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SEROLOGIC SURVEY OF SELECTED PATHOGENS IN WHITE-TAILED AND MULE DEER IN WESTERN NEBRASKA

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ABSTRACT: Exposure of free-ranging white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) in western Nebraska to selected livestock pathogens was determined by serology and attempted virus isolation. Antibodies to bluetongue virus, epizootic hemorrhagic disease virus, and bovine respiratory syncytial virus were present in both species of deer. No serologic reactors to *Brucella* or *Anaplasma* were found. Attempts to isolate bluetongue virus were negative.

INTRODUCTION

Wild ruminants and other wildlife may serve as sentinels or reservoirs for cattle diseases (Trainer, 1970; Adrian and Keiss, 1977; Kocan et al., 1982), and since the deer population in Nebraska is free-ranging throughout the state, mingling with cattle is common.

Infections with bluetongue virus (BTV) (Jaggers, 1984) and bovine respiratory syncytial virus (BRSV) (Johnson et al., 1982; Frey, 1983) are problems in cattle of western Nebraska. Brucellosis and anaplasmosis still occur in isolated herds of cattle throughout Nebraska and other parts of the United States (Kruse, pers. comm.). Epizootic hemorrhagic disease (EHD) is known to be a sporadic problem in deer in Nebraska (Wilhelm and Trainer, 1966; Schildman, 1983), and cattle in Colorado have been reported to be infected with EHD virus (Barber and Jochim, 1975; Foster et al., 1980). The purpose of this serological survey was to determine the prevalence of antibodies to selected disease agents in white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) in Nebraska.

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MATERIALS AND METHODS

Collection of samples

During the 1982 and 1983 hunting seasons in Nebraska, blood was collected from deer in sterile vacutainers by wildlife biologists. Eight different check stations were utilized to sample West Central and the Panhandle of Nebraska. The samples were collected from the body cavity or heart, stored in ice chests, and shipped or hand-carried to the West Central Research and Extension Center in North Platte. Collection times were the second and third weekends of November.

In 1983 approximately 125 hunters were sent blood sample collecting kits containing two vacutainer tubes, one heparinized and one without anticoagulant, with instructions and a return mailing address. The clotted samples were for serology and heparinized blood was utilized for virus isolation. These volunteers were obtained through radio announcement or personal communication. Hunter permit numbers were written on the mailing carton which was either to be mailed back to the West Central Research and Extension Center in North Platte or deposited at the Game and Parks check stations. The permit numbers were then used to retrieve kill data stored by the Game and Parks Department. Samples were received, processed and frozen at the West Central Research and Extension Center and then forwarded to the appropriate laboratory for testing.

Serology

Samples from the hunter-killed deer were tested for antibody to BTV and EHDV by the BT immunodiffusion (BTID) test (Pearson and Jochim, 1979) at the Arthropod-borne Animal Diseases Research Laboratory in Denver, Col-

TABLE 1. Results of immunodiffusion tests for antibody to bluetongue virus in deer in Nebraska by age and species, 1982 and 1983.

	Number positive/total number tested (% positive)								
	Age (yr)								
Deer	Fawn (<1)	1	2	3	4	5	6–9	Unknown	Total
Mule deer	0/13 (0%)	21/143 (15%)	21/68 (31%)	11/23 (48%)	6/16 (38%)	2/6 (33%)	5/9 (56%)	4/12 (33%)	70/290 (24%)
White-tailed deer	4/26 (15%)	27/121 (22%)	13/45 (29%)	6/24 (25%)	3/9 (33%)	3/5 (60%)	1/3 (33%)	1/5 (20%)	58/238 (24%)
Total	4/39 (10%)	48/264 (18%)	34/113 (30%)	17/47 (36%)	9/25 (36%)	5/11 (45%)	6/12 (50%)	5/17 (29%)	128/528 (24%)

orado. Samples for BRSV were tested at the Veterinary Science Department at the University of Nebraska, Lincoln, Nebraska by microtitration serum-virus neutralization test. It differed from standard virus neutralization tests, primarily in having increased buffers (10 mmol/ml each of HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid), EPPS (N-[2-hydroxethyl]-piperazine-N'-3-propanesulfonic acid; HEPPS), and MOPS (3-[N-morpholino]-propanesulfonic acid), and 2 mmol of $NaHCO_3$), and serum (final, 10% horse serum) in the maintenance medium, and increased virus (approximately 10^3 plaque-forming units per well) (Frey, unpubl. data) in each milliliter.

