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BOVINE TUBERCULOSIS IN WHITE-TAILED DEER IN NORTHWEST MINNESOTA: A 7-YEAR EFFORT TO RESTORE MINNESOTA'S DISEASE-FREE ACCREDITATION

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SUMMARY OF FINDINGS

A total of 10,667 white-tailed deer (*Odocoileus virginianus*) were tested for bovine tuberculosis (bTB) in northwest Minnesota from 2005 to 2012. Fall 2012 marked the 7th consecutive year that the Minnesota Department of Natural Resources (MNDNR) conducted surveillance for this disease in deer since 2005, when bTB was first detected in a northwest cattle farm. The disease has since been found in a total of 12 cattle operations and 27 free-ranging white-tailed deer. Both deer and cattle had the same strain of bTB, which has been identified as one that is consistent with the disease found in cattle in the southwestern United States and Mexico. The Board of Animal Health (BAH) has been leading efforts to eradicate the disease in Minnesota's cattle, which have included the depopulation of all infected herds, a buy-out program that removed 6,200 cattle from the affected area, and mandatory fencing of stored feeds on remaining farms. No new infections have been detected in either cattle or deer since 2009. A total of 323 hunter-harvested deer were sampled in fall 2012, with no positive cases of bTB detected. This marked the 3rd consecutive year that MNDNR has conducted bTB surveillance in hunter-harvested deer in the bTB outbreak area and failed to find any new cases of the disease. The state regained its bTB-Free accreditation in October 2011; however, some testing requirements remained on cattle herds within the endemic area until the infection in deer could be determined as nonexistent. While MNDNR is unable to declare the local deer herd entirely disease-free, the cumulative years of intensive surveillance efforts aimed at bTB detection of prevalence >0.5% with 99% confidence, provided solid evidence that this disease is no longer within these detectable levels in the deer population. MNDNR has now suspended any futures efforts to monitor for bTB in the state.

INTRODUCTION

Bovine tuberculosis (bTB) is an infectious disease that is caused by the bacterium *Mycobacterium bovis*. Bovine tuberculosis primarily affects cattle; however, other mammals may become infected. The disease was first discovered in 5 cattle operations in northwest Minnesota in 2005. Since that time, 7 additional herds were found infected; resulting in a reduction of the state's bTB accreditation to Modified Accredited in early 2008. In fall 2008, Minnesota was granted a split-state status for bTB accreditation that maintained only a small area (2,670mi^2) in northwest Minnesota as "Modified Accredited," allowing the remainder of the state to advance to "Modified Accredited Advanced." In total, 27 wild deer have been found infected with the disease in northwest Minnesota, which can be attributed to a spillover of the disease from infected cattle. In 2010, The United States Department of Agriculture (USDA) upgraded Minnesota's bTB accreditation to Modified Accredited Advanced within the split-state zone and bTB-Free throughout the remainder of the state. With no new infections discovered in MN cattle in 2009 and 2010, USDA upgraded the split-state portion to bTB-Free in October 2011. Although bTB was once relatively common in U.S cattle, it has historically been a very rare disease in wild deer. Prior to 1994, only 8 wild white-tailed and mule deer (*O. hemionus)* had been reported with bTB in North America. In 1995, bTB was detected in wild deer in Michigan and do serve as a reservoir of the disease in that state.

Bovine tuberculosis is a progressive, chronic disease. It is spread primarily through the exchange of respiratory secretions between infected and uninfected animals. This transmission usually happens when animals are in close contact with each other. Animals may also become infected with bTB by ingesting the bacteria from contaminated feed. Incubation periods

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can vary from months to years from time of infection to the development of clinical signs. The lymph nodes in the animal's head usually show infection first and as the disease progresses, lesions (yellow or tan, pea-sized nodules) will begin to develop throughout the thoracic cavity. In severely infected deer, lesions can usually be found throughout the animal's entire body.

Hunters do not always readily recognize small lesions in deer, as they may not be visible when field dressing deer. In fact, most infected deer appear healthy. While it is possible to transmit bTB from animals to people, the likelihood is extremely low. Most human tuberculosis is caused by the bacteria *M. tuberculosis*, which is spread from person to person and rarely infects animals.

