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Exposure to Select Pathogens in an Expanding Moose (*Alces alces*) Population in North Dakota, USA

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ABSTRACT: Forty female moose (*Alces alces*) captured in North Dakota, US, in March 2014 were tested for antibodies to a variety of pathogens. Antibodies to West Nile virus (WNV) were detected in 39 (98%) moose following a year with a high number of human cases, suggesting the population accurately reflects WNV activity. Fifteen percent of moose (6/40) had antibodies to *Borrelia burgdorferi*, implying expansion of the tick vector into the area. Antibodies to *Anaplasma* spp. were detected in 55% of moose (22/40), a higher rate than previously detected in cattle from the region. Low titers (100–400) to one or more serovars of *Leptospira* spp. were detected in 23% of moose (9/40), a common finding in wild ruminants. Exposure to other pathogens was uncommon (<8%; <3/40) or not documented. Survival and recruitment were high during the study period, suggesting a limited population-level impact at current levels of exposure and environmental co-stressors.

Key words: *Alces alces*, *Anaplasma*, *Borrelia*, *Leptospira*, moose, North Dakota, serology, West Nile virus.

Moose (*Alces alces*) populations have been declining in areas of the contiguous US and southern Canada, partly attributed to the impact of *Parelaphostrongylus tenuis* (PT; Wünschmann et al. 2015; Lankester 2018). In contrast, the number of moose has increased in recent years in northwest North Dakota, US, an area outside the established PT range (Maskey et al. 2015; Maskey and Smith 2017). In the absence of this health threat, the rate of exposure to other agents provides an indication of pathogen presence in the region that may be of concern for human, wildlife, and domestic animal health.

In March 2014, 40 female moose were captured using aerial net-gunning for a population study designed in accordance with guidelines established by the American Society of Mammologists (Sikes et al. 2016) and approved by the North Dakota Game and Fish Department (study no. C-XIII). Twenty were captured along the Missouri River bottoms near Williston, North Dakota, and 20 along and west of the Des Lacs River, near Kenmare, North Dakota. Blood was collected at the time of capture and centrifuged into cellular and serum fractions that were respectively discarded and frozen at –80 C. While the exact ages of moose were not determined, moose do not reach sexual maturity as calves (Schwartz 1992); based on pregnancy status determined by the presence of pregnancy-specific protein B levels (BioTracking LLC, Moscow, Idaho, USA), 38 moose were ≥22 months old. For our opportunistic study, serum was submitted to the National Veterinary Services Laboratory (Ames, Iowa, USA) and the University of Minnesota Veterinary Diagnostic Laboratory (St. Paul, Minnesota, USA), where it was tested for antibodies to a variety of pathogens of potential concern to human or animal health (Table 1).

Antibodies to West Nile virus (WNV) were detected in 39 of 40 moose (98%; Wilson confidence interval [CI] 87–100%) with titers ranging from 10 to ≥100 using the plaque reduction neutralization test (Table 1; Ostlund 2008). Antibodies to *Anaplasma* spp. (ANA) were detected in 22 moose (55%; 95% CI 40–69%) using a commercially available competitive ELISA kit (VMRD Inc., Pullman, Washington, USA; Scoles et al. 2008). Six moose (15%; CI 7–29%) had titers ≥160 to *Borrelia burgdorferi* (BBD) via the immunofluorescent antibody test (Artsob et al. 1993). Nine moose (23%; CI 12–38%) were seropositive to one or more serovars of *Leptospira* sp. with the microscopic agglutination test, with titers of 100–400 (Bolin 2008). Antibodies to

TABLE 1. Prevalence of antibodies to pathogens in 40 adult and yearling female moose captured in northwest North Dakota, USA, in 2014. Serum was submitted to the National Veterinary Services Laboratory in Ames, Iowa, USA, to test for antibodies to West Nile virus and eastern and western equine encephalitis viruses. Serum was also submitted to the University of Minnesota Veterinary Diagnostic Laboratory in St. Paul, Minnesota, USA, to test for antibodies to the other pathogens listed. No antibodies were detected to the following pathogens (test): *Brucella abortus* (Card Test), bluetongue virus (competitive enzyme linked immunosorbent assay; ELISA), infections bovine rhinotracheitis (serum neutralization), *Mycobacterium avian paratuberculosis* (ELISA), *Neospora caninum* (ELISA), and *Leptospira interrogans* serovars Canicola (modified agglutination test; MAT), Hardjo (MAT), and Pomona (MAT). Titers are given as the reciprocal of the final dilution when applicable.

Pathogen[a]	No. positive	% Prevalence	95% Confidence interval	Titer range[b]
WNV	39	98	87–100	10–100
ANA	22	55	40–69	NA
BBD	6	15	7–29	160–320
L ICT	8	20	11–35	100–400
L BRAT	1	2.5	0–13	200
L GRIP	1	2.5	0–13	100
EHDV	3	7.5	3–20	NA
BPIV3	3	7.5	3–20	20–40
BVDV1, BVDV2	2	5	1–17	16–128
EEEV, WEEV	1	2.5	0–13	10

[a] Pathogen, assay are as follows: WNV = West Nile virus, Plaque Reduction Neutralization Test (PRNT); ANA = *Anaplasma* spp., competitive ELISA; BBD = *Borrelia burgdorferi*, immunofluorescent antibody; L ICT = *L. interrogans* serovar Icterohaemorrhagiae, MAT; L BRAT = *L. interrogans* serovar Bratislava, MAT; L GRIP = *L. interrogans* serovar Grippotyphosa, MAT; EHDV = epizootic hemorrhagic disease virus, agar gel immunodiffusion; BPIV3 = bovine parainfluenza virus 3, hemagglutination inhibition; BVDV1 and BVDV2 = bovine viral diarrhea virus 1 and 2, serum neutralization; EEEV = eastern equine encephalitis virus, PRNT; WEEV = western equine encephalitis virus, PRNT.

