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BRUCELLOSIS IN ELK OF EASTERN IDAHO

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ABSTRACT: Brucellosis occurs in free-ranging elk (*Cervus elaphus*) and bison (*Bison bison*) in the Greater Yellowstone Area, which includes portions of Idaho, Wyoming, and Montana. *Brucella abortus* was first detected in elk in Idaho in 1998, and from 1998 to 2002, serologic surveillance of hunter-killed elk was conducted in northeastern and southeastern Idaho. Prevalence of antibodies in these elk varied annually, but averaged between 2% and 3%. Elk were also trapped in northeastern Idaho from 1998–2002 and tested for brucellosis using serology and tissue culture. In areas where artificial feeding of elk was done, antibody prevalence ranged from 12% to 80% depending on site, age, and sex. At one feeding site (Rainey Creek), a decline in the prevalence of antibodies (from 56.8% in 1999 to 13.5% in 2002) was detected after the removal of seropositive elk over 4 yr. Seropositive elk removed from two artificial winter feeding sites (Rainey Creek and Conant Creek) were euthanized and sampled or held in captivity and allowed to calve prior to euthanasia and necropsy. At necropsy, *B. abortus* biovar 1 and *B. abortus* biovar 4 were isolated from both cows and calves; however, biovar 4 was predominant. A dual infection with both biovars was found in one calf born to a seropositive cow from which biovar 4 was isolated. Abortions (16%), stillbirths (8%), and weak calves (4%) were observed in these elk. These findings confirm the presence of brucellosis in elk in eastern Idaho and provide information on disease management options.

Key words: *Brucella abortus*, brucellosis, *Cervus elaphus*, disease management, elk, Idaho.

INTRODUCTION

Brucellosis affects both bison (*Bison bison*) and elk (*Cervus elaphus*) in the Greater Yellowstone Area (GYA), which includes portions of Idaho, Montana, and Wyoming (Mohler, 1917; Creech, 1930; Rush, 1932; Turncliff and Marsh, 1935; Lee and Turner, 1937). These species represent the only known wildlife foci of *Brucella abortus* in the United States (Meyer, 1994; Thorne et al., 1997; Williams et al., 1997; Hollingsworth, 1998). The long-term economic, trade, and management problems associated with brucellosis in bison and elk in the GYA are complex and controversial, especially when set against the impending eradication of brucellosis in cattle in the United States (Ragan, 2002).

The Idaho Department of Fish and Game (IDFG) began collecting data on the health status of elk in 1988. A concerted effort was begun in 1998 to determine the status of brucellosis in elk in eastern Idaho as part of a brucellosis management plan in elk and to minimize the possibility of disease transmission between elk and cattle. This paper documents the presence of brucellosis in elk in eastern Idaho and defines the current status of infection in elk within the state.

MATERIALS AND METHODS

Elk were captured and sampled from winter feeding sites using corral traps with bull excluders or chemical immobilization. Trapping efforts were concentrated at four sites in northeastern Idaho: Rainey Creek, Victor, Teepee Creek, and Conant Creek. The Rainey Creek feeding site is located on US Forest Service land and used by IDFG to prevent depredation of stored hay by elk. The Victor, Teepee Creek, and Conant Creek sites are located on private property. Blood from the trapped animals was collected by jugular venipuncture and placed into sterile tubes and allowed to clot. Blood was centrifuged and the serum decanted and refrigerated or frozen until analysis.

Serum samples from elk trapped at Rainey Creek and Conant Creek in 1999–2001 were initially screened at the trap site using the card test and the standard plate agglutination test

(SPT), but in 2002, the card test was replaced by the buffered acidified plate agglutination test (BAPA) test. A positive card test or positive BAPA and ≥1+100 SPT were used to classify animals as seropositive for management decisions. All samples were submitted to the Idaho State Department of Agriculture (ISDA) Animal Health Laboratory, Boise, Idaho for serologic testing. Samples were initially screened using the card, BAPA and/or the SPT. Samples with positive reactions were tested with rivanol agglutination and/or complement fixation (CF) tests to confirm serologic results. Test methodology and interpretation followed the US Department of Agriculture Uniform Methods and Rules for Brucellosis in Cervidae (US Department of Agriculture, 1998).

