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Michael A. Cover
University of Nebraska-Lincoln

Scott E. Hygnstrom
University of Nebraska-Lincoln, shygnstrom1@unl.edu

David W. Oates
Nebraska Game and Parks Commission

Kit M. Hams
Nebraska Game and Parks Commission

Kurt C. Vercauteren
USDA/APHIS/WS National Wildlife Research Center, kurt.c.vercauteren@aphis.usda.gov

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Michael A. Cover, Scott E. Hygnstrom, Scott R. Groepper

School of Natural Resources
University of Nebraska–Lincoln
Lincoln, NE 68583-0819
scott.groepper@yahoo.com

David W. Oates

Genetics and Forensics Analysis Laboratory
Wildlife Research Section
Nebraska Game and Parks Commission
Lincoln, NE 68503-0370

Kit M. Hams

Wildlife Division
Nebraska Game and Parks Commission
Lincoln, NE 68503-0370

and

Kurt C. VerCauteren

USDA-APHIS-WS-National Wildlife Research Center
Fort Collins, CO 80521-2154

ABSTRACT—Sera samples were collected from 21 free-ranging, captured female elk (*Cervus elaphus nelsoni*) in 1995–96, and tissue and sera samples were collected from 415 hunter-harvested elk from 1995 to 2006 and tested for selected diseases. Titers for *Anaplasma marginale* were detected in 81 of 436 (19%) elk. Occurrence of antibodies to anaplasmosis increased from 4 to 40 elk from 2002 to 2006. Titers for bovine viral diarrhea virus (BVDV) were detected in 18 of 346 (5%) samples. Titers for *Leptospira interrogans* serovars were detected in 21 of 289 (7%) of samples from 1995 to 2004. Titers for bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) were detected in 65 of 370 (18%) sampled elk during 1995–2006. Biologists collected obex tissues from 566 elk from 1997 to 2009 and found evidence of chronic wasting disease (CWD) in one elk in 2009. No brucellosis was detected. Due to the prevalence of several diseases in elk in Nebraska, we recommend that surveillance efforts continue.

Key Words: anaplasmosis, bluetongue virus, bovine viral diarrhea virus, brucellosis, chronic wasting disease, elk, epizootic hemorrhagic disease virus, leptospirosis

INTRODUCTION

Transmission of diseases from livestock to wildlife, and vice versa, is an ongoing and increasing problem (Ward et al. 2008). Free-ranging elk (*Cervus elaphus nelsoni*) in Nebraska had never been tested or monitored for diseases. No records are available for elk to compare or evaluate the prevalence of *Anaplasma marginale*, brucellosis, bovine viral diarrhea virus (BVDV), bluetongue virus (BTV), epizootic hemorrhagic disease virus (EHDV), *Leptospira interrogans*, or chronic wasting disease (CWD). Prior

