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Serological Survey and Pathogen Exposure of Adult Female White-tailed Deer in the Western Dakotas

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ABSTRACT Establishing baseline values for pathogen exposure and nutritional indices is necessary to monitor population health. However, little is known about white-tailed deer (*Odocoileus virginianus*) pathogen exposure and nutritional condition in the Northern Great Plains. Our objective was to assess pathogen exposure and establish nutritional indices for female white-tailed deer in Dunn and Grant counties, North Dakota and Perkins County, South Dakota. During 2014, we collected blood serum from 150 adult female white-tailed deer. Pathogens with the highest antibody prevalence included West Nile Virus (WNV; 85%), epizootic hemorrhagic disease (48%), and malignant catarrhal fever (32%). Serum values for creatine kinase, globulin, glucose, potassium, and lactate dehydrogenase in all three study areas were higher than reference ranges while sodium was low in Grant County relative to Dunn and Perkins counties. We speculate that high exposure of WNV and high potassium values combined with low sodium values may affect neonate survival in Grant County. However, regional differences in pathogen exposure, their connection to serum values, and their potential interactive effects on survival are not well understood.

KEY WORDS: disease, epizootic hemorrhagic disease, livestock pathogens, nutritional indices, Northern Great Plains, *Odocoileus virginianus*, white-tailed deer, West Nile virus.

Nutritional indices and pathogen exposure rates are important components when assessing wildlife health. Nutritional indices are used to assess forage and habitat quality as well as reproductive state of white-tailed deer (*Odocoileus virginianus*; White and Cook 1974, Seal et al. 1981, Gill et al. 2001). Also, disease antibodies provide an assessment of past exposure to pathogens (e.g., bovine viral diarrhoea virus, bluetongue virus, epizootic hemorrhagic disease; Gilbert et al. 2013). Further, white-tailed deer are sentinels for human and livestock related diseases (Gill et al. 1994, Wolf et al. 2008, Sherrill et al. 2012) and can facilitate disease transmission (Roug et al. 2012, Myers et al. 2015). Therefore, monitoring health factors and establishing baseline nutritional indices and pathogen exposure provides essential herd health information that may help explain population trends (Myers et al. 2015).

Antibody prevalence indicates previous exposure to an antigen but does not indicate current infection (Gilbert et al. 2013). Pathogen exposure can impact wildlife populations, domestic livestock, and human health (Wolf et al. 2008, Billinis 2012, Roug et al. 2012, Sherrill et al. 2012) by affecting factors such as reproduction and survival. For example, epizootic hemorrhagic disease and bluetongue virus are diseases that could impact ungulate population dynamics as infection often occurs during the breeding season and can be

lethal (Dubay et al. 2006). Monitoring antibody prevalence in ungulate species is important in the western United States because livestock roam large tracts of land, which increases risk of disease transmission when compared to areas where cattle are confined (Wolf et al. 2008). Likewise, humans can become infected with pathogens carried by white-tailed deer such as *Anaplasma* and *Borrelia* (Wolf et al. 2008). Although pathogen exposure can have wide ranging effects, no pathogen exposure information has been reported for white-tailed deer inhabiting the rangelands of the western Dakotas.

Nutritional indices are used to monitor trace elements and minerals present in blood to evaluate seasonal health and nutrition (Seal et al. 1981, DelGiudice et al. 1987, DeLiberto et al. 1989) and are helpful when investigating forage nutritional value, reproduction, and survival (DelGiudice et al. 1991). For example, comprehensive nutritional analyses have been reported for white-tailed deer in Kansas (Klinger et al. 1986), southern Texas (White and Cook 1974), and North Carolina (Chitwood et al. 2013). Also, DelGiudice et al. (1991) investigated seasonal hematological differences of white-tailed deer in northern Minnesota, while Wolf et al. (2008) reported selenium values in female white-tailed deer in southern Minnesota. Seal et al. (1981) stressed the need for reference ranges for specific populations of white-tailed

deer to accurately assess population health and to compare health across white-tailed deer populations in the United States. Although Zimmerman (2004) investigated impacts of burning on nutritional indices of white-tailed deer and mule deer (*O. hemionus*) in the southern Black Hills, South Dakota, USA, there are no published nutritional indices for white-tailed deer inhabiting the grasslands region of the western Dakotas.