Seropositive animals from the trap sites were transported to the University of Idaho's Caine Veterinary Teaching Center in Caldwell, Idaho (CVTC), or the Idaho Department of Fish and Game Wildlife Health Laboratory in Caldwell, Idaho (WHL). Animals sent to the CVTC were euthanized by captive bolt pistol and/or exsanguination after induction of anesthesia. Animals sent to the WHL were allowed to calve and the calves were allowed to interact with their dams for 24–72 hr prior to euthanasia. Euthanasia was administered in cows by captive bolt pistol after chemical immobilization and in calves by intravenous barbiturates after physical restraint. After euthanasia, a complete necropsy was conducted and tissue samples were collected for culture of *Brucella* spp. Tissue samples were refrigerated for up to 72 hr prior to freezing at −20 C or immediately frozen at −20 C. Culture was attempted from thawed samples at the ISDA Animal Health Laboratory as described (Carter, 1990).

In 2000, 13 elk (12 calves and one yearling) were vaccinated with *B. abortus* RB51 at the Rainey Creek feeding site as a disease management strategy. However, three of these elk tested seropositive at the trap site and were removed. Two vaccinated calves were euthanized and necropsied at the CVTC, and the vaccinated yearling was transported to the WHL for further observation.

To determine the prevalence of brucellosis in elk not directly associated with artificial winter feeding sites, blood sampling kits were sent to elk hunters who successfully drew a controlled hunt tag in selected Game Management Units (GMU) in Idaho from 1998 to 2002. Kits consisted of 15-ml conical plastic tubes with screw-tops (Falcon Blue Max Jr., Fischer Scientific, Houston, Texas, USA) for blood collection, a data sheet, and a prepaid mailer for shipping the blood sample to the ISDA Animal Health Laboratory. Total numbers of sampling kits mailed to hunters varied by year, ranging from 900 to 5,000.

RESULTS

From 1989 to 1997, 242 elk were trapped and tested from northern and central Idaho; no elk were trapped from eastern Idaho. All tested negative for antibodies to *B. abortus*.

Serologic test results from elk at the four winter feeding sites in northeastern Idaho from 1998 to 2002 are shown in Tables 1, 2, and 3. Antibody prevalence varied by site, year, age, and sex. Antibodies were detected in 35%, 11%, 33%, and 0% at Rainey Creek ($n=322$), Conant Creek ($n=38$), Teepee Creek ($n=6$), and Victor ($n=20$), respectively. Antibody prevalence was significantly greater in 1999 (57%) than in 2002 (14%) at Rainey Creek (Mann-Whitney *U*-test: $U=1.675$, df=95, $P<0.001$; paired *t*-test: $T=5.934$, df=95, $P<0.001$) and was significantly higher in 1999 and 2000 than in other years (analysis of variance: $F=12.59$, df=124, $P<0.001$) (Table 1). At Rainey Creek, females had higher mean seroprevalence ($n=205$, 47%) than males ($n=107$, 15%) (Table 1). For adult females, antibody prevalence varied by year and location (Tables 1, 2, and 3) with mean seroprevalence of 60%, 7%, and 50% at Rainey Creek, Conant Creek, and Teepee Creek, respectively.

Based on on-site testing, 70 elk were removed from Rainey Creek ($n=65$) and Conant Creek ($n=5$) from 1999 to 2002. All but two elk from Conant Creek were taken to either the CVTC or the WHL. In 2002, two animals from Conant Creek, a yearling female and a male calf, were sent to a local slaughter plant and tissue samples were collected for culture. *Brucella abortus* biovar 1 was isolated from the calf.

Nineteen animals from Rainey Creek were transported to the CVTC, euthanized, and necropsied. Of these, eight

TABLE 1. Antibody prevalence for *Brucella abortus* in elk trapped at Rainey Creek, Idaho, 1998–2002.