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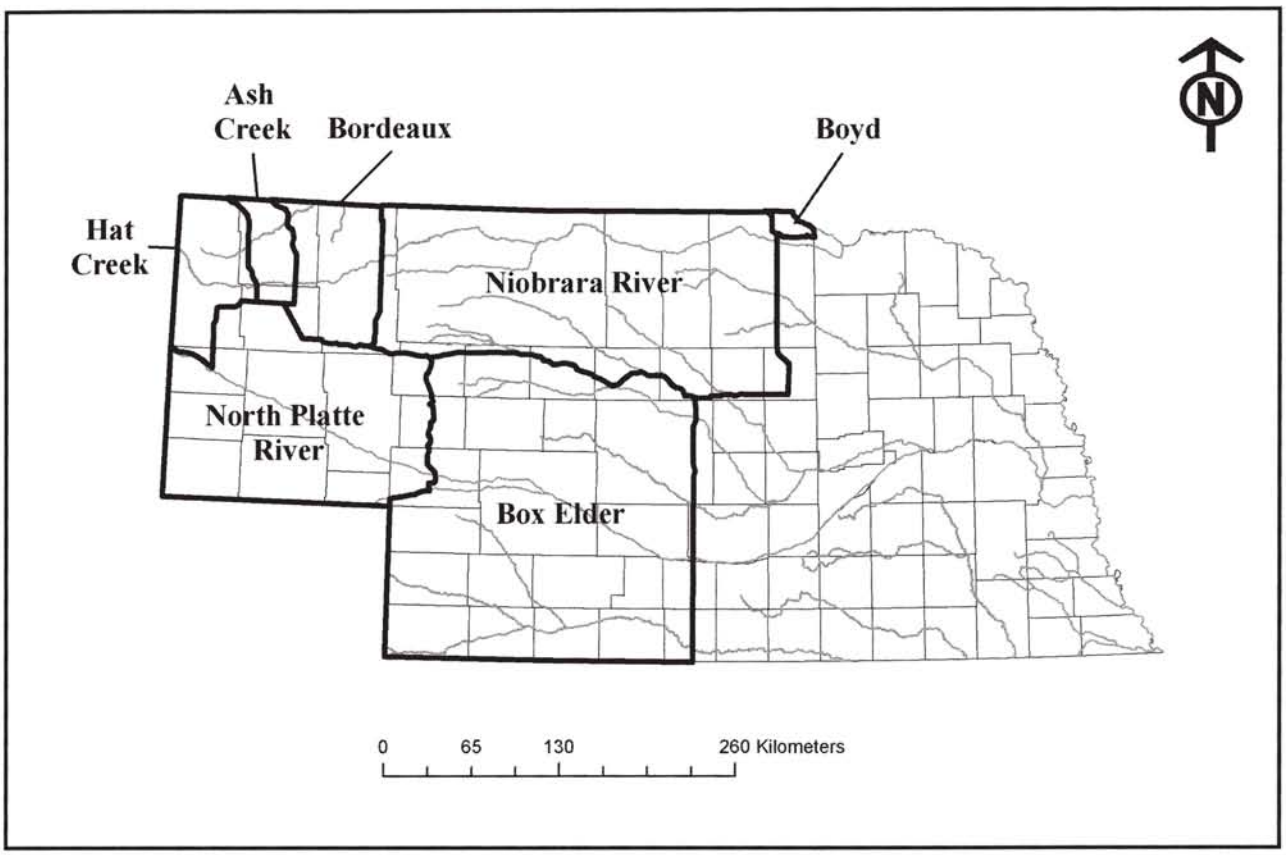

Figure 1. Elk management units (EMUs) in Nebraska, 1995–2010.

serologic surveys of wildlife in Nebraska have addressed anaplasmosis, brucellosis, BTV, EHDV, and bovine respiratory syncytial virus in free-ranging white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*; Wilhelm and Trainer 1966), and pronghorn (*Antilocapra americana*; Johnson et al. 1986).

In the mid-1990s, the population of elk in Nebraska was estimated at 150–200 individuals (Fricke et al. 2008). The current population is approximately 1,400 and increasing at 15%–20% annually, with over half located in the three elk management units (EMUs) in the Pine Ridge region of northwestern Nebraska (NGPC 2007a; Fig. 1). Nebraska has the second-largest beef cattle herd in the United States, with 6.7 million head in 2007. Two of the top five cattle-producing counties in the state—Cherry and Sheridan, producing 166,000 and 56,000 head, respectively (Small et al., 2007)—are located within the Bordeaux and Niobrara River EMUs (Fig. 1). The risk of disease transmission between livestock and elk prompted us to carry out a long-term serologic study to determine prevalence of disease in elk. We tested for *Anaplasma marginale*, brucellosis, BVDV, BTV, EHDV, *Leptospira interrogans*, and CWD.

Sera samples were collected from 21 captured, free-ranging elk during 1995 and 1996, and tissue and sera samples were collected from 415 hunter-harvested elk at mandatory hunter check stations in six EMUs in Nebraska from 1995 to 2006. In 1995, the Nebraska Game and Parks Commission established Bordeaux and Hat Creek EMUs (Fig. 1). In 1996 Boyd EMU was established in response to depredation complaints associated with elk migrating back and forth from South Dakota (Cover 2000:151–82). The Box Elder, Ash Creek, and North Platte River EMUs were established in 2002, 2004, and 2005, respectively. The Niobrara River EMU was established in 2007, in recognition of the importance of elk management in the Sandhills region of Nebraska.