Our objectives were to establish baseline information on nutritional indices and pathogen exposure for adult female white-tailed deer in western North Dakota and northwestern South Dakota. We measured nutritional indices for several minerals including sodium (Na), phosphorus (P), and magnesium (Mg), given their potential impacts on spatial distribution and carrying capacity (McNaughton 1988, Freeland and Choquenot 1990), seasonal movements (McNaughton 1990), and diet selection (Furness 1988) of ungulates. We then chose to compare our baseline information from the Dakotas to similar information provided by Seal et al. (1981; Minnesota), Tumbleson et al. (1968; Missouri), and Chitwood et al. (2013; North Carolina). Similarly, we measured exposure to several pathogens that can have population level impacts on white-tailed deer, including epizootic hemorrhagic disease (Fischer et al. 1995, Gaydos et al. 2004) and chronic wasting disease (CWD; Edmunds et al. 2016), as well as pathogens that are transmissible between domestic livestock and white-tailed deer (e.g., malignant catarrhal fever [MCF; Li et al. 2013, Palmer et al. 2013]).

STUDY AREA

We assessed female white-tailed deer pathogen exposure and nutritional indices in Grant and Dunn counties, North Dakota, and Perkins County, South Dakota (Fig. 1), during 2014. The three study areas were located in the Northwestern Great Plains Level III Ecoregion (Bryce et al. 1998).

In Dunn County, we captured white-tailed deer in a 1,492 km² area in the southwestern portion of the county. Grasslands, cropland, and forested areas comprised 60, 20, and 9% of the land cover, respectively (U. S. Department of Agriculture 2015), and white-tailed deer density was estimated at 1.0 deer/km² in 2011 (Stillings et al. 2012). Thirty-year mean annual precipitation was 41.4 cm, and thirty-year mean monthly temperature ranged from -15.1°C to 29.3°C (North Dakota State Climate Office 2016). Cattle and sheep densities were 14.8 cattle/km² and 0.3 sheep/km² during 2012 (U. S. Department of Agriculture 2014). Oil and natural gas development was prevalent, with ~1,800 active oil wells in Dunn County that produced about 64 million barrels of oil and 35 million cubic feet of natural gas annually (Department of Mineral Resources 2016).

In Grant County, we captured white-tailed deer in a 1,865 km² area in the southwestern portion of the county. Grasslands, cropland, and forested areas comprised 68, 26,

and 1% of the land cover, respectively (U.S. Department of Agriculture 2015), and white-tailed deer density was estimated at 1.8 deer/km² in 2011 (Stillings et al. 2012). Thirty-year mean annual precipitation was 41.2 cm, and thirty-year mean monthly temperature ranged from -14.4°C to 29.7°C (North Dakota State Climate Office 2016). Cattle and sheep densities were 17.8 cattle/km² and 0.5 sheep/km² during 2012 (U.S. Department of Agriculture 2014). From 2009 to 2016, chronic wasting disease was detected in 1 white-tailed deer and 8 mule deer in Grant County. There was no active oil and natural gas development in Grant County during our study.

In Perkins County, we captured white-tailed deer in a 1,492 km² area in the central portion of the county. Grasslands, cropland, and forested areas comprised 86, 11, and 0.01% of the land cover, respectively (U.S. Department of Agriculture 2015), and white-tailed deer density was estimated at 1.2 deer/km² in 2015 (K. Robling, South Dakota Game, Fish and Parks, personal communication). Thirty-year mean annual precipitation was 44.9 cm, and mean thirty-year monthly temperature ranged from -12.1°C to 30.3°C (North Dakota State Climate Office 2016). Cattle and sheep densities were 14.1 cattle/km² and 2.0 sheep/km² during 2012 (U.S. Department of Agriculture 2014). There was no active oil and natural gas development in Perkins County during our study.

METHODS

We captured female (≥ 9 month-old) white-tailed deer via helicopter net guns (Native Range Capture Services, Elko, NV, USA) from 24 February to 2 March 2014. We hobbled, blindfolded, radio-collared, and collected blood at capture locations; we collected about 20 ml of blood from each white-tailed deer via jugular venipuncture from all study areas. All capture and handling methods were approved by the Institutional Animal Care and Use Committee at South Dakota State University (13-091A) and followed guidelines for care and use of mammals established by the American Society of Mammalogists (Sikes et al. 2016).