METHODS

During falls 2005–2012 a hunter-harvested surveillance strategy was developed to collect samples from the bTB Management Zone, which is approximated by Deer Management Area (DMA) 101. Sampling goals varied by year, dependent on frequency of new infections as well as existing Memorandums of Understanding (MOU) with United States Department of Agriculture, signed by both MNDNR and BAH. To that end, MNDNR was committed to ensuring the disease is not present in wild deer within the bTB Management Zone at >1.0% with 99% confidence.

At the registration stations, hunters were asked to voluntarily submit lymph node (LN) samples for bTB testing. Hunter information was recorded, including the hunter's name, telephone number, MNDNR number, and location of kill. Maps were provided to assist the hunters in identifying the location (Township, Range, Section, and Quarter-section) of the kill. Cooperating hunters were given a cooperator's patch and entered into a raffle for a firearm donated by the Minnesota Deer Hunter's Association (MDHA). In addition, the Roseau River chapter of MDHA raffled additional firearms and a life-time deer hunting license for hunters that submitted samples from within the bTB Management Zone or bTB Core Area.

Sampling procedures included a visual inspection of the chest cavity of the hunter-killed deer. Three pairs of cranial LNs (parotid, submandibular, and medial retropharyngeal) were visually inspected for presence of gross lesions and collected for further testing. Samples were submitted to the Veterinary Diagnostic Laboratory (VDL) at the University of Minnesota for histological examination and acid-fast staining (when lesions are present only). All samples were then pooled in groups of 5 and sent to the National Veterinary Services Laboratories (NVSL) in Ames, IA for culture. Any suspect carcasses (e.g., obvious lesions in chest cavity or head) were voluntarily surrendered at the registration stations and the hunter was issued a replacement deer license at no charge. Suspect carcasses were transported in their entirety to the VDL for further testing.

Additionally, MNDNR implemented efforts to further reduce deer numbers in the post-hunting season in the bovine 140-mi_2 TB-infected Core Area, through the use of ground sharpshooters (USDA-Wildlife Services). During Feb–April of 2007–2010, sharpshooter-harvested deer were transported intact to a central processing facility at Thief Lake Wildlife Management Area. Sample collection and handling was similar to that described above. Carcasses were salvaged for venison and available to the public. The ground sharpshooting effort was augmented with the addition of aerial gunning during winters 2008 and 2009.

In early winter of 2007–2012, MNDNR conducted an aerial survey of the bTB Core Area to assess deer numbers and distribution. This information was used to guide future management activities and estimate the percentage of deer removed from the area through hunting and agency culling.

RESULTS AND DISCUSSION

In 2005, Minnesota began a 7-year effort to attempt to eradicate bTB from the state's cattle and free-ranging deer in the northwest corner of the state. Minnesota DNR, having

learned valuable lessons from Michigan's bTB outbreak, adopted an aggressive surveillance and management effort to prevent an establishment of a wildlife reservoir (Carstensen and Doncarlos 2011). To date, our efforts appear successful and the "Minnesota Model" of bTB response as received both national and international attention.

From 2005 to 2012, a total of 10,667 white-tailed deer were tested for bTB in northwest Minnesota, including hunter-harvested (*n* = 7,839), sharpshooting (*n* = 2,613), and landowner shooting permits (*n* = 215) (Table 1). There have been a total of 27 confirmed bTB-positive deer, all within a limited geographic area that extended in a 10-mile radius from Skime, MN (Fig 1). In fall 2012, MNDNR collected 323 samples from hunter-harvested deer in DPA 101 (including deer tested just outside this area). Testing of all lymph node samples at NVSL has confirmed that there were no positive cases of bTB, marking 2012 as the 3rd consecutive year that no new cases of bTB have been detected in wild deer. Apparent prevalence of bTB in the local deer population, sampled throughout a 1,730 to 2,670mi^2 Surveillance Zone, indicates a significant decreasing trend from 2006–2012 (Table 1, Figure 2). Further, disease prevalence in the bTB Core Area has decreased dramatically from 2007 to 2010 (Table 1, Figure 2). Although disease prevalence estimates in the TB Core Area are biased due to the limited geographic distribution of bTB-positive deer and the increased probability of detecting a positive individual, the decreasing trend is consistent with the large-scale surveillance of the local deer population.