Age and sex[a]	1998		1999		2000		2001		2002		Total	
	n	+ (%)[b]	*n*	+ (%)	*n*	+ (%)	*n*	+ (%)	*n*	+ (%)	*n*	+ (%)
AF	10	5 (50)	61	49 (80)	11	10 (83)	7	1 (14)	36	10 (28)	125	75 (60)
AM	4	2 (50)	3	1 (33)	1	0 (0)	0	0	2	1 (50)	10	4 (40)
YF	2	1 (50)	5	3 (60)	3	1 (33)	0	0	7	1 (14)	17	6 (35)
YM	2	1 (50)	1	0 (0)	4	1 (25)	0	0	5	0	12	2 (17)
CF	6	1 (17)	14	5 (36)	14	5 (43)	12	1 (8)	27	2 (7)	73	14 (19)
CM	7	0 (0)	27	5 (19)	12	3 (25)	5	1 (20)	34	1 (3)	85	10 (12)
Total	31	10 (32)	111	63 (57)	45	20 (44)	24	3 (13)	111	15 (14)	322	111 (35)

[a] AF = adult female; AM = adult male; YF = yearling female; YM = yearling male; CF = calf female; CM = calf male.
[b] Number seropositive (% seropositive).

TABLE 2. Antibodies to *Brucella abortus* in elk trapped at Conant Creek, Idaho, 1998–2002.

Age and sex	1998		2001		2002		Total	
	n	+ (%)[a]	*n*	+ (%)	*n*	+ (%)	*n*	+ (%)
Adult female	2	0	7	1 (14)	6	0	15	1 (7)
Adult male	0	0	0	0	0	0	0	0
Yearling female	1	0	0	0	4	1 (25)	5	1 (20)
Yearling male	0	0	0	0	0	0	0	0
Calf female	2	1 (50)	0	0	5	0	7	1 (14)
Calf male	8	0	0	0	3	1 (33)	11	1 (9)
Total	13	1 (8)	7	1 (14)	18	2 (11)	38	4 (11)

[a] Number seropositive (% seropositive).

TABLE 3. Seroprevalence of brucellosis in elk captured at Victor, 1998, and Teepee Creek, 2000–2001, Idaho.

Age and sex	Victor		Teepee Creek					
	1998		2000		2001		Total	
	n	+ (%)[a]	*n*	+ (%)	*n*	+ (%)	*n*	+ (%)
Adult female	12	0	2	1 (50)	2	1 (50)	4	2 (50)
Adult male	1	0	0	0	0	0	0	0
Yearling female	2	0	0	0	0	0	0	0
Yearling male	2	0	0	0	0	0	0	0
Calf female	3	0	0	0	0	0	0	0
Calf male	0	0	2	0 (0)	0	0	2	0 (0)
Total	20	0 (0)	4	1 (25)	2	1 (50)	6	2 (33)

[a] Number seropositive (% seropositive).

(42%) were culture positive for *B. abortus*. *Brucella abortus* biovar 1 was isolated from three adult cows and one calf and *B. abortus* biovar 4 was isolated from three adult cows and one calf. *Brucella abortus* RB51 was isolated from an additional calf that had been vaccinated at Rainey Creek 14 days prior to euthanasia.

Forty-nine elk trapped at Rainey Creek ($n=46$) and Conant Creek ($n=3$) were sent to the WHL from 1999 to 2002 for further testing and observations. However,

in 2001, the card test results were determined to be unreliable based on repeated testing of 17 elk (14 cows and three male calves) from Rainey Creek and three adult cows from Conant Creek that were classified as seropositive using the card test and the SPT. Subsequent supplemental and repeated full battery testing of these animals revealed consistent abnormal agglutination on the card test, which made interpretation impossible. Two animals (one cow and one calf) from Rainey Creek were determined to be consistently seropositive based on multiple testing at ISDA and were kept at the WHL. The remaining 15 elk from Rainey Creek were transported and released near the original trap site in northeastern Idaho. Repeated testing of the three cows from Conant Creek revealed the same abnormal agglutination on the card test. One of the cows was euthanized and other two were transported and released near the original trap site.

