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Selenium Status and Antibodies to Selected Pathogens in White-tailed Deer (*Odocoileus virginianus*) in Southern Minnesota

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ABSTRACT: To determine exposure to a variety of infectious diseases potentially important for native ungulates, livestock, and humans, serum samples from 114 (94 adults, 20 fawns) female white-tailed deer (*Odocoileus virginianus*) were collected during January 2000–03 from multiple locations in southeast (SE) and southwest (SW) Minnesota. Antibody prevalence was determined for the following pathogens: *Mycobacterium avium* subsp. *paratuberculosis, Leptospira interrogans* (six serovars), *Anaplasma marginale, Borrelia burgdorferi, Brucella abortus,* epizootic hemorrhagic disease virus, and bovine viral diarrhea virus (BVDV) types 1 and 2. Samples collected in 2001 were screened for antibodies against *Anaplasma phagocytophilum,* and whole blood was submitted for polymerase chain reaction (PCR) testing for *A. phagocytophilum* and *B. burgdorferi.* In addition, serum selenium concentrations were evaluated for samples collected during 2001–03. Antibody prevalence and selenium concentration were compared by age-class and geographic region. Antibodies to all of the infectious agents except *A. marginale* and *B. abortus* were detected; when detected, antibody prevalence was highest in adults. Deer collected from SE Minnesota had a higher antibody prevalence to *B. burgdorferi* than SW deer. Blood culture and PCR results for *A. phagocytophilum* and *B. burgdorferi* were negative. Antibodies against BVDV (combined types 1 and 2) were more prevalent ($\chi^2=3.617$, $P\leq0.029$) in deer collected in SW (41%) than in SE (25%) Minnesota. No statistically significant differences in serum selenium concentrations were detected when data were analyzed by age-class or by geographic location.

Key words: Infectious disease, Minnesota, *Odocoileus virginianus*, selenium, serology, white-tailed deer.

Development of appropriate management recommendations requires identification of factors affecting health of white-tailed deer (*Odocoileus virginianus*) populations. White-tailed deer can serve as sentinels for livestock and human diseases, making epidemiologic surveillance helpful in identifying potential exposure to diseases of economic and public health concern. Much of the information on white-tailed deer in Minnesota (MN) is based on data obtained from populations in the northern part of the state (Brinkman et al., 2004a, 2005; Swanson, 2005). Comparatively little is known about southern populations (Brinkman, 2003; Brinkman et al., 2004a, 2005; DePerno et al., 2000, 2003; Swanson, 2005). The geography and predominant land use differ markedly between the southwest (SW) and southeast (SE) portions of MN. The SW is intensively managed for agriculture with less than 10% permanent cover (Brinkman, 2003; DePerno et al., 2003), whereas the SE is largely forested with slopes and hillsides dominated by deciduous forests (Bigalke et al., 2002; DePerno et al., 2003). As habitat composition and land use influence movement of individuals and diseases across a landscape (Wiens 2001), these differences may impact deer health and survival. The potential impact of selenium deficiency, which may differ between geographic areas, also is of key interest in the upper

TABLE 1. Collection sites for white-tailed deer southeastern and southwestern Minnesota, 2000–2003.

Site (Map coordinates)	Region	Years sampled	Age group (No. adult, no. fawn)	Total no.
Chatfield (43°89′S, 92°22′E)	SE	2000	(2, 0)	2
Pleasant Grove (43°89′S, 92°35′E)	SE	2000, 2001	(4, 1)	5
Dumfries (44°29′S, 92°16′E)	SE	2000, 2003	(16, 2)	18
Rushford (43°85′S, 91°70′E)	SE	2000	(10, 1)	11
Zumbro Falls (44°24′S, 92°47′E)	SE	2000, 2001	(9, 4)	13
Lake Benton (71°50′S, 49°02′W)	SW	2001, 2002	(16, 4)	20
Lamberton (31°28′S, 48°98′W)	SW	2001	(4, 2)	6
Redwood Falls (33°69′S, 49°36′W)	SW	2001, 2002, 2003	(29, 5)	34
Walnut Grove (30°05′S, 48°98′W)	SW	2001	(4, 1)	5

Midwest (Custer et al., 2004). Deficiencies in selenium concentrations can lower deer productivity by negatively impacting overall herd health, fecundity, and survival (Ullrey et al., 1981; Fielder, 1986; McDowell et al., 1995). Selenium requirements of free-ranging white-tailed deer are not well documented, and baseline data from disparate wild deer populations are needed to understand the impact of selenium on white-tailed deer productivity.