Aerial survey results from 2007 to 2012 demonstrated an overall decline in the deer population in the bTB Core Area (Table 2, Figure 3). This was expected, given the intensive removal efforts that occurred through ground and aerial sharpshooting; however, it was surprising that it took 4 consecutive years (2007 to 2010) of these removal operations to detect a significant difference in deer numbers (55% decline from the 2007 estimate) (Figure 3). Also of interest, is that more deer were shot in the bTB Core Area during winter removal efforts than counted in both 2008 and 2009 aerial surveys (Tables 1 and 2). There are a couple reasons for this apparent discrepancy. First, the aerial survey occurs at one point in time, typically at the end of January or beginning of February, prior to the start of the deer removal operations in mid-late February that occurred over 8-10 weeks. Second, it is likely that the bTB Core Area is home to both migratory and resident deer, some of which may move out of the zone to spring-summer-fall or winter ranges during the year. It is further likely that deer from the surrounding area are immigrating into the bTB Core Area as deer numbers are reduced and habitat availability increases. In a pilot study involving radio-collared deer south of the bTB Management Zone, mean home range size for deer (*n* = 9) surviving through the end of the study was 46.7 km^2 (SE = ±10.1); 7 of these deer were migratory, traveling 4–20 km to distinct winter ranges over 2-3 day periods (Carstensen et al. 2011). This fluid environment within the bTB Core Area suggests deer move in and out of this zone relative to season, food resources, and predator pressure. Thus, a deer survey that generates a point estimate of deer abundance at one point in time only cannot account for these changes that occur over time. Deer are also very prolific and mild winters, in combination with abundant food resources (natural or artificial) will grow a herd rapidly. We have demonstrated that continued pressure on a deer herd is needed to reduce overall deer densities and yield a significant long-term reduction in the local population.

Another interesting question is whether or not the intensive deer removal efforts in DPA 101 affected hunter effort and success? While MNDNR-sponsored removal efforts (sharpshooting and aerial gunning) were very effective at reducing deer numbers and targeting bTB-infected individuals, it was also expensive ($390 and $960 per deer, respectively) and unpopular with the local residents. Eventhough we were told by local hunters that many would discontinue hunting in DPA 101 as a result of unpopular bTB management efforts, hunting number remained stable from 2007 to 2011, at >1,500 hunters/year (Figure 4). Total harvest and hunter success rates did decline after 2007, which suggests less deer were available to hunters in DPA 101 as a result of the intensive bTB management efforts.

Recent work being conducted at NVSL on genetic isolates from bTB-positive deer and cattle is helping to further explain Minnesota's outbreak. The proximity of the bTB-infected deer

to infected cattle herds, the strain type, and the fact that disease prevalence (<0.1%) was low, supports our theory that this disease spilled-over from cattle to wild deer in this area of the state. Further, the lack of infected yearlings or fawns and limited geographic distribution of infected adults further supports that deer are not a wildlife reservoir for this disease in Minnesota (Carstensen and DonCarlos, 2011). MNDNR is currently working with BAH, USDA, and NVSL on 2 manuscripts that detail the epidemiology of the outbreak in the northwest and magnitude of the response that was necessary to regain the state's TB-Free accreditation within 7 years.

With the recent upgrade in status to bTB-Free across the state and a lack of available funding to continue support payments to farms that participated in the buy-out program, BAH allowed farms to repopulate with cattle within the bTB Management Zone beginning July 1, 2012. Now that MNDNR completed its 3rd consecutive year with no new cases of the disease detected in deer and announced the suspension of further surveillance efforts, BAH discontinued whole-herd testing within the bTB Management Zone.