We maintained blood vials at room temperature and allowed them to clot before centrifugation. Following centrifugation, we separated serum from cells via pipette and placed serum in cryovial tubes. We sent serum samples to the North Dakota State University (NDSU) Veterinary Diagnostic Laboratory for analysis (NDSU, Fargo, ND, USA). We prioritized which nutritional indices to run based on previous literature (Seal et al. 1981, Tumbleson et al. 1968, Chitwood et al. 2013). We analyzed serum samples for alkaline phosphatase (IU/L), aspartate aminotransferase (IU/L), albumin (ALB, g/dL), blood urea nitrogen (BUN, mg/dL), calcium (Ca, mg/dL), chloride (Cl, mEq/L), creatinine kinase (CK, md/dL), gamma-glutamyl transpeptidase (GGT, IU/L), globulin (GLOB, g/dL), glucose (GLU, mg/dL),

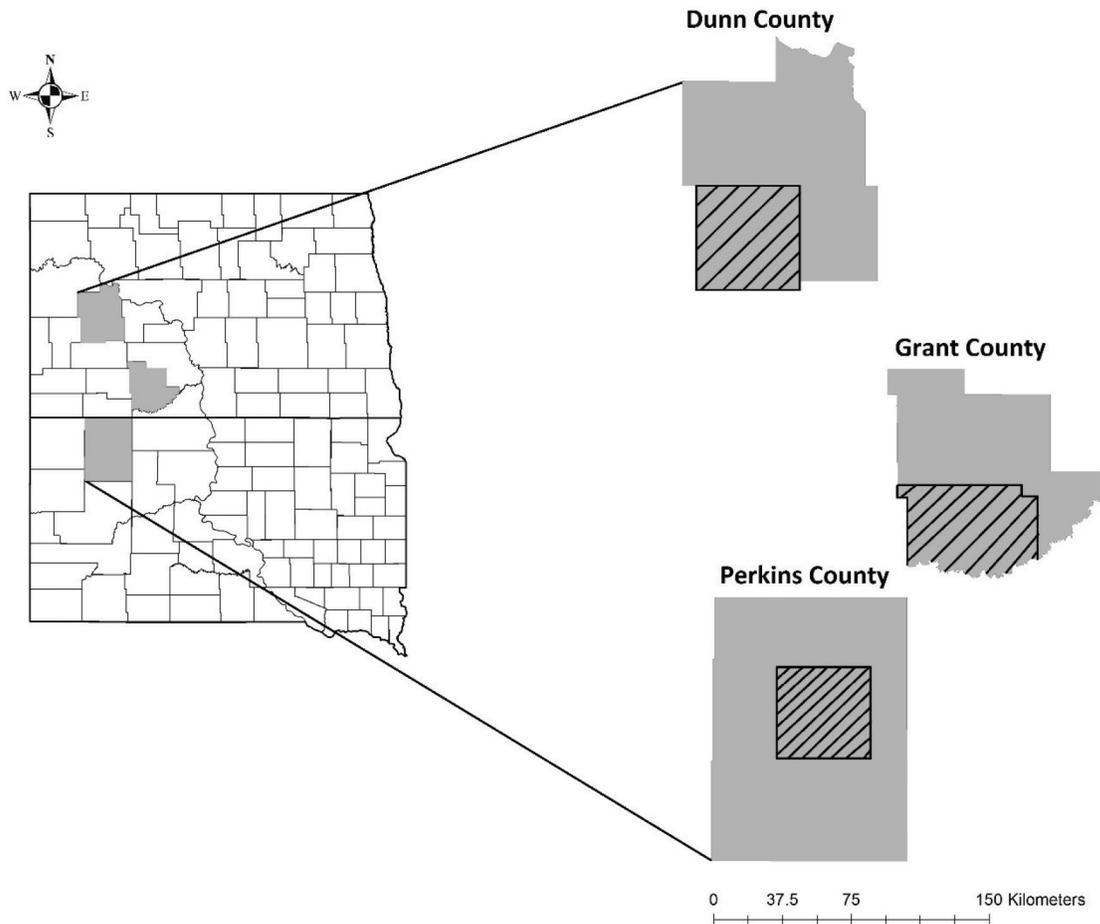


Figure 1. Study areas where adult female white-tailed deer (*Odocoileus virginianus*) were captured and radio-collared in Dunn and Grant counties, North Dakota, and Perkins County, South Dakota, USA. Dashed lines indicate deer capture areas within each county.

lactate dehydrogenase (LDH, IU/L), magnesium (Mg, mg/dL), phosphorus (P, mg/dL), potassium (K, mEq/L), sodium (Na, mEq/L), and total protein (TP, g/dL).