Serum samples from female white-tailed deer ($n=114$; 94 adults, 20 fawns) were collected during January 2000–03 from five locations in SE MN and four locations in SW MN (Table 1). The SW study sites were comprised of ≥85% agricultural lands characterized by intensive cultivation consisting mainly of corn and soybeans (Brinkman, 2003; DePerno et al., 2003). Less than 10% of the area had either permanent forest or grassland cover (Brinkman, 2003; DePerno et al., 2003). The topography of the SE sites consisted predominantly of rolling hills, stream-cut valleys, and forested hillsides (Bigalke et al., 2002; DePerno et al., 2003). Mean cattle (beef and dairy) densities within study sites in SW MN and SE MN were 19.38 animals/km^2 (SE=2.11) and 36.18 animals/km^2 (SE=2.93), respectively (National Agricultural Statistics Service, 2002).

White-tailed deer were captured using helicopter net-guns in January of each sampling year and transported approximately 2 km to a processing site where physical condition was assessed; blood samples were collected within 15 min of capture via routine jugular venipuncture. Deer were blindfolded and rectal temperature was monitored throughout the restraint. Captured deer were assigned to age cohorts (adult [>1 yr] or a fawn [~8 mo]) based on body size, measured (chest and neck circumference), ear-tagged, and administered a broad-spectrum antibiotic. Radiocollars (Advanced Telemetry System, Isanti, Minnesota, USA) equipped with activity and mortality sensors were placed around the neck of each deer. Deer were chemically immobilized with ketamine hydrochloride and xylazine hydrochloride and administered yohimbine hydrochloride prior to release (Swanson, 2005).

Serologic testing for the selected pathogens was conducted at the Minnesota

TABLE 2. Diagnostic test utilized by the Minnesota Veterinary Diagnostic Laboratory and antibody prevalence estimates for white-tailed deer sampled in southern Minnesota, 2000–2003.

Agent	Test procedure[a] (Positive antibody titer)	No. antibody positive/total tested (%)		
		Fawn	Adult	Total
Anaplasma marginale	Card test (NA)	0/16	0/76	0/92
Bovine viral diarrhea virus type 1	SN (1:16)	5/20 (25%)	40/94 (43%)	45/114 (39%)
Bovine viral diarrhea virus type 2	SN (1:16)	0/3	8/37 (22%)	8/40 (20%)
Brucella abortus	Ag (1:25)	0/16	0/81	0/97
Epizootic hemorrhagic disease virus	AGID	0/16	1/77 (1%)	1/93 (1%)
Anaplasma phagocytophilum	ELISA/Western blot (0.32)	0/14	1/45 (2%)	1/59 (2%)
Mycobacterium avium subsp. *paratuberculosis*	ELISA (≥0.25)	0/20	2/94 (2%)	2/114 (2%)
Leptospira spp.[b]	MAT (1:100)	4/16 (25%)	14/80 (18%)	18/96 (19%)
Borrelia burgdorferi	IFA (1:320)	3/20 (15%)	27/94 (29%)	30/114 (26%)

[a] AGID = agar gel immunodiffusion; ELISA = enzyme-linked immunodiffusion assay; SN = serum neutralization; MAT = microscopic agglutination test; IFA = immunofluorescent antibody test; Ag = standard plate agglutination test.

[b] *Leptospira interrogans* serovars *bratislava, canicola, grippotyphosa, hardjo, icterohemorrhagica,* and *pomona.*

Veterinary Diagnostic Laboratory (University of Minnesota, St. Paul, Minnesota, USA). Serum antibody prevalence was determined from samples collected each year (2000–03) for the following pathogens: *Mycobacterium avium* subsp. *paratuberculosis* (MAP), *Leptospira interrogans* (serovars *canicola, grippotyphosa, hardjo, pomona, bratislava, icterohemorrhagica*), *Anaplasma marginale, Borrelia burgdorferi,* epizootic hemorrhagic disease virus (EHDV), *Brucella abortus,* and bovine viral diarrhea virus type 1 (BVDV1). Test procedure positive threshold titers are listed in Table 2. Serologic testing for antibodies to bovine viral diarrhea virus type 2 (BVDV2) was performed using samples collected in 2002 and 2003. Samples collected in 2001 were screened for antibodies against *Anaplasma phagocytophilum,* which causes human granulocytic ehrlichiosis (HGE), and whole blood was submitted for polymerase chain reaction (PCR) to detect DNA for *A. phagocytophilum* and *B. burgdorferi.* For PCR positive deer, whole blood was used to inoculate Barbour-Stoenner-Kelly medium and HL 60 cells for culture of *B. burgdorferi* and *A. phagocytophilum,* respectively.