Thirty-one elk from Rainey Creek (29 cows and one male and one female calf) were maintained at the WHL for further testing. Of the 29 cows, 25 were pregnant. Abortions (4/25, 16%), stillbirths (2/25, 8%), and weak calves that died within 24 hr (1/25, 4%) were observed. Of the 18 live calves, 13 (72.2%) were seropositive for brucellosis at 24–72 hr of age. One cow was pregnant with a late-term fetus at the time of euthanasia.

At necropsy, nine cows and six calves were culture positive for brucellosis. *Brucella abortus* biovar 1 was isolated from two cows and *B. abortus* biovar 4 was isolated from seven cows. *Brucella abortus* biovar 1 was isolated from one calf and *B. abortus* biovar 4 was isolated from four calves. One calf had a mixed infection of *B. abortus* biovars 1 and 4. Four cows and their calves were both culture positive for *B. abortus*. In two of these, *B. abortus* biovar 4 was isolated from both the cow and the calf and in one, the cow was positive for *B. abortus* biovar 1 and the calf was positive for *B. abortus* biovar 4. One cow was culture positive for *B. abortus* biovar 4 but the calf had a mixed infection of biovars 1 and 4. Of the two calves trapped at Rainey Creek and held at the WHL, *B. abortus* biovar 4 was isolated from one and the other was negative on culture.

The number and quality of blood samples returned by hunters in eastern Idaho was highly variable. The return rate was generally less than 20% of the total number of kits sent to hunters. Hemolysis was the primary reason samples were unsuitable for testing. Of 923 usable samples received from hunters in northeastern Idaho from 1998 to 2002, only 20 (2%) were seropositive (Table 4). Until 2001, seropositive elk were found only in a distinct area composed of seven GMUs in northeastern Idaho (GMUs 60, 60A, 62, 62A, 64, 65, and 67) (Fig. 1), which was defined as the brucellosis core area (Table 4). In 2001, antibodies were detected in one of 37 samples (3%) from GMU 76 in southeastern Idaho (Fig. 1). An antibody prevalence of 3% was observed after additional samples from GMUs 76 and 66A were collected in 2002 (Table 4). Seroprevalence to brucellosis in elk harvested by hunters in northeastern Idaho appeared to decline over time (Table 4).

Results of brucellosis testing of elk in the field versus ISDA are shown in Table 5. Both testing methods detected seropositive animals (suspect and reactors), but field testing classified more animals as reactors than did testing at ISDA in most years. Replacing the card test with the BAPA test after 2001 resulted in elk being classified as suspect on field testing in 2002. Of the 63 animals classified as seropositive based on on-site testing and removed from Rainey Creek, 49 were also classified as seropositive at ISDA. A total of 17 of 63 (27%) and 17 of 49 (35%) seropositive elk based on field and IDSA testing, respectively, were culture positive for *B. abortus*.

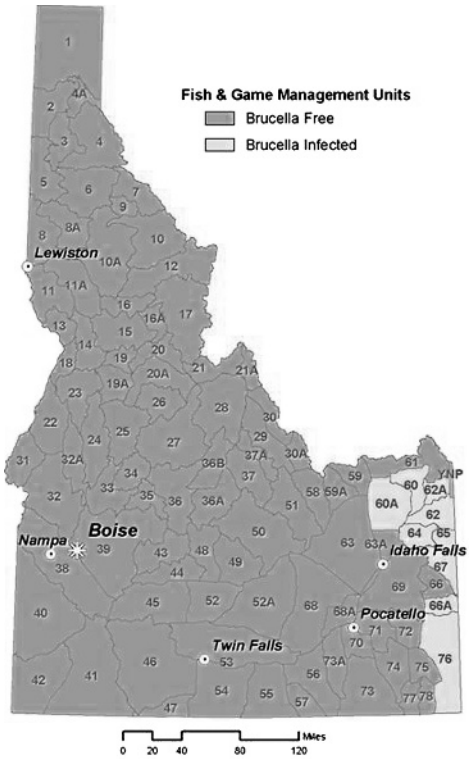

FIGURE 1. Map of elk Game Management Units (GMUs) in Idaho. Brucellosis is present in elk in light colored GMUs.