Serum samples for antibody to *Anaplasma marginale* were tested at the State–Federal Laboratory in Lincoln, Nebraska using the complement fixation test and serum rapid card agglutination test (Amerult and Roby, 1968).

Serum samples were tested for antibody to *Brucella* spp. at the State–Federal Laboratory in Lincoln, Nebraska using the acidified plate agglutination method (Alton et al., 1975).

Viral assay

Heparinized blood samples were assayed for BTV and EHDV by subculture in embryonated chicken eggs and BHK-21 cell culture as previously described (Barber and Collisson, 1983).

RESULTS

Collecting blood from the body cavity or heart of hunter-killed deer was not an ideal way to collect samples for serology. Many of the samples were not suitable for testing and were discarded. However, it allowed for a rapid means of collection of numerous samples for testing deer from a large portion of the state. Hunter cooperation in the counties where serum collection was requested in 1983 was excellent and 69 samples of adequate quality for virus isolation were obtained.

Five hundred twenty-eight deer samples collected in 1982 and 1983 were tested for antibody to BTV; of the total deer tested, 24% were positive. Of the 290 mule deer tested, 24% were positive, while 24% of the 238 white-tailed deer were positive (Table 1).

Five hundred twenty-five deer samples were tested for antibody to EHDV; 28% were positive. Of the 288 mule deer tested, 30% were positive, while 27% of the 237 white-tailed deer were positive (Table 2).

Seventy-six of the serum samples collected by hunters who were furnished blood collection kits were usable for BRSV serology. Twenty-five (33%) of the 76 deer samples for BRSV had titers above 1:5 (Table 3).

One hundred fifty-two samples collected in 1983 were usable for testing for *Brucella* spp. All were negative using the acidified plate method.

The same 152 samples were tested for antibodies to *Anaplasma marginale*. One of 152 was suspect (1:5) using the complement fixation test. All tested negative with the serum rapid card agglutination test.

TABLE 2. Results of immunodiffusion tests for antibody to epizootic hemorrhagic disease virus in deer in Nebraska by age and species, 1982 and 1983.

	Number positive/total number tested (% positive)								
	Age (yr)								
Deer	Fawn (<1)	1	2	3	4	5	6–9	Unknown	Total
Mule deer	0/13 (0%)	29/142 (20%)	27/68 (40%)	11/22 (50%)	6/16 (38%)	2/6 (33%)	7/9 (78%)	4/12 (33%)	86/288 (30%)
White-tailed deer	3/26 (12%)	31/121 (26%)	14/45 (31%)	6/23 (26%)	3/9 (33%)	4/5 (80%)	1/3 (33%)	1/5 (20%)	63/237 (27%)
Total	3/39 (8%)	60/263 (23%)	41/113 (36%)	17/45 (38%)	9/25 (36%)	6/11 (55%)	8/12 (67%)	4/17 (24%)	149/525 (28%)

Results of virus isolation from 69 samples were negative for BT and EHD viruses.

DISCUSSION

The presence of serologic reactors indicated that some animals had been exposed to agents which are related to or identical to the four livestock pathogens used as standards in these serological tests. Documentation of reactors alone does not provide evidence of pathogenicity or transmission of pathogens between wildlife and livestock.

Our BTV serologic results in Nebraska were similar to those in Wyoming and Colorado (Trainer and Jochim, 1969) in that the prevalences of antibody in deer correlated with those of the cattle population. A 1983 cattle survey of 4% of the herds in the Panhandle area revealed 28% of the animals positive (+1 or greater) by the bluetongue immunodiffusion (BTID) test (Jaggers, 1984) compared to the 24% in deer. The prevalences of BTV and EHDV antibodies in deer were similar to the prevalences found in pronghorns (*Antilocapra americana*) in Nebraska in a 1983 survey (Johnson et al., 1986). The prevalence of EHDV antibody was higher than expected and did not correlate with confirmed EHD/HD mortalities (Schildman, 1983). The percentage of animals serologically positive for BTV/EHDV was higher in our study than reported in Oregon (Kistner et al., 1975), Wyoming, Michigan, Illinois, and Iowa, but lower than New Mexico and Texas (Trainer and Jochim, 1969). The mule deer results were similar to those positives reported from New Mexico (Trainer and Jochim, 1969; Couvillion et al., 1980). Age-specific antibody prevalence occurred. Twenty-one percent of the fawns and 1-yr-old deer had antibody to EHDV, while 39% of the 2–9-yr-olds were seropositive. BTV antibody was present in 17% of the fawns and 1-yr-olds and 34% of the 2–9-yr-olds. The results may indicate a 2–3-yr positive antibody cycle indicating enzootic transmission. The age-specific antibody prevalence was similar to our findings on 2–8-yr-old pronghorns and other studies where the highest percentages of positive animals were in 2–6-yr-old adults (Trainer and Jochim, 1969; Couvillion et al., 1980). Antibodies to BTV and EHDV in deer were prevalent throughout the western part of the state. The highest prevalence was from areas surrounding the Platte River Valley where the rivers and creeks provided the greatest wooded protection, but low-level flooding occurs regularly providing excellent habitats for *Culicoides variipennis*, the biological vector for BTV and EHDV. Our results indicate