Serum selenium concentrations were evaluated for samples collected during 2001–03 by the Analytical Sciences Laboratory (University of Idaho, Moscow, Idaho, USA). Serum selenium concentrations between 0.06 and 0.15 ppm were considered adequate (McDowell et al., 1995).

For each pathogen, antibody prevalence was compared by age-class (adult, fawn) and geographic region (SE, SW) using Chi-square analysis with Yates correction. Fisher's exact test was performed if any cells had <5 individuals. A P value ≤ 0.05 was considered statistically significant. Serum selenium concentrations were compared by age-class and geographic location using the Mann-Whitney Rank sum test with values of $P \leq 0.05$ considered statistically significant.

Antibody titers were detected for all infectious agents except *Anaplasma marginale* and *Brucella abortus* (Table 2). Although not statistically significant, antibody prevalence was consistently higher in adults compared to fawns for all agents except *Leptospira* spp. (Table 2). Antibodies against BVDV, *B. burgdorferi,* and *Leptospira* spp. were most prevalent, whereas numbers of deer with antibodies against EHDV, *A. phagocytophilum,* and MAP were low (Table 2). All tested deer were culture and PCR negative for *B. burgdorferi* and *A. phagocytophilum.*

Statistical differences in antibody prev-

TABLE 3. Prevalence of antibodies to select agents in white-tailed deer in southeastern and southwestern Minnesota, 2000–03.

Agent	No. antibody positive/total tested (%)	
	Southwestern deer	Southeastern deer
Bovine viral diarrhea virus type 1	30/65 (46%)	15/49 (31%)
Bovine viral diarrhea virus type 2	7/25 (28%)	1/15 (7%)
Epizootic hemorrhagic disease virus	0/65	1/28 (4%)
Anaplasma phagocytophilum	0/47	1/12 (8%)
Mycobacterium avium subsp. *paratuberculosis*	1/65 (2%)	1/49 (2%)
Leptospira interogans	11/65 (17%)	7/31 (23%)
L. grippotyphosa	8/65 (12%)	7/31 (23%)
L. bratislava	1/65 (2%)	0/31
L. pomona	3/65 (5%)	0/31
Borrelia burgdorferi	6/65 (9%)	24/49 (47%)[a]

[a] Significant differences ($P<0.05$) detected between antibody prevalence estimates for southeastern and southwestern deer.

alence between age-classes were not detected for any of the pathogens. Regional differences in antibody prevalence were detected for *B. burgdorferi* ($\chi^2=20.94$, $P\leq0.001$; Table 3); deer collected from the SE had a higher prevalence of antibody than SW deer. Positive antibody titers ranged from 320 to >1,280. Antibodies against BVDV (combined types 1 and 2) were more prevalent ($\chi^2=3.617$, $P\leq0.029$) in deer collected in the SW (41%) than in SE deer (25%; Table 3). However, significant regional differences were not present when each BVDV type was evaluated independently. All deer that had antibodies against both BVDV1 and BVDV2 were from the SW. Although not significantly different, deer from the SW population had antibodies against three different *Leptospira interrogans* serovars (*L. bratislava, L. pomona, L. grippotyphosa*), whereas SE deer had antibodies detected against only one serovar (*L. grippotyphosa*) (Table 3).

No statistically significant differences in serum selenium concentrations were detected when data were analyzed by age-class or by geographic location. Median serum selenium concentrations for SE deer ($n=24$) and SW deer ($n=63$) were 0.08 and 0.09 ppm, respectively. However, 11% of SW deer ($n=7$) had serum selenium concentrations below 0.06 ppm (considered deficient in livestock; McDowell et al., 1995). None of the samples from SE deer had selenium concentrations below this value.

In this study, we documented that deer from southern MN have antibodies to several infectious agents that have relevance to white-tailed deer, domestic animal, and public health. As expected, adult deer tended to have higher antibodies against these agents than fawns, presumably due to increased contact opportunity over time.

The pronounced regional difference in seroprevalence of antibodies against *B. burgdorferi* was likely related to the variability in habitat type between the two regions. The SE collection sites were more forested and had abundant ground cover that would provide suitable habitat for *Ixodes scapularis.* This is consistent with a previous study in east central MN that demonstrated dramatic variation in prevalence of *B. burgdorferi* antibodies in white-tailed deer based on the habitat suitability of the collection sites for *Ixodes* spp. (Gill et al., 1994).