Biologists recorded information including identification number, sex, and date, time, and location of the kill. Three milliliters of serum from each elk was sent to the Veterinary Diagnostic Center, Nebraska State Brucellosis Laboratory, in Lincoln, NE, for analysis. Sera samples were tested for brucellosis antibodies using a particle concentration fluorescence immunoassay (PCFIA) test, Card test, Rivanol test, and plate agglutination

test. Five-milliliter samples of blood were centrifuged, and serum was forwarded to the South Dakota Veterinary Diagnostic Laboratory in Brookings (1995–1997), Wyoming State Veterinary Laboratory in Laramie (1998), National Veterinary Services Laboratories in Ames, IA (1999–2001), and the Veterinary Diagnostic Center in Lincoln, NE (2002–2006). Tests for antibodies to *Anaplasma marginale* were performed using a complement fixation or polymerase chain reaction enzyme-linked immunosorbent assay (PCR ELISA) test. In addition, tests for BVDV antibodies were performed using a serum neutralization test, BTV and EHDV antibodies using an Agar-Gel immunodiffusion and immunofluorescent antibody test, and *Leptospira interrogans* using a micro-agglutination test. Obexes from 566 hunter-harvested elk were collected at check stations from 1997 to 2009. Tissues were fixed in formalin, embedded in paraffin, and stained for a modified protease resistant protein (PrP^{CWD}) test. Tissue samples were tested for CWD using immunohistochemistry (IHC; Miller and Williams 2002) and ELISA tests (Hibler et al. 2003).

The University of Nebraska Institutional Animal Care and Use Committee (IACUC #94-09-075) approved all live animal procedures, and Nebraska Game and Parks Commission biologists conducted tissue collection on hunter-harvested animals.

RESULTS AND DISCUSSION

No antibodies to *Anaplasma marginale* were detected in elk from 1995 to 2001 (n = 182, $\bar{x}$ = 30). In 2002 antibodies were detected in four elk; however, we were unable to determine the sex of these elk and EMU where they were harvested, due to incomplete information (Table 1). Titers to anaplasmosis increased after 2002, with 38 male (20%, n = 187), 39 female (16%, n = 249) and four unknown elk with antibodies to *Anaplasma marginale* during the study. The number of elk exposed increased annually from 12% (n = 4) in 2002 to 61% (n = 40) in 2006. The number of areas affected increased from two EMUs in 2003 to five EMUs in 2006. Active infection can lead to abortions, low milk production, and death in cattle (Palmer 1989). Anaplasmosis has been identified as a major production-limiting disease in cattle in the United States in 2002–2006 (U.S. Department of Agriculture 2006), costing producers from $100 million to $300 million (Falkner 2001; Coetzee et al. 2005). The increase we detected may be due to expanding populations of elk in Nebraska. Distinct herds that expand in range may come into contact with other herds, increasing the probability of disease transmission. Additionally, we conducted sampling in areas where no sampling was previously conducted.

Five percent (18 of 346) of elk tested seropositive for BVDV during 1995 to 2001, 2003, 2004, and 2006 (Table 2), with titers ranging from 1:4 to 1:1,024. The prevalence of BVDV was relatively low and varied, from 0% in 1996, 1997, and 2001 to a maximum of 12% in 1995. Approximately 84% of cattle in western Nebraska have been exposed to BVDV (Jaggers 1984:36–56). Cattle producers in Nebraska supplement livestock with high-energy feed rations to increase weight gain and prepare for calving during the winter and early spring. Supplemental feeding is done in protected areas to avoid adverse weather. Elk may become conditioned to using cattle feed lines in winter, bringing them in contact with infected cattle or contaminated feed. Transmission of BVDV between cattle and elk has not been documented in Nebraska. Cattle persistently infected with BVDV are more likely to suffer abortions, stillbirth, or birth of weak calves (Carman et al. 2005).