TABLE 3. Prevalence of positive titers to bovine respiratory syncytial virus in deer in Nebraska by age and species, 1983.

	Number positive/total number tested (% positive)						
	Age (yr)						
Deer	Fawn (<1)	1	2	3	4	5–8	Total
Mule deer	0/2 (0%)	3/15 (20%)	2/8 (25%)	1/3 (33%)	3/5 (60%)	1/2 (50%)	10/35 (29%)
White-tailed deer	2/6 (33%)	9/21 (43%)	2/8 (25%)	1/2 (50%)	0/1 (0%)	1/3 (33%)	15/41 (37%)
Total	2/8 (25%)	12/36 (33%)	4/16 (25%)	2/5 (40%)	3/6 (50%)	2/5 (40%)	25/76 (33%)

a strong association between BTV and EHDV in cattle and the prevalence of antibodies to these viruses in wild ruminants. The situation in Nebraska is thus similar to that reported for these viruses in wild and domesticated ruminants at or near sea level in California (Jessup, 1985).

The prevalences of BRSV antibodies in deer were similar to those of the cattle population in Nebraska (Johnson et al., 1982; Frey, 1983) but lower than pronghorns in Nebraska (Johnson et al., 1986). The fawns and 1-yr-olds had an antibody prevalence of 32%, while 34% of the 2–8-yr-olds were serologically positive. These age-specific antibody prevalences may indicate recent exposure to BRSV and possibly epizootic type of transmission in 1983. The positive findings were not surprising as previous serological investigations in wild ruminants have given positive results for other respiratory viruses (Couvillion et al., 1980; Stauber et al., 1980).

The negative findings on *Anaplasma* and *Brucella* are lower than those of the domestic cattle in West Central and the Panhandle of Nebraska. The negative findings on *Brucella* were similar to those in Colorado and Missouri (Adrian and Keiss, 1977; Jones et al., 1983).

The negative finding on *Anaplasma* is lower than the 4% prevalence in cattle in Nebraska and below the 6.9% prevalence found in white-tailed deer in Illinois (Smith et al., 1982). The negative findings would indicate that in Nebraska white-tailed deer and mule deer are not a major reservoir of *Anaplasma*. This is similar to information on white-tailed deer in the southeastern United States (Kuttler, 1984). Since anaplasmosis has been reported in various wild ruminants (Kuttler, 1984), one would suspect that there is little difference in species susceptibility, but that vector species and population densities are perhaps more important.

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LITERATURE CITED

ADRIAN, W. J., AND R. E. KEISS. 1977. Survey of Colorado's wild ruminants for serologic titers to brucellosis and leptospirosis. J. Wildl. Dis. 13: 429–431.

ALTON, G. G., L. M. JONES, AND D. E. PIETZ. 1975. Laboratory Techniques in Brucellosis, 2nd Ed. World Health Organization, Geneva, Switzerland, Monograph Series No. 55, pp. 64–112.

AMERAULT, T. E., AND T. O. ROBY. 1968. A rapid card agglutination test for bovine anaplasmosis. J. Am. Vet. Med. Assoc. 153: 1828–1834.

BARBER, T. L., AND E. W. COLLISSON. 1983. Implications of a new bluetongue serotype for the U.S. livestock industry. Proc. U.S. Anim. Health Assoc. 87: 90–104.