Antibodies to both BVDV1 and BVDV2 were relatively high in both regions, indicating that deer are routinely exposed to this pathogen of cattle. Regional prevalence estimates for BVD in cattle from Minnesota are lacking; however,

antibody prevalence in cattle in the United States ranges from 29% to 90%, with similar prevalence rates reported for beef and dairy cows (Houe, 2005). This variability may be due to differences in stocking density, management strategies, and vaccination practices. Experimentally, white-tailed deer are susceptible to BVDV infection (Van Campen et al., 1997) and can become persistently infected (Passler et al., 2007). Detection of antibodies in the serum, however, does not indicate a persistent infection.

Deer in SW MN were more likely to have antibodies to BVDV even though more cattle and cattle farms are present in SE MN (National Agricultural Statistics Service, 2002). In a similar analysis in Europe, BVDV antibody prevalence in wild cervids also was not related to cattle density (Frölich, 1995), but the type of cattle operation may be an important factor to consider. Management practices often differ considerably between beef and dairy herds. In this study, although cattle density was highest in the SE, it was predominately composed of dairy herds where cows are typically managed under more confined conditions (National Agricultural Statistics Service, 2002). In SW MN, there were more beef cattle, and with these animals, movements are generally less restricted to maximize grazing opportunities. The greater range of beef cattle could have allowed more opportunities for contact with deer in the SW despite the lower overall cattle density. The movement behavior of deer also may have contributed to the greater BVDV antibody prevalence seen in SW MN deer. Deer in SW MN have higher migration rates and longer dispersal distances compared to SE MN; it has been shown that dispersal distances of deer increase with decreasing forest cover (Brinkman et al., 2005; Long et al., 2005).

This study provided evidence of widespread exposure of southern MN white-tailed deer to *L. interrogans.* Previous antibody prevalence estimates for *L. interrogans* in MN white-tailed deer have ranged from 3% (Ingebrigtsen et al., 1986) to 43% in NW populations (Goyal et al., 1992). The latter estimate is similar to the 49% prevalence rate in an extensive study of mature cattle in the United States (Miller et al., 1991). In our study, 19% of deer were seropositive for *L. interrogans;* and this is similar to an overall antibody prevalence of 17.9% reported in cattle, pigs, and horses submitted to the Minnesota Veterinary Diagnostic Laboratory between 1984 and 1986 (Kahn et al., 1991).

In previous studies of samples collected from NW MN deer populations, antibodies to serovars *L. pomona* and *L. bratislava* were most common (Goyal et al., 1992). However, in our study antibodies against *L. grippotyphosa* were observed most frequently, and only SW deer had antibodies against serovars *L. pomona* and *L. bratislava.* These variations may reflect regional differences between populations that could be related to disparities in land use. Overall antibody titers from the deer in this study were low, and there were no apparent differences between adults and fawns.

Antibodies to *A. phagocytophilum* were detected in only one deer. This low prevalence of antibodies is consistent with results from Wisconsin and Iowa where antibodies were reported in 8% and 0% of white-tailed deer tested (Belongia et al., 1997). Minnesota was classified as a brucellosis-free state in 1985 (Anonymous, 1985). The lack of antibodies against *B. abortus* is consistent with the brucellosis-free designation and with results from previous surveillance of white-tailed deer in MN (Ingebrigtsen et al., 1986) and other states (Davis, 1990). We did not detect evidence of exposure to *A. marginale,* and the low prevalence of antibody titers to EHD was not surprising given that this disease occurs infrequently in deer populations in northern latitudes (Nettles and Stallknecht, 1992). The low percentage of deer with antibodies against

M. avium subsp. *paratuberculosis* is consistent with a previous study that examined fecal cultures from MN white-tailed deer living in proximity to dairy farms (Raizman et al., 2005).

The median serum selenium concentration for deer in this study appeared to be similar in both regions (SE, SW) and was in the acceptable range for livestock (McDowell et al., 1995). However, seven deer in the SW had serum selenium concentrations that would be considered deficient in cattle. The criteria used to define the selenium status of livestock may not be directly applicable to white-tailed deer. Farmed red deer (*Cervus elaphus*) in New Zealand appear to have lower selenium requirements than cattle in the same region (Grace et al., 2000). Furthermore, measurement of serum selenium concentration is most useful for identifying immediate deficiencies, whereas whole blood or liver are more suitable for determining selenium status over time (Wichtel, 1998).

The results from this study provide evidence that white-tailed deer in southern MN are exposed to a variety of pathogens, including those that can affect cattle and humans. Baseline data can provide valuable information on temporal changes that can be useful for disease surveillance.

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