The Minnesota Veterinary Diagnostic Laboratory (University of Minnesota, St. Paul, MN, USA) determined disease status from serum samples. We tested serum for the following pathogens: *Anaplasma marginale*, *Borrelia* spp., *Brucella abortus*, bovine parainfluenza – 3 virus (PI3), bovine viral diarrhea virus (BVDV) type 1 and 2, bluetongue virus (BTV), epizootic hemorrhagic disease (EHD), infectious bovine rhinotracheitis (IBR), six serovars of *Leptospira interrogans* (*bratislava*, *canicola*, *grippotyphosa*, *hardjo*, *icterohemorrhagica*, and *pomona*), and *Neospora* spp. We sent additional serum samples to the National Veterinary Services Laboratory (U.S. Department of Agriculture, Ames, IA, USA) to test for the following pathogens: malignant catarrhal fever (MCF), West Nile Virus (WNV), and eastern and western equine encephalitis (EEE and WEE, respectively). The Diagnostic Center for Population and Animal Health (currently known as

Michigan State University Diagnostic Laboratory; Michigan State University, Lansing, MI, USA) tested lymph nodes from hunter-harvested radio-collared white-tailed deer for chronic wasting disease (CWD).

We used card agglutination to determine positive *A. marginale* titers at 1:320 and used indirect immunofluorescence assay (IFA) to determine positive *Borrelia* titers at 1:320. We used hemagglutination inhibition (HI) to determine positive PI3 titers at 1:10 and used serum neutralization (SN) to determine positive BVDV 1 and 2 and IBR titers at 1:8. We used a microscopic agglutination test (MAT) to determine positive *L. interrogans* (including serovars *bratislava*, *canicola*, *grippotyphosa*, *hardjo* *icterohemorrhagica*, and *pomona*) titers at 1:100. We used peroxide linked assay (PLA) to determine MCF positive titers at 1:20 and used immunoglobulin M (IgM) and immunoglobulin G (IgG) to detect WNV titers at 1:10. We interpreted no agglutination in a sample to indicate a negative reaction for *B. abortus*.

We used an enzyme-linked immunosorbent assay (ELISA) to detect EEE and WEE titers at 1:10. We used

ELISA to detect *Neospora* spp. titers when sample to positive ratios (S:P) were greater than 0.50 and also used ELISA to test lymph nodes from mortalities for CWD. We used polymerase chain reaction (PCR) to detect BTM and EHD DNA presence.

Statistical analysis

We quantified antibody prevalence and nutritional index values to establish baseline information for female white-tailed deer in western North Dakota and northwestern South Dakota. We used a proportions analysis using the `prop.test` function in Program R (R Development Core Team 2017; version 3.3.1) to assess if pathogen exposure varied by study area. We used descriptive statistics and qualitative comparisons with other published values to assess whether or not white-tailed deer in North and South Dakota were in or out of normal ranges for nutritional index values.

RESULTS

We captured and collected blood from 50 adult female white-tailed deer in each county (totaling 150) and collected lymph nodes from nine hunter-harvested radio-collared deer.

In Dunn County, antibodies for WNV (79%), EHD (40%), and MCF (24%) were most prevalent (all other antibodies were $\leq 12\%$; Table 1). Similarly, in Perkins County, antibodies for WNV (86%), EHD (81%), MCF (62%) were most prevalent (all other antibodies were $\leq 37\%$; Table 1). In Grant County, antibodies for WNV (89%), PI3 (45%), and IBR (22%) were most prevalent (all other antibodies were $\leq 10\%$; Table 1). We detected antibodies for all infectious agents except *Brucella* spp., *L. interrogans* serovars *canicola*, *hardjo*, and *icteroheorrhagica*, and eastern and western equine encephalitis. Observed titer levels were low for most pathogens except one individual with titers of 1:128 for BVDV 1, one individual with titer levels of 1:1600 for *L. interrogans* serovar *pomona*, and one individual with titer levels of 1:320 for PI3. None of the hunter-harvested radio-collared individuals tested positive for CWD ($n = 9$).