DISCUSSION

Brucellosis has been present in free-ranging bison and elk in the GYA since the early 1900s (Mohler, 1917; Creech, 1930; Rush, 1932; Turncliff and Marsh, 1935; Lee and Turner, 1937). The management of this disease in the GYA has proven difficult given the relative inaccessibility of the animals, multiple land use agency jurisdictions, and lack of ecosystem scale management options. Idaho was declared free of brucellosis in cattle in 1990. Because of the proximity of Yellowstone National Park, surveillance for brucellosis in elk of Idaho was started at about the same time.

In cattle, brucellosis is associated with abortion, low-viability calves, and poor reproductive performance (Nicoletti and Gilsdorf, 1997); however, the abortion rate can vary greatly. In cattle, abortion occurs during the first pregnancy following infection (Smith, 1990). Brucellosis can also cause joint infections and epdidymitis (Smith, 1990). The primary means of transmission in ruminants is ingestion of contaminated fetal tissue or fluids expelled during abortion or parturition. The disease in elk and bison appears similar to that in cattle (Thorne et al., 1997; Williams et al., 1997).

The documentation of brucellosis in elk in Idaho in 1998 resulted in the formation of a task force to develop a comprehensive plan for dealing with brucellosis in elk while maintaining the brucellosis-free status of cattle in the state. A Wildlife Brucellosis Task Force Report was submitted and approved by the governor in October 1998. This report formed the basis for the current ISDA and IDFG

TABLE 4. Test results from samples collected by elk hunters in eastern Idaho, 1998–2002.

	Northeastern Idaho samples[a]			Core brucellosis area samples[b]			Southeastern Idaho samples[c]		
Year	*n*	No. seropositive	% seropositive	*n*	No. seropositive	% seropositive	*n*	No. seropositive	% seropositive
1998	173	12	6.8	173	12	6.8	0	—	—
1999	46	4	8.7	46	4	8.7	0	—	—
2000	107	1	0.9	37	1	2.7	6	0	0
2001	81	1	1.2	47	1	2.1	37	1	2.8
2002	96	2	2.1	92	2	2.2	184	5	2.7
Total	923	20	2.2	395	20	5.1	227	6	2.6

[a] Samples from GMUs 60, 60A, 62, 62A, 63A, 64, 65, 66, and 67 (see Fig. 1).
[b] Samples from GMUs 60, 60A, 62, 64, 65, and 67 (see Fig. 1).
[c] Samples from GMUs 66A and 76 (see Fig. 1).

TABLE 5. Comparison of brucellosis serological results for elk in Idaho tested in the field and laboratory, 1998–2002.

			Field test results[a]				Laboratory test results[a]			
Site	Year	*n*	N	S	R	H	N	S	R	H
Rainey	1998	31[b]					21	4	6	0
Creek	1999	111	84	0	27	0	47	25	39	0
	2000	45	28	0	16	1	24	6	14	1
	2001	24	7	0	17	0	21	1	2	0
	2002	111	79	24	8	0	96	4	11	0
Total		322	198	24	68	1	209	40	72	1
Conant	1998	13[b]					12	0	1	0
Creek	2001	7	0	0	7	0	6	0	1	0
	2002	18	16	0	2	0	16	1	1	0
Total		38	16	0	9	0	34	1	3	0

[a] N = negative; S = suspect; R = reactor; H=hemolysis.
[b] These samples were not tested in the field.

management plan for brucellosis in elk. The Wildlife Brucellosis Plan called for continued surveillance and removal of seropositive elk from herds with a history of 3 or more years of continual winter feeding. In addition, significant effort was placed on development of winter habitat to provide adequate wintering areas for the approximately 600–1,000 elk in areas of northeastern Idaho affected by brucellosis.

