	54	25	22	101	1	35	37	73	174	79	13	16.5
	2008	159	28	78	265	12	28	50	90	355	154	21	13.6
	2009	119	13	94	226	4	51	51	106	332	114	8	7.0
	2010	115	26	38	179	11	13	39	63	242	141	7	5.0
	Subtotal	595	102	306	1003	31	136	246	413	1416	646	107	
Fall Creek	2008	209	31	54	294	27	24	37	88	382	191	21	11.0
	2009	159	13	50	222	6	9	38	53	275	158	11	7.0
	Subtotal	368	44	104	516	33	33	75	141	657	349	32	
Scab Creek	2009	135	20	90	245	7	17	64	88	333	149	31	20.8
	2010	120	21	28	169	7	16	26	49	218	141	27	19.1
	Subtotal	255	41	118	414	14	33	90	137	551	290	58	
	TOTAL	1218	187	528	1933	78	202	411	691	2624	1285	197	

A total of 1,285 yearling and adult female elk were bled at all sites during the project, which was 43% (1,285/2,959) of those cumulatively attending the feedgrounds as counted during annual elk surveys by WGFD biologists. Percent captured of available adult and yearling females ranged from a high of 65% (191/296) on the Fall Creek feedground in 2008, to a low of 29% (149/513) at the Scab Creek feedground in 2009. Target females comprised 53% (1,405/2,624) of all elk captured, although they made up 67% (2,959/4,393) of all elk cumulatively attending the feedgrounds.

Brucellosis seroprevalence of elk captured on the Muddy Creek feedground decreased significantly ($P = 0.0001$; Fisher's Exact test) from 37% ($n = 158$) in 2006 to 5% ($n = 141$) in 2010 following the slaughter of 107 seropositive females. Antibody prevalence of elk captured from the Fall Creek feedground fell from 11% ($n = 191$) to 7% ($n = 158$) after removal of 32 seropositive females, however the decrease was not significant ($P = 0.1$). Prevalence of antibodies decreased the least (1.7 percentage points) in elk captured from the Scab Creek feedground after 58 seropositive elk were slaughtered (also not significant, $P = 0.59$).

Culture Results

A total of 197 seropositive elk were cultured for *B. abortus* during the project to estimate the percentage of elk demonstrating antibodies to the bacteria that were actually infected (Table 2). Culture prevalence ranged from 36% ($n = 31$) at Scab Creek feedground in 2009 to 77% ($n = 13$) at Muddy Creek feedground in 2007. Pregnancy rate of slaughtered elk was 75% ($n = 210$), and *B. abortus* was successfully cultured from 45% ($n = 146$) of pregnant seropositive elk. Among the 144 fetuses cultured, only 16 (11%) were positive, and the bacteria was recovered from 92% ($n = 12$) of yearlings.

Table 2. Culture prevalence of total, pregnant, and yearling elk and fetuses of seropositive elk slaughtered from three feedgrounds in the Pinedale Elk Herd, 2006-2010.

Feedground	Year	Culture Prevalence	Culture Prevalence-Pregnant Elk	Culture Positive Fetuses	Culture Positive Yearlings
Muddy Creek	2006	31/58 (53.4%)	24/46(53.3%)	10/45 (22.2%)	2/2(100%)
	2007	10/13 (76.9%)	6/9 (66.7%)	1/8 (12.5%)	2/2 (100%)
	2008	13/21 (61.9%)	10/18(55.6%)	0/18	1/1(100%)
	2009	5/8 (62.5%)	4/6 (66.7%)	0/6	0/0
	2010	5/7 (71.4%)	3/4 (75%)	0/4	0/0
	Subtotal	64/107 (59.8%)	47/83 (57.3%)	11/81 (13.6%)	5/5 (100%)
Fall Creek	2008	10/21 (47.6%)	8/19 (38.1%)	1/19 (4.8%)	1/2 (50%)
	2009	5/11 (45.5%)	1/6 (16.7%)	0/6	0/0
	Subtotal	15/32 (46.9%)	9/25 (29%)	1/25 (3.7%)	1/2 (50%)
Scab Creek	2009	11/31 (35.5%)	3/17 (17.6%)	0/17	3/3 (100%)
	2010	16/27 (59.3%)	12/21 (57.1%)	4/21 (19%)	2/2 (100%)
	Subtotal	27/58 (46.6%)	15/38 (39.5%)	4/38 (26.7%)	5/5 (100%)
	TOTAL	106/197 (53.8%)	71/146 (45.3%)	16/144 (11.1%)	11/12 (91.7%)

Project Costs

Table 3 reports all costs associated with the Test and Slaughter project as tracked by WGFD Fiscal personnel by a unique job reporting code. Over the five years of the project, a total of $1.2 million was spent. A considerable amount of funds were also incurred prior to the establishment of the code in 2006, mostly devoted to the design, construction, and eventual erection of the first portable elk trap on the Muddy Creek feedground, thus actual total costs are $30,000-$40,000 higher than reported. In addition, costs to plow access roads into the feedgrounds were incurred by the Sublette County Road and Bridge Department. Actual road-plowing costs are unknown, but would have been substantial if contracted with a private entity.