ACKNOWLEDGMENTS

There is no way to complete a project of this scale without the assistance and leadership from St. Paul and regional staff (some recently retired), including Ed Boggess, Mike DonCarlos, Dennis Simon, Paul Telander, and John Williams. For all the help with field collections, we'd like to thank area staff from Thief Lake, Red Lake, Roseau River, and Thief River Falls, as well as students and faculty from the University of Minnesota, College of Veterinary Medicine. Also thanks to Bob Wright and John Heineman for conducting a deer survey within the bTB Core Area. Also thanks to John Giudice for analyzing the survey data. GIS support was provided by Julie Adams, Bob Wright, and Chris Scharenbroich. Lastly, we thank USDA-Wildlife Services for their excellent work during sharpshooting operations.

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Table 1. Number of deer sampled for bovine tuberculosis (bTB) and testing results listed by sampling strategy, 2005 to 2012, northwestern Minnesota.

Sampling strategy	2005	2006	2007	2008	2009	2010	2011	2012	Totals
Hunter-harvested (Oct-Jan)	474	942	1,166	1,246	1,488	1,639	561	323	7,839
# bTB-positive	1	5	5	0	1	0	0	0	
Apparent prevalence	0.21%	0.53%	0.43%	0.0%	0.07%	0.0%	0.0%	0.0%	
Sharpshooting (Feb-May)	n/a	n/a	488	937	738	450	n/a	n/a	2,613
# bTB-positive			6	6	2	0			
Apparent prevelance			1.23%	0.64%	0.27%	0.0%			
Landowner/tenant	n/a	90	n/a	125	n/a	n/a	n/a	n/a	215
# bTB-positive		1		0					
Total deer tested	474	1,032	1,654	2,308	2,226	2,089	561	323	10,667
Total # bTB-positive	1	6	11	6	3	0	0	0	27

Table 2. Population estimates[a] and 95% confidence intervals[b] of deer within the Bovine Tuberculosis Core Area, 2007–2012, northwest Minnesota.

Year	Aircraft	Design	Var.est	n	N	Srate	Svar	SE	Xbar	SE	95%CI	**PopEst**	SE	**95% CI**	CV(%)	RP(%)
2007	OH-58	StRS3	SRS	72	164	0.439	NA	NA	5.7	0.46	4.9-6.5	**935**	76.0	**784-1086**	8.1	16.2
2008	OH-58	GRTS.SRS	Local	72	164	0.439	21.94	4.53	4.9	0.56	3.8-6.0	**807**	75.2	**659-954**	9.3	18.3
2009	Enstrom	GRTS.stRS2	Local	79	164	0.482	20.63	2.56	4.1	0.27	3.5-4.6	**664**	44.4	**577-751**	6.7	13.1
2010	OH-58	GRTS.SRS	Local	72	164	0.439	29.30	6.70	2.6	0.39	1.8-3.3	**422**	64.4	**296-548**	15.3	30.0
2011	OH-58	GRTS.SRS	Local	72	164	0.439	21.01	2.80	3.2	0.30	2.7-3.8	**531**	48.6	**436-627**	9.2	18.0
2012	OH-58	GRTS.SRS	Local	72	164	0.439	3.06	0.57	1.0	0.14	0.7-1.3	**160**	22.3	**120-210**	13.6	26.7

[a]**Population estimate = estimated *minimum* number of deer present during the sampling interval.** Estimates are not adjusted for detectability (but intensive survey is designed to minimize visibility bias) and deer movement between sample plots is assumed to be minimal or accounted for via survey software.
[b]**95%CI's** based on sampling variance only (adjusted for spatial correlation in 2008-2011); they do not include uncertainty associated with detectabilty or animal movements (temporal variation due to animals moving onto or off the study area).