The distribution of brucellosis in elk appears to be limited to a small area of eastern Idaho and appears to be concentrated in areas with active winter feeding of elk. Based on testing of trapped or captured elk and sampling of elk killed by hunters, brucellosis in elk is limited to areas of the state that border Yellowstone and Grand Teton national parks or areas adjacent to elk feed grounds maintained by the Wyoming Game and Fish Department. Elk from both national parks are known to be infected with brucellosis (Meyer, 1994; Thorne et al., 1997; Williams et al., 1997; Hollingsworth, 1998). Some elk from the affected herds in northeastern Idaho migrate to Yellowstone in summer and return to Idaho in winter. However, the extent of interchange of elk between Idaho, Yellowstone, and the winter feed grounds in western Wyoming is unclear.

The data collected from seropositive elk in eastern Idaho show that *B. abortus* biovars 1 and 4 are present in these animals; however, biovar 4 is predominant. In addition, nearly 25% of the infected animals that were removed from Rainey Creek and allowed to calve aborted or produced stillborn calves, which is similar to elk from western Wyoming that were infected with field strain *B. abortus* (Thorne, 1978). If this rate of reproductive loss is typical of infected free-ranging elk in northeastern Idaho, the presence of brucellosis may significantly impact population dynamics of elk in these herds.

The presence of *B. abortus* biovar 4 in a large proportion of the elk from northeastern Idaho makes this situation different from that in Wyoming, where biovar 1 is more common. (Thorne et al., 1997). Idaho likely has its own nidus of infection, which appears to be centered on elk that winter at Rainey Creek and at least two private feeding sites in northeastern Idaho.

Based on samples collected by hunters, the background seroprevalence to brucellosis in elk in eastern Idaho is 2–3%. Comparative data from Montana and

Wyoming indicate a background antibody prevalence for elk that are not associated with feedgrounds of 1.8% (Aune et al., 2002) and 1.7–3% (Thorne et al., 1997; Clause et al., 2002), respectively. In Idaho, there appears to be a declining trend in seroprevalence over time, but the data are limited and the cause of the trend is unknown. Removal of seropositive animals, randomly through hunting and deliberately through trapping and testing at winter feeding sites, may be related to the decline. Continued data collection and sustained harvest and disease management actions are warranted to determine if the trend continues and if antibody prevalence can be further reduced.

In Idaho, the prevalence of antibodies to brucellosis in elk at sites where winter feeding occurs (Rainey Creek, Conant Creek, and Teepee Creek) is two to four times higher than in elk that are not fed in winter. These data are consistent with data from Wyoming (Thorne, 1982; Thorne et al., 1997; Clause et al., 2002) and strongly suggest that supplemental winter feeding of elk in areas where brucellosis is known to occur enhances transmission because of the artificial concentrations of elk in these situations. Cessation of winter feeding of elk in the GYA would be a prudent disease management action provided that adequate quality winter habitat is available.

Field testing classified more animals as seropositive (reactors) than did laboratory testing at ISDA, likely because of the use of the full complement of primary and supplemental tests at ISDA. In 2001, large numbers of elk were classified as seropositive for brucellosis in the field based on the card test; cross reactions with *Yersinia* spp. were the most likely explanation for this (Drew, unpubl.). If removal of infected animals is used as a management tool, animals must either be tested on site or held in captivity for several days until the results of the full battery of testing are known. For field operations in Idaho, the SPT and card or the SPT and BAPA tests allowed for quick identification of seropositive animals; *B. abortus* was confirmed by culture in 17 of 49 seropositive elk. The removal of infected animals may have decreased the seroprevalence of brucellosis in elk at Rainey Creek and management decisions using liberal seropositive criteria may result in faster reduction of seroprevalence in free-ranging elk populations in Idaho and elsewhere.

Efforts to manage brucellosis in elk in eastern Idaho must aim to reduce the risk of disease transmission between elk and cattle. Potential management actions may include reductions of elk populations, reduction or elimination of artificial winter feeding operations, improvement of or acquisition of wintering habitat for elk, mandatory vaccination of cattle, and testing of cattle herds that interact with elk in winter. By decreasing the number of elk on winter feeding sites, disease transmission would be minimized. In addition, the temporal and spatial separation of cattle and elk during time periods when infected elk abort or during elk calving season will minimize interspecies transmission. Long-term management of brucellosis in elk in Idaho will likely involve all of these management options, but solutions may have to be site specific.

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