Seven percent (21 of 289) of elk tested seropositive for *Leptospira interrogans* serovars (*L. i. bratislava, L. i. hardjo, L. i. pomona, L. i. grippotyphosa,* and *L. i. icterohemmorrhagiae*) during 1995 to 2002 and 2004 (Table 2), with titers ranging from 1:100 to 1:400. Prevalence of leptospirosis varied from 0% in 1997, 2001, and 2002 to 42% in 1996. Fifteen male elk (13%, n = 115) and six females (3%, n = 174) had titers. Leptospirosis is caused by a bacterial agent and cattle can act as a principal reservoir (Davidson and Nettles 1997:49–59). Elk and cattle sharing common pasture, rangelands, or watering areas may share the disease (Adrian and Keiss 1977).

Eleven percent (39 of 370) of elk had titers for BTV during 1995 to 2005 and 12% (26 of 214) had titers for EHDV during 1995 to 2001 and 2006. Prevalence of BTV varied from 0% in 1995–97 to 30% in 2005. Prevalence of EHDV varied from 0% in 1996–97 to 15% in 2006. Thirty-five male elk (19%, n = 187) and 30 female elk (12%, n = 249) were seropositive to BTV or EHDV (Table 2). Outbreaks of BTV and EHDV in cervids and cattle in Nebraska are limited to warmer months because transmission occurs through biting midges (*Culicodes* spp.) and ceases with the first killing frost (Kistner et al. 1982; Gibbs et al. 1983). Periodic outbreaks of BTV and EHDV have been reported in white-tailed deer in the Bordeaux and Niobrara River EMUs. Both diseases are considered enzootic in northern regions of the Sandhills and Platte River valley of Nebraska (Wilhelm and Trainer 1966). Prevalence of antibodies is higher (66%)

TABLE 1
SEROLOGIC TESTS INDICATING NUMBER OF POSITIVE RESULTS (*N*) FOR ANAPLASMOSIS AND THEIR LOCATION IN SAMPLED ELK IN NEBRASKA, 2002–2006

			Anaplasmosis	
Year	**N tested**	**Sex**	**N**	**Location (EMU)**
2002	15	M	4	*
	18	F		*
2003	12	M	6	Bordeaux, Hat Creek
	12	F	4	Bordeaux, Hat Creek
2004	26	M	3	Bordeaux, Hat Creek
	26	F	3	Ash Creek
2005	30	M	11	Ash Creek, Bordeaux, Hat Creek
	27	F	10	Ash Creek, Hat Creek, North Platte
2006	30	M	18	Ash Creek, Bordeaux, Box Elder, Hat Creek, North Platte
	36	F	22	Bordeaux, Hat Creek, North Platte

Note: Asterisk (*) indicates incomplete data on sex and location.

in deer in western and central Nebraska, compared to deer in eastern Nebraska (35%, Frost 2009). In the past, significant BTV prevalence (28%) has been detected in cattle in western Nebraska (Jaggers 1984). Experimental infections of cattle with EHDV derived from a deer led only to subclinical infections (Gaydos and Nettles 1998).

Chronic wasting disease was detected in 1 of 566 (<0.2%) samples collected from hunter-harvested elk from 1997 to 2009. The CWD-infected female elk was harvested in 2009 in the Hat Creek EMU (Fig. 1). In 1997, a captive elk infected with CWD was detected in the Hat Creek EMU. Chronic wasting disease was first observed in wild mule deer in Nebraska in the North Platte River EMU in 2000. One hundred ninety-eight positives were documented in mule deer and white-tailed deer in Nebraska from 2000 to 2009 (NGPC 2007b; D. Oates, pers. comm. 2010). Effective strategies for controlling CWD have yet to be discovered.

All 403 samples tested for brucellosis from 1995 to 2006 were negative. Elk are susceptible to brucellosis, as are bison (*Bison bison*; Jessup and Boyce 1996), mule deer, white-tailed deer (Trainer and Hanson 1960), moose (*Alces alces*; Forbes et al. 1996), and pronghorn (Thorne et al. 1979), all of which, except moose, have established populations in western Nebraska. Transmission of brucellosis from elk to cattle under free-range conditions was documented in cattle in Idaho and Wyoming in 2005 (Donch et al. 2006). Nebraska has been considered brucellosis free for ≥12 years (Donch and Gertonson 2008).