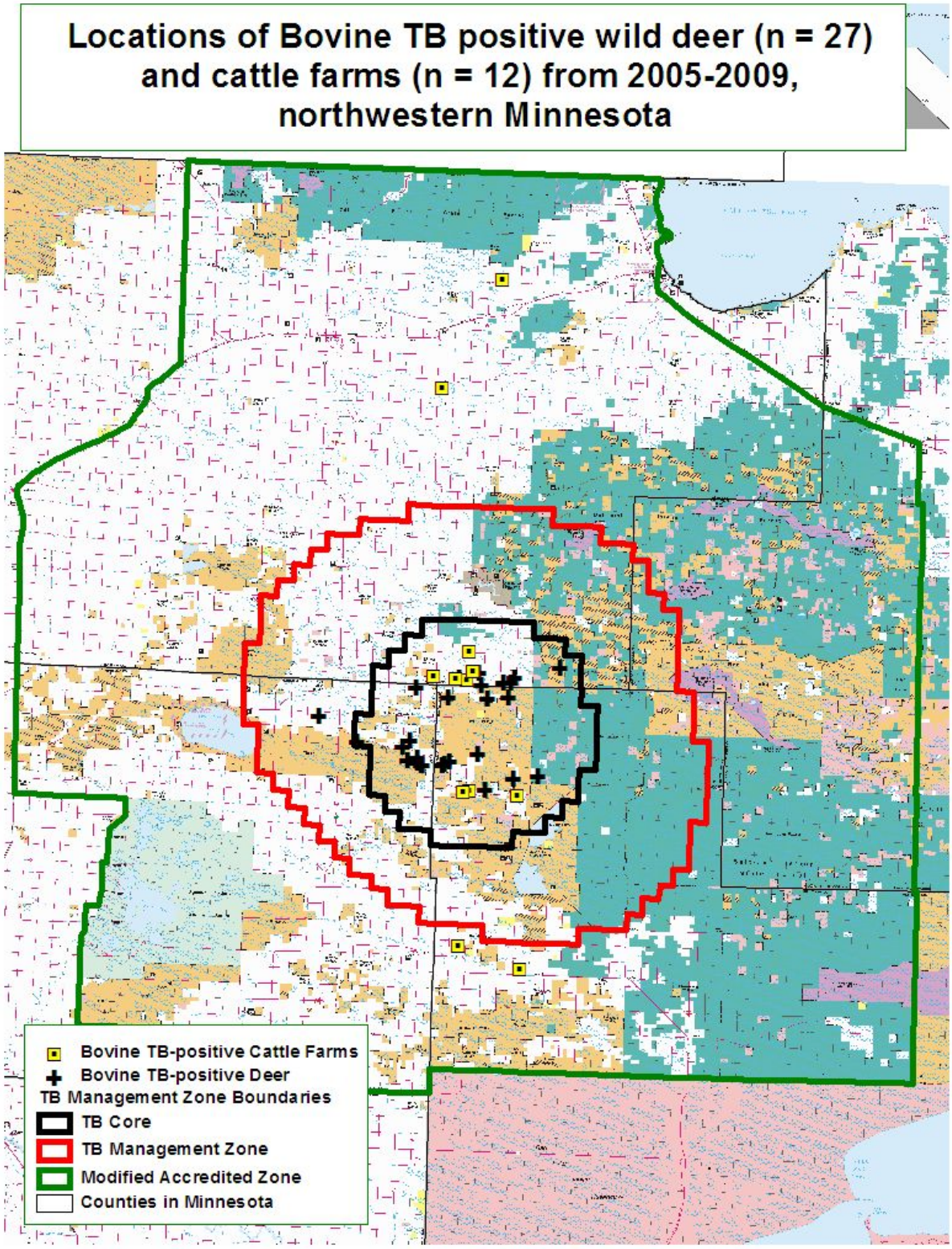
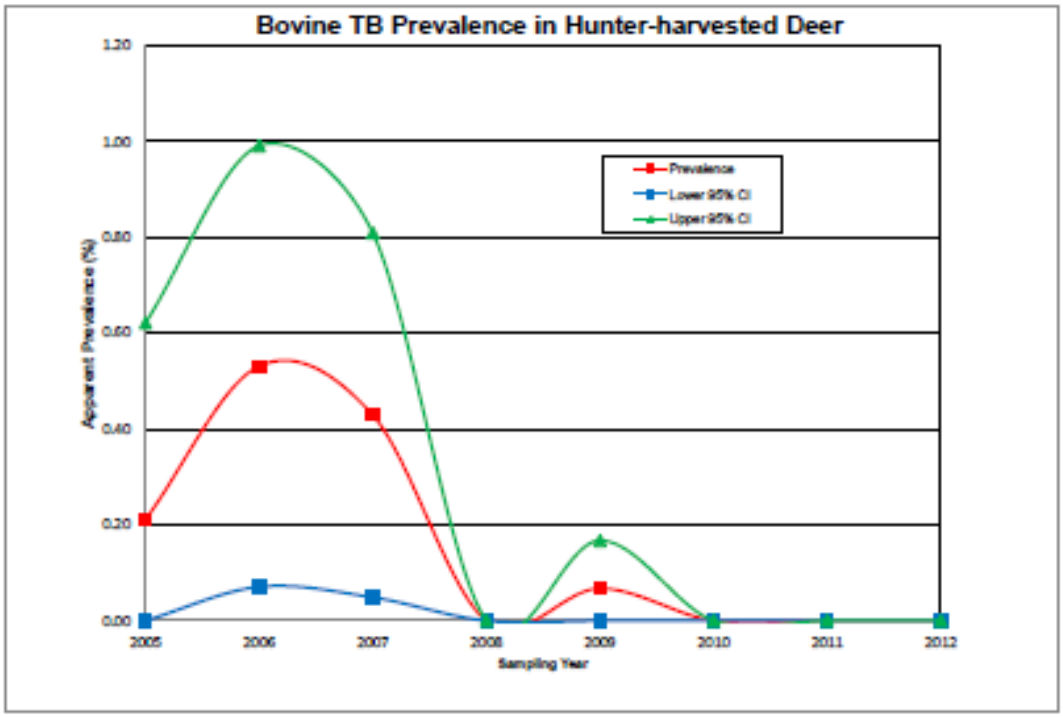