[b] NA = not applicable.

the following pathogens were detected in three or fewer animals (<8%): eastern and western equine encephalitis viruses, epizootic hemorrhagic disease virus, bovine parainfluenza virus 3, and bovine viral diarrhea virus 1 and 2. No antibodies were detected to *Brucella abortus*, bluetongue virus, infectious bovine rhinotracheitis, *Mycobacterium avium* subsp. *paratuberculosis*, *Neospora caninum*, or *Leptospira interrogans* serovars Canicola, Hardjo, or Pomona.

Following its introduction into the US in 1999, WNV became an important cause of morbidity and mortality in humans, horses, and many species of birds (Murray et al. 2010). However, the importance of WNV in wild mammals remains poorly understood (Root and Bosco-Lauth 2019). Exposure to WNV is relatively common in moose in Minnesota, as 50% (115 of 330) hunter-harvested moose were found to be seropositive (Butler et al. 2010). By comparison, only 6% of white-tailed deer (*Odocoileus virginanus*) surveyed from several states across the US had antibodies to WNV (Pedersen et al. 2017). Human cases of WNV in North Dakota peaked in 2007, 2013, and 2018 (North Dakota Department of Health 2020). Our results indicate that moose sampled in spring 2014 accurately reflected the high WNV activity in 2013. Additional years of serology and a better understanding of antibody dynamics in this species will help to determine the full value of moose as an indicator of WNV activity.

Anaplasma marginale and *Anaplasma phagocytophilum* are recognized causes of disease in cattle and humans. Direct or serologic evidence of infection with ANA has been documented in many free-ranging ruminants, with few reports of clinical disease (Davidson and Goff 2000). In 2002–03, less than 2% of cattle exported from Montana, US, were seropositive for ANA (Van Donkersgoed et al. 2006). However, bovine anaplasmosis, caused by *A. marginale*, appears to be increasing in range and frequency, possibly attributable to movement of asymptomatic, persistently infected cattle, and changes in climate that favor tick hosts (Kocan et al. 2010).

White-tailed deer have been found to be poor sentinels of BBD; a low apparent antibody prevalence was found among deer heavily infested with BBD-infected *Ixodes scapularis* in Ontario (Gallivan et al. 1998).

Interpretation of BBD serology is complicated by weak cross-reaction with treponemes, leptospires, and other *Borrelia* spp. (Magnarelli et al. 1986). Two of the four moose with BBD titers ≥160 also had *Leptsospira* sp. titers of 100, while the two moose with BBD titers ≥320 did not react to *Leptospira* sp. The study area is on the edge of the known *I. scapularis* range, but this tick is expanding westward, and BBD-infected ticks were detected in Grand Forks County in northeast North Dakota in 2010 (Russart et al. 2014). Although cross-reactions cannot be ruled out, our results suggest that BBD and *I. scapularis* may be established in northwest North Dakota.

The *Leptospira* serology results are consistent with other reports showing common and widespread exposure in wildlife across the US (Pedersen et al. 2018). In Quebec, Canada, only one of 208 moose was antibody positive to *Leptospira* sp., while 12% of moose from Alaska, US, had antibodies (Bourque and Higgins 1984; Kocan et al. 1986). In our study, one moose had a titer of 400 to *Leptospira interrogans* serovar *icterohaemorrhagiae*, six had titers of 100 to serovar *icterohaemorrhagiae*, one had a titer of 200 to serovar *bratislava*, and one had titers of 100 to both serovars *grippotyphosa* and *L. icterohaemorrhagiae*. *Leptospira* sp. serology is confounded by extensive cross-reactivity among serovars and can be used as only a weak qualitative index of exposure (Leighton and Kuiken 2000). Thus, our results suggest that exposure to *Leptospira* sp. occurs in northwest North Dakota, but additional implications of this finding are unknown.

Serology is retrospective in nature and provides only a crude prediction of future exposure, particularly when only one timepoint is included in a study. It is important to note that moose were sampled in March 2014, approximately 6 mo after freezing temperatures begin to regularly occur in the region (National Oceanic and Atmospheric Administration 2010). Therefore, exposure to pathogens through vectors (WNV, ANA, and BBD) or contaminated surface water (*Leptospira*) likely occurred long before moose were sampled. Except for ANA, which latently infects erythrocytes, titers were probably nearing or falling below the threshold of detection. Thus, our findings may underrepresent the true levels of exposure in the moose population. Additionally, the assays used have not been validated in moose, so their true sensitive and specificity is unknown.

Regardless, these results indicate that several pathogens of concern to human and animal health are actively transmitted in northwest North Dakota. The annual survival in these moose was 97.5% during the 2-yr population study, and the calf:cow ratio was 93:100, which is consistent with an expanding population (Maskey and Smith 2017). For two radiocollared moose cows, one died of unknown causes, while the other drowned after falling through ice on a river. Thus, there is no current evidence suggesting overt population-level impacts resulting from the pathogens evaluated in our study at this level of exposure. However, such impacts are subject to co-stressors influenced by land use, species composition, and climate (Murray et al. 2006). As these factors are highly dynamic, so too is the potential impact of the selected pathogens on moose population health.

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