CONCLUSIONS

We recommend that the Nebraska Game and Parks Commission conduct annual serologic surveys of harvested elk in Nebraska. The prevalence and distribution of *Anaplasma marginale* increased considerably from 2002 to 2006, and it would be appropriate to continue monitoring. Bovine viral diarrhea and leptospirosis have all been detected in Nebraska, so it is important to monitor the status of these transmissible diseases, considering that elk are highly mobile (Fricke et al. 2008). In addition, elk may have been infected with chronic wasting disease due to their sympatric relationship with infected populations of mule deer and white-tailed deer in western Nebraska. We encourage continued surveillance of CWD in elk. We do not recommend further surveillance for EHDV or BTV in Nebraska. Both diseases are considered enzootic and cause little or no risk to cattle. While brucellosis has yet to be observed in elk in Nebraska, it is important to continue monitoring because of the social behavior

TABLE 2
SEROLOGIC TESTS INDICATING NUMBER OF POSITIVE TESTS FOR FOUR DISEASES AND THEIR LOCATION IN ELK SAMPLED IN NEBRASKA, 1995–2006

			BVDV		Leptospirosis		Bluetongue virus		EHDV	
Year	*N*	Sex	*N*	Location (EMU)	*N*	Location (EMU)	*N*	Location (EMU)	*N*	Location (EMU)
1995	5	M	1	Bordeaux	1	Bordeaux	—		1	Bordeaux
	28	F	3	Ash Creek, Bordeaux	1	Hat Creek	—		1	Hat Creek
1996	9	M	—		6	Bordeaux, Boyd, Hat Creek	—		—	
	10	F	—		2	Bordeaux, Boyd	—		—	
1997	15	M	—		—		—		—	
	14	F	—		—		—		—	
1998	21	M	—		2	Bordeaux	—		1	Bordeaux
	24	F	2	Bordeaux	—		1	Bordeaux	1	Bordeaux
1999	10	M	—		—		1	Bordeaux	4	Bordeaux, Boyd, Hat Creek
	17	F	1	Boyd	3	Bordeaux	—		1	Bordeaux
2000	9	M	1	Hat Creek	1	Bordeaux	1	Hat Creek	1	Bordeaux
	20	F	—		—		2	Bordeaux, Hat Creek	4	Bordeaux, Hat Creek
2001	5	M	—		—		3	Bordeaux, Hat Creek	—	
	17	F	—		—		1	Hat Creek	2	Bordeaux
2002	15	M	NT		—		5*		NT	
	18	F	NT		—		*		NT	
2003	12	M	—		NT		3	Bordeaux, Hat Creek	NT	
	12	F	1	Bordeaux	NT		—		NT	
2004	26	M	2	Bordeaux	5	Ash Creek, Bordeaux, Hat Creek	3	Ash Creek, Bordeaux	NT	
	26	F	2	Bordeaux, Hat Creek	—		2	Bordeaux, Hat Creek	NT	
2005	30	M	NT		NT		8	Ash Creek, Bordeaux, Hat Creek, North Platte	NT	
	27	F	NT		NT		9	Bordeaux, Hat Creek, North Platte	NT	
2006	30	M	2	North Platte	NT		NT		4	Ash Creek, Bordeaux, Box Elder, North Platte
	36	F	3	North Platte	NT		NT		6	Bordeaux, Box Elder, North Platte

Notes: Dash (—) indicates disease not found; NT indicates disease not tested; asterisk (*) indicates incomplete data on sex and location.

and mobility of elk and the economic significance of brucellosis-free status for livestock in Nebraska. We did not test for bovine tuberculosis in our study; however, it was detected in cattle in the Niobrara EMU and in captive elk east of the EMUs in Nebraska in 2009 (D. Oates, pers. comm. 2010), so surveillance is merited in elk.

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