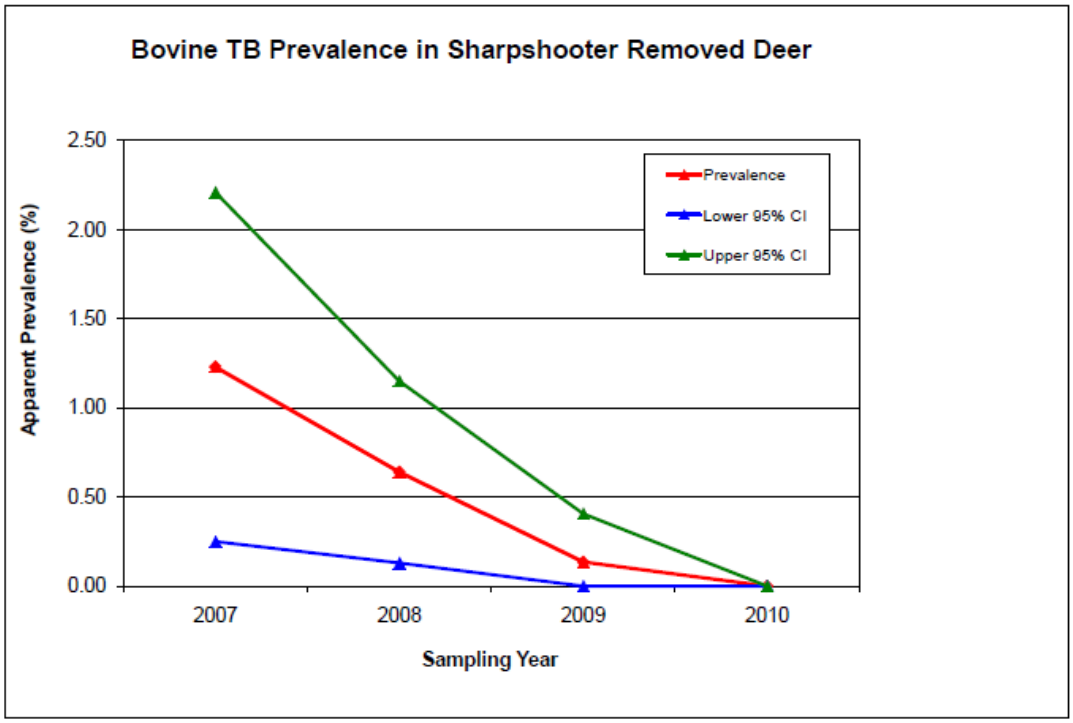