———, AND M. M. JOCHIM. 1975. Serotyping bluetongue and epizootic hemorrhagic disease virus strains. Proc. Am. Assoc. Vet. Lab. Diag. 18: 149–162.

COUVILLION, C. E., E. W. JENNEY, J. E. PEARSON, AND M. E. COKER. 1980. Survey of antibodies to viruses of bovine virus diarrhea, bluetongue and epizootic hemorrhagic disease in hunter killed mule deer in New Mexico. J. Am. Vet. Med. Assoc. 177: 790–791.

FOSTER, N. M., H. E. METCALF, T. L. BARBER, R. H. JONES, AND A. J. LUEDKE. 1980. Bluetongue and epizootic hemorrhagic disease virus isolations from vertebrate and invertebrate hosts at a common geographic site. J. Am. Vet. Med. Assoc. 176: 126–129.

FREY, M. L. 1983. Bovine respiratory syncytial virus and acute respiratory distress syndrome in cattle. Bovine Pract. 18: 73–77.

JAGGERS, R. 1984. Everything you ever wanted to know about bluetongue and less. Proc. Neb. Vet. Med. Assoc. Annu. Mtg., pp. 66–73.

JESSUP, D. A. 1985. Epidemiology of two orbiviruses in California's native wild ruminants: Preliminary report. *In* Bluetongue and Related Orbiviruses, T. L. Barber and M. M. Jochim (eds.). A. R. Liss, New York, pp. 53–65.

JOHNSON, J. L., T. L. BARBER, M. L. FREY, AND G. NASON. 1986. Serosurvey for selected pathogens in hunter-killed pronghorns in western Nebraska. J. Wildl. Dis. 22: 87–90.

———, J. L. POMMER, D. B. HUDSON, AND A. R. DOSTER. 1982. Pulmonary emphysema in weaned calves: Laboratory diagnosis. Proc. Annu. Conv. Am. Assoc. Bovine Pract. 14: 122–126.

JONES, R. L., R. I. TAMAYO, W. PORATH, N. GIESSMAN, L. S. SELBY, AND G. M. BUENING. 1983. A serologic survey of brucellosis in white-tailed deer (*Odocoileus virginianus*) in Missouri. J. Wildl. Dis. 19: 321–323.

KISTNER, T. P., G. E. REYNOLDS, L. D. KOLLER, C. E. TRAINER, AND D. L. EASTMAN. 1975. Clinical and serological findings on the distribution of bluetongue and epizootic hemorrhagic disease virus in Oregon. Proc. Am. Assoc. Vet. Lab. Diag., 18: 135–148.

KOCAN, A., A. E. CASTRO, B. ESPE, R. T. DOYLE, AND S. K. OLSEN. 1982. Inapparent bluetongue in free-ranging white-tailed deer. J. Am. Vet. Med. Assoc. 181: 1415–1416.

KUTTLER, K. L. 1984. *Anaplasma* infections in wild and domestic ruminants: A review. J. Wildl. Dis. 20: 12–20.

PEARSON, J. E., AND M. M. JOCHIM. 1979. Protocol for the immunodiffusion test for bluetongue. Proc. Am. Assoc. Vet. Lab. Diag. 22: 463–471.

SCHILDMAN, G. 1983. Wildlife Disease and Mortality Investigations. Nebraska Game and Parks Comm. Work Plan S-83, Pittman-Robertson Proj. W-15-R-40, 17 pp.

SMITH, R. D., A. WOOLF, L. L. HUNGERFORD, AND J. P. SUNDBERG. 1982. Serologic evidence of *Anaplasma marginale* infection in Illinois white-tailed deer. J. Am. Vet. Med. Assoc. 181: 1254–1256.

STAUBER, E. K., R. AUTENRIETH, O. D. MARKHAM, AND U. WHITEBECK. 1980. A seroepidemiologic survey of three pronghorn (*Antilocapra americana*) populations in southeastern Idaho 1975–1977. J. Wildl. Dis. 16: 109–115.

TRAINER, D. O. 1970. The use of wildlife to monitor zoonoses. J. Wildl. Dis. 6: 397–401.

———, AND M. M. JOCHIM. 1969. Serologic evidence of bluetongue in wild ruminants in North America. Am. J. Vet. Res. 30: 2007–2011.

WILHELM, A. R., AND D. O. TRAINER. 1966. A serological study of EHD of deer. J. Wildl. Manage. 30: 777–780.