We documented variation in the nutritional indices that fell above, within, and below reference ranges. When comparing to Seal et al. (1981), mean CI, CK, GGT, GLOB, LDH, and K were above reference ranges for all counties (Table 2). Mean P was above reference ranges in Dunn and Perkins counties and mean ALB was also above Seal et al. (1981) reference range in Perkins County. Mean BUN, Ca, GLU, Na, and TP were all within reference ranges

Table 1. Antibody prevalence (# positive/# tested) in female white-tailed deer in Dunn and Grant Counties, North Dakota, and Perkins County, South Dakota, during 2014.

Agent	No. positive/total tested (%)	No. positive/total tested (%)		
		Dunn	Grant	Perkins
<i>Anaplasma marginale</i>	5/118 (4%)	3/44 (7%)	2/29 (7%)	0/45
<i>Borrelia</i> spp.	14/146 (10%)	1/47 (2%)	3/49 (6%)	10/50 (20%)
<i>Brucella abortus</i>	0/131	0/36	0/49	0/46
Bovine Parainfluenza – 3 Virus (PI3)	33/114 (29%)	3/39 (8%)	13/29 (45%)	17/46 (37%)
Bovine Viral Diarrhea Virus Type 1 (BVDV 1)	3/150 (2%)	0/50	3/50 (6%)	0/50
Bovine Viral Diarrhea Virus Type 2 (BVDV 2)	2/150 (1%)	0/50	2/50 (4%)	0/50
Bluetongue Virus (BTV)	2/150 (1%)	0/50	0/50	2/50 (4%)
Epizootic Hemorrhagic Disease (EHD)	62/128 (48%)	20/50 (40%)	3/30 (10%)	39/48 (81%)
Infectious Bovine Rhinotracheitis (IBR)	28/150 (19%)	6/50 (12%)	11/50 (22%)	11/50 (22%)
<i>L. i. grippotyphosa</i>	1/150 (1%)	0/50	0/50	1/150 (2%)
<i>L. i. bratislava</i>	12/150 (8%)	3/50 (6%)	4/50 (8%)	5/50 (10%)
<i>L. i. pomona</i>	7/150 (5%)	5/50 (10%)	1/50 (1%)	1/50 (1%)
Malignant Catarrhal Fever (MCF)	33/103 (32%)	7/29 (24%)	3/37 (8%)	23/37 (62%)
<i>Neospora</i> spp.	5/117 (4%)	2/43 (5%)	2/29 (7%)	1/45 (2%)
West Nile Virus (WNV)	87/102 (85%)	23/29 (79%)	32/36 (89%)	32/37 (86%)
Eastern and Western Equine Encephalitis (EEE and WEE)	0/118	0/29	0/52	0/37
Chronic Wasting Disease (CWD)	0/9	0/1	0/7	0/1

Table 2. Nutritional indices for radio-collared female white-tailed deer in Dunn and Grant Counties, North Dakota, and Perkins County, South Dakota, during 2014.