Figure 1. Locations of all white-tailed deer found infected (*n* = 27) with bovine tuberculosis (bTB) since fall 2005 in northwest Minnesota, with the 12 previously-infected cattle operations are also included.



a)



b)

Figure 2. Prevalence of bovine tuberculosis (bTB) in hunter-harvested deer from 2005–2012 in the bTB Surveillance Zone (a) and disease prevalence from sharpshooter removed deer from 2007–2010 in the bTB Core Area (b), northwest Minnesota.

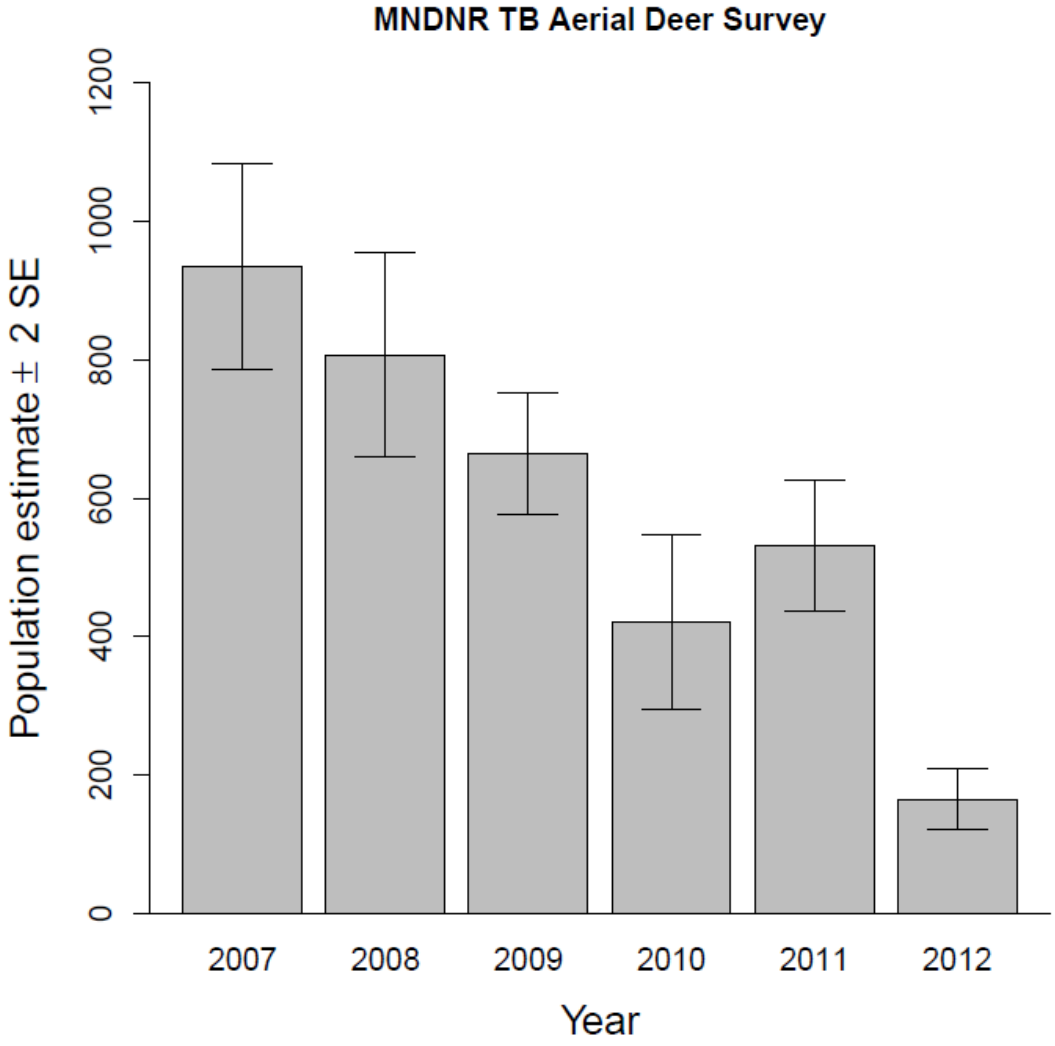


Figure 3. Population estimate of deer within the Bovine Tuberculosis Core Area, winters 2007–2012, northwest Minnesota.