Table 3. Direct costs, personnel hours worked, and miles driven coded to the Pinedale elk herd pilot test and slaughter project, 2006-2010.

Fiscal Year	Direct Costs ($)							Personnel	
	Personnel	Vehicle	Travel	Elk Slaughter	Lab, Supplies*	Corral Traps**	Total	Hours	Miles
2006	96,495	12,923	17,387	12,409	17,148	151,218	307,580	3,906	34,095
2007	91,353	16,179	15,771	3,104	22,027	148,812	297,246	3,434	40,680
2008	118,808	23,610	19,178	9,250	13,636	0	184,482	3,722	38,273
2009	172,593	23,609	32,334	15,083	34,766	3,486	281,871	5,097	43,774
2010	121,115	18,908	18,150	9,895	15,959	499	184,526	3,314	34,014
Total	**600,364**	**95,229**	**102,820**	**49,741**	**103,536**	**304,015**	**1,255,705**	**19,473**	**190,836**

*includes snow removal at traps

**includes purchase, construction of, and major repair costs

DISCUSSION

The serologic data presented here supports that capturing nearly one-half of available yearling and adult female elk attending a feedground, screening them for exposure to *B. abortus,* and slaughtering those testing positive can reduce antibody prevalence of brucellosis in captured elk by over 30 percentage points in 5 years. Antibody prevalence of elk captured from the Muddy Creek feedground declined most precipitously (~20%) among all sites between the first and second years of the project, when 60% of available females were captured and tested and 58 seropositive individuals were slaughtered. Although a higher proportion of target females were captured at the Fall Creek feedground during the first year of the project (65%), seroprevalence only decreased 4 percentage points, suggesting that test and slaughter is less efficient when applied to populations with relatively low prevalence.

Antibody prevalence of elk captured from the Scab Creek feedground only decreased 2 percentage points, despite the removal of 31 seropositive individuals in 2009. Capturing and testing of only 30% of available females does not appear to appreciably reduce prevalence in a single year. However, the estimated antibody prevalence of the population of elk attending the Scab Creek feedground during 2009 was only based on 30% of the total elk. Thus, the true prevalence of the entire population of elk attending the Scab Creek feedground during 2009 was likely within some range of the observed; although we found a decrease of 2 percentage points between years, this could have been due to sampling error. Additionally, the amount of annual fluctuation in antibody prevalence is unknown; natural oscillations in seroprevalence could mask affects of the treatment.

In fact, WGFD has observed dramatic changes in brucellosis seroprevalence in elk captured from other feedgrounds where test and slaughter has not been implemented. Prevalence among elk captured from the Grey's River feedground dropped from 59% (*n* = 39) in 2004 to 14% (*n* = 36) in 2007 (Figure 3). Although sampling error may have played a role in this observed fluctuation, similar patterns in brucellosis prevalence have been observed on other feedgrounds (Dell Creek; Figure 3), indicating prevalence of antibodies to brucellosis may fluctuate naturally among elk attending feedgrounds. However, prevalence trends on other state feedgrounds do not mimic the steady decrease in seroprevalence observed from elk captured from Muddy Creek feedground during the test and slaughter project, indicating slaughtering seropositive individuals reduces antibody prevalence beyond natural oscillations (see figure 3).