Blood Chemistry Parameter	Dunn		Grant		Perkins		Reference Ranges	
	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Seal et al. (1981)	Chitwood et al. (2013)
Albumin (g/dL)	4.11 (0.05)	3.50-4.90	4.14 (0.05)	3.10-4.80	4.34 (0.18)	2.50-12	2.50-4.20	2.10-3.30
Alkaline Phosphatase (IU/L)	54.32 (3.04)	12-147	56.50 (2.04)	17-132	63.18 (3.66)	29-145	n/a	24-267
Aspartate Aminotransferase (IU/L)	154.96 (7.23)	75-344	175.30 (7.65)	72-317	198.58 (25.30)	93-1384	n/a	47-166
Blood Urea Nitrogen (mg/dL)	23.38 (1.01)	13-43	20.62 (0.85)	8.50-11.10	23.24 (0.97)	13.57	15-45	6-35
Calcium (mg/dL)	10.39 (0.61)	8.70-40.10	9.79 (0.07)	8.50-11.10	9.74 (0.12)	6.60-12	8.80-10.80	8.70-11.60
Chloride (mEq/L)	113.60 (0.52)	108-133	112.92 (1.25)	53-117	113.80 (0.38)	109-123	100-110	97-119
Creatinine Kinase (md/dL)	414.88 (38.10)	103-1486	614.92 (30.32)	196-1041	730.06 (46.70)	13-2007	20-400	63-1883
Gamma-Glutamyl Transpeptidase (IU/L)	118.84 (4.34)	50-231	116.36 (4.25)	50-247	111.92 (3.73)	80-227	40-100	n/a
Globulin (g/dL)	2.67 (0.04)	2.30-3.60	2.95 (0.06)	2.40-4.30	2.74 (0.04)	2.2-3.40	0.40-1.00	2.70-5.30
Glucose (mg/dL)	157.08 (4.64)	82-243	161.26 (4.89)	90-243	139.64 (5.77)	23-212	60-320	85-409
Lactate Dehydrogenase (IU/L)	1152.42 (49.93)	112-2377	1437.76 (76.73)	866-3486	1160.08 (46.25)	590-2800	100-300	n/a
Phosphorus (mg/dL)	8.92 (0.23)	5.80-12	8.15 (0.19)	5.00-10.50	8.63 (0.30)	2.94-13.80	4.50-8.50	5.60-15.50
Potassium (mEq/L)	12.70 (0.38)	8.90-14.80	13.50 (0.00)	13.5	25.19 (3.18)	12.90-50.81	3.40-5.00	5.80-12.00
Sodium (mEq/L)	152.50 (0.81)	133-161	140.34 (2.03)	61-157	146.38 (1.20)	127-161	132-156	139-171
Total Protein (g/dL)	6.78 (0.06)	6-7.90	7.09 (0.07)	5.80-8.50	7.12 (0.15)	6-13.50	5.00-7.80	5.30-8.20

reported by Seal et al. (1981) for all counties. Mean ALB was within reference ranges for Dunn and Grant counties, while mean P was within reference range for Grant County only. When comparing to Chitwood et al. (2013), only mean ALB and K were above reference ranges for all counties, while mean aspartate aminotransferase was above the reference range for Grant and Perkins counties, only. Mean alkaline phosphatase, Ca, Cl, CK, GLU, P, Na, and TP for all 3 counties were all within the reference range reported by Chitwood et al. (2013), while mean GLOB was within range for Grant and Perkins counties and mean aspartate aminotransferase was within range for Dunn County only. No mean nutritional indices were reported below reference ranges reported by Seal et al. (1981), while mean GLOB was the only nutritional index reported below the reference range for Chitwood et al. (2013). Mean Mg was greater in Dunn (2.81 mg/dL), Grant (2.94 mg/dL), and Perkins (3.04 mg/dL) compared to the reference range reported by Tumbleson et al. (1968; range = 2.2–2.6). Sufficient serum was available for most samples ($n \geq 146$) for assessing nutritional indices; however, given that we prioritized nutritional indices, some that were of lower priority had fewer samples. For example, samples available for assessing K were low for Dunn ($n = 22$), Grant ($n = 1$), and Perkins ($n = 14$) counties.

DISCUSSION

Pathogen exposure

Exposure of EHD ranged from 10% (Grant County) to 81% (Perkins County). Although exposure rates in Grant County were comparable to historic EHD exposure rates reported for North Dakota (7%; Sohn and Anderson 1991), we report greater exposure rates in Dunn (40%) and Perkins (81%) counties. North Dakota observed high white-tailed deer mortality attributed to EHD during 2008, 2011, and 2013; epizootics caused high mortality in Grant County with few reports in Dunn County, indicating differences in intensity of exposure across the landscape (North Dakota Game and Fish Department). Naïve white-tailed deer populations exposed to new strains of EHD may display increased mortality compared to white-tailed deer populations previously exposed to the same strain (Shope et al. 1960, Gaydos et al. 2002). Individuals in Grant County may not have been exposed to the strain(s) of EHD present on the landscape in 2008, 2011, and 2013, causing them to perish at an increased rate and removing them from the landscape during sampling. Conversely, if white-tailed deer in Dunn and Perkins counties were previously exposed to those strains and developed immunity allowing them to survive until our sampling effort, then they would have displayed increased antibody prevalence compared to white-tailed deer sampled from Grant County.

Our results indicate white-tailed deer are exposed

to a number of livestock pathogens that are potentially influenced by farm operation type (Wolf et al. 2008). For example, most farm operations in the western Dakotas allow livestock grazing, which facilitates increased white-tailed deer exposure to livestock and disease transmission compared to farm operations that keep livestock contained. Exposure of MCF was highest in Perkins County compared to Dunn and Grant counties, which could be explained by its relatively higher sheep density (sheep were also allowed to graze; 2.0 sheep/km²) compared to Dunn (0.3 sheep/km²) and Grant (0.5 sheep/km²) counties. Exposure of PI3 and IBR were higher in Perkins County than Dunn County but there was no difference in exposure between Perkins and Grant counties. We hypothesize that white-tailed deer in the western Dakotas come in contact with livestock and/or their feces on the landscape, increasing exposure to livestock pathogens.

We observed exposure to *Borrelia* spp. in all study areas with a relatively high exposure rate in Perkins County (20%) compared to Dunn (2%) and Grant (6%) counties. The high exposure rate in Perkins County was similar to levels detected in Minnesota (29%; Wolf et al. 2008). Wolf et al. (2008) attributed differences in *B. burgdorferi* antibody prevalence between study areas to one area providing more suitable habitat for *Ixodes scapularis*, but surveys in North and South Dakota show that *I. scapularis* is only present in eastern portions of the states (Russart et al. 2014, Maestas et al. 2016). The presence of *Borrelia* spp. may indicate that *B. burgdorferi* or *B. mayonii* were present; however, we did not specifically test for either species. Additionally, *B. mayonii* is relatively new to the landscape and its distribution is unknown (Pritt et al. 2016). Further investigation will help to clarify the cause of the *Borrelia* spp. antibody presence in the western Dakotas.

Although our results indicate that white-tailed deer in the western Dakotas are exposed to a variety of viruses, WNV exposure was consistently high (> 56%). White-tailed deer have tested positive for WNV in New Jersey, USA (Farajollahi et al. 2004) and Georgia, USA (Miller et al. 2005), but only one white-tailed deer mortality was linked to WNV (Miller et al. 2005). While avian species are affected severely, effects of WNV on ungulate species are not well understood, though Miller et al. (2005) suggested that WNV was not a threat to white-tailed deer populations. High WNV exposure could be related to the low neonate survival reported in Grant County (35%; Moratz 2016); however, we did not collect blood samples from dead neonates to verify cause of death. Nevertheless, we hypothesize that WNV infections could be related to neonate mortality if WNV acts as an additive stressor.

Nutritional indices

Several nutritional indices were above the reference

ranges reported by Seal et al. (1981) and Chitwood et al. (2013). We observed greater than 42% of white-tailed deer across all sites with ALB, AST, and K values above reference ranges reported by Chitwood et al. (2013), while 35.6% and 17.6% of individuals displayed GLOB and Na values, respectively, below the reference ranges reported by Chitwood et al. (2013). We observed greater than 60% of individuals with Cl, CK, GGT, GLOB, K, LDH, and Mg values above reference ranges established by Seal et al. (1981), whereas less than 50% of individuals had ALB, Ca, Na, P, and TP values above reference ranges (Seal et al. 1981). We observed less than 10% of individuals with BUN, Ca, CK, Cl, GLU, Na, and P values below reference ranges (Seal et al. 1981).

There are several minerals that are not considered to be limiting in the environment. For example, Cl is generally not thought to be limited in the environment while Ca and Mg are readily available in forage (Barboza et al. 2009, Hewitt 2011) and wild ungulates are rarely deficient (Barboza et al. 2009). Our results support this as we observed over 80% of females with Cl and Mg values above reference ranges and more than 90% of females had Ca values within reference range (Seal et al. 1981). Although, P can be a limiting nutrient for herbivores because levels can be limited in forage (Hewitt 2011) we determined that 50% of females had P values within reference ranges (Seal et al. 1981) suggesting that P was not limited to females in our study. Winter Cl and P values in white-tailed deer in the southern Black Hills were similar to observed Cl and P values in white-tailed deer in our study areas, and Mg values for Grant and Perkins counties were similar to winter Mg values in the southern Black Hills (Zimmerman 2004). Calcium values in all study areas were higher than winter Ca values in white-tailed deer in the southern Black Hills (Zimmerman 2004). Therefore, our results suggest that forage availability likely varies among the reference area in Minnesota (Seal et al. 1981), the southern Black Hills (Zimmerman 2004), and western North Dakota and northwestern South Dakota.

High K values for free-ranging white-tailed deer are reported in the literature (DeLiberto et al. 1989, Zimmerman 2004, Chitwood et al. 2013), with K values varying considerably (although not in a predictable manner) due to K concentrations in available forage (DeLiberto et al. 1989, Zimmerman 2004), capture methodology (DeLiberto et al. 1989, Stringer et al. 2011), and blood sample handling (Stringer et al. 2011). Potassium values reported by Seal et al. (1981) ranged from 3.40 – 5.00 mEq/L and values reported by Chitwood et al. (2013) ranged from 5.80 – 12.00; however, in our study individuals ranged from 8.90 – 50.81, though we obtained small sample sizes for some study areas. Regardless, average winter K values in the southern Black Hills were also higher than average K values in our study (Zimmerman 2004). Intracellular K concentrations are important for cardiac excitability and neurotransmission (Carlson 1997),

while extracellular K concentrations are tightly regulated within the body. The physiological impacts of high K values in white-tailed deer are unclear as individuals do not show negative effects at high levels (Stringer et al. 2011). Therefore, white-tailed deer appear to be able to consistently maintain high levels of K in free-ranging populations.

Growth and reproduction increases Na demands in female ungulates (Hellgren and Pitts 1997, Barboza et al. 2009). For instance, female Na requirements double those of males during gestation and lactation (Hewitt 2011). White-tailed deer females seek mineral licks in spring and summer to supplement deficiencies in dietary Na during gestation and lactation (Kennedy et al. 1995). However, we observed high K values in all study areas, and high K intake can prevent absorption of Na, exacerbating low Na levels (Weeks and Kirkpatrick 1976, Barboza et al. 2009). White-tailed deer fawn survival was lower in Grant County (35%) compared to Dunn and Perkins counties (93%; Moratz 2016). It is possible that increased K levels may be reducing absorption of Na, potentially becoming a limiting factor for reproduction in Grant County.

Our capture methods may have influenced the nutritional indices CK, GLOB, GLU, and LDH, which were above our comparative reference ranges (Seal et al. 1981). Individuals that are immobilized for handling often have lower CK and stress levels than those not immobilized (Montané et al. 2003); however, we did not immobilize individuals during capture, which potentially explains our high CK values. High GLOB, LDH, and GLU levels also can be attributed to high levels of stress in individuals (Rosef et al. 2004) and therefore, our high levels may be attributed to chase time and capture from helicopter net-gunning (Klinger et al. 1986, Smith 2011). However, GLU concentrations can also be highly variable in wild populations of white-tailed deer (Jenks et al. 1991, DePerno et al. 2015). Regardless, capture methods need to be considered before using CK, GLOB, LDH, and GLU as nutritional indices for white-tailed deer.

High GGT levels may indicate liver injury, which can result in reduced weight and performance in cattle (Moreira et al. 2012). Mean GGT values were similar among areas, but more than 45% of individuals displayed values outside of the reference range (Seal et al. 1981). Winter GGT in the southern Black Hills were lower than observed GGT values in all of our study areas. Effects of high GGT levels on white-tailed deer are unknown.

Our results provide new reference range data for white-tailed deer that can be used for comparison to other white-tailed deer populations across North America and for future herd health evaluation in the western Dakotas. Collecting blood samples from individual white-tailed deer over time and using a variety of capture methods would better provide information needed to determine the relationship between our results and herd health. Additional research is needed to identify potential differences in forage quality

and availability among study areas that may be responsible for differences in nutritional indices documented during our study. Finally, more information is needed to better understand the transmission of many livestock pathogens between cattle and wildlife populations. Future research could also evaluate the potential impacts of WNV on white-tailed deer survival and reproduction.

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