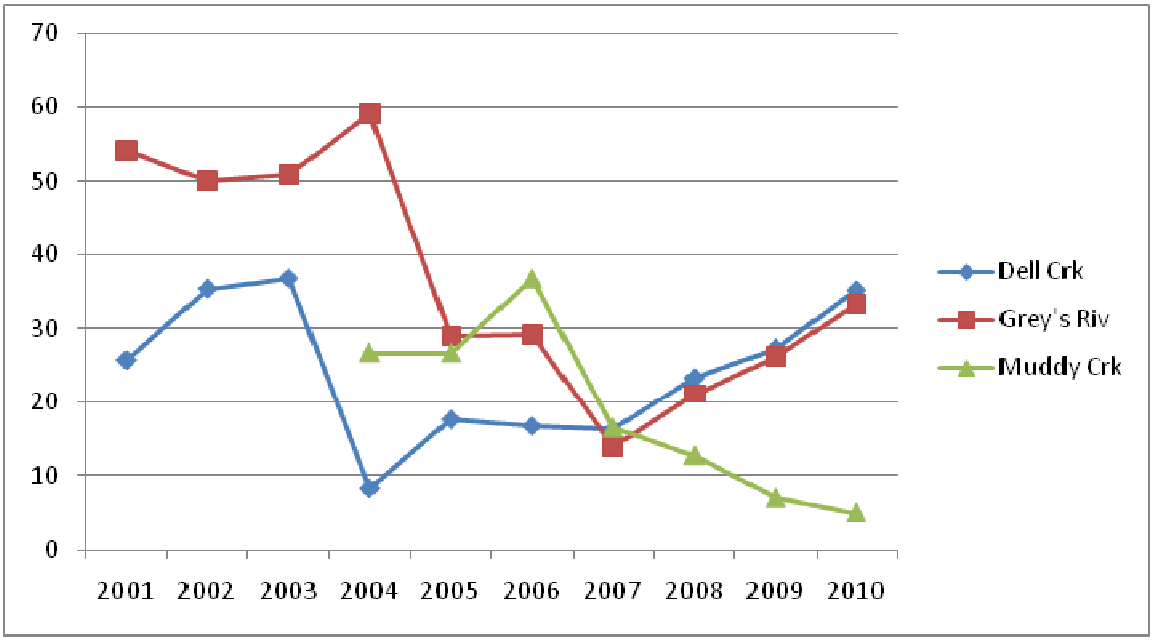

Figure 3. Brucellosis seroprevalence trend of elk captured from the Dell Creek, Grey's River and Muddy Creek feedgrounds, 2001-2010.

Less than 7% (n = 183) of all yearling females demonstrated antibodies to *B. abortus*. However, the bacteria was cultured from 92% (n = 12) of seropositive yearlings; much higher than the overall culture prevalence of 54% (n = 197). Increased culture prevalence observed in yearlings is likely due to recent exposure (Cheville et al., 1998), thus seroprevalence of yearlings may indicate intensity of transmission events the prior winter/spring. Antibody prevalence in yearlings captured from the Muddy Creek feedground fell from 20% (n = 10) in 2006 to 0% in 2009 and 2010 (n = 13 and 26, respectively), possibly indicating reduced abortion events during the brucellosis transmission periods of 2008 and 2009.

However, seroconversion was observed in four elk recaptured on the Muddy Creek feedground in 2010. Each of these animals had been captured between one and three times from 2007 to 2009, and consistently tested seronegative. Although latent infection is a possibility, it is more likely that these elk contacted *B. abortus* in the environment during 2009. Thus, capturing 35% to 60% of cow elk attending a feedground and removing seropositive individuals over a 5 year period does not appear to prevent transmission events.

In consideration of the total *B. abortus* culture positive animals, relatively few (11%) had culture positive fetuses. We believe this is probably due to the timing of trapping (late January/early February), as most elk were slaughtered during their second trimester of pregnancy. In 2006, one trapping occurred later in the year (16 February), and 100% (8/8) of fetuses were culture positive. The highest proportion of culture positive fetuses was only 29% on any trap date prior to February 16th. Our results agree with Nielsen and

Duncan (1990), who reported fetal infection was more likely to occur during the third trimester which corresponds with mid February in most elk.

The proportion of culture positive elk was expected to increase as test and slaughter progressed on each feedground, as culture success is generally higher in animals recently infected with *B. abortus* opposed to those chronically infected for many years (Cheville et al., 1998). We assumed that the proportion of naive (i.e., seronegative) animals in the population would increase during test and slaughter as chronically infected animals were gradually removed. If any of these animals were subsequently infected and trapped the following year, culture success would be greater in these recently infected animals. Although a slight increase in culture positive animals was observed between the first and second year on Muddy Creek and Scab Creek feedgrounds, Fall Creek remained basically the same. In addition, we did not observe an incremental increase in culture prevalence across the five years of test and slaughter on Muddy Creek, possibly due to the inability to capture the majority of yearling and adult female elk in the feedground population.

The Pinedale elk herd pilot test and slaughter project was successful in reducing the prevalence of antibodies to *B. abortus* among elk attending feedgrounds, however capturing only half of the elk available did not appear to prevent brucellosis transmission events. The project was extremely labor intensive and very expensive. Longevity of observed decreases in brucellosis seroprevalence among elk attending the feedgrounds in the Pinedale elk herd is unknown; future surveillance is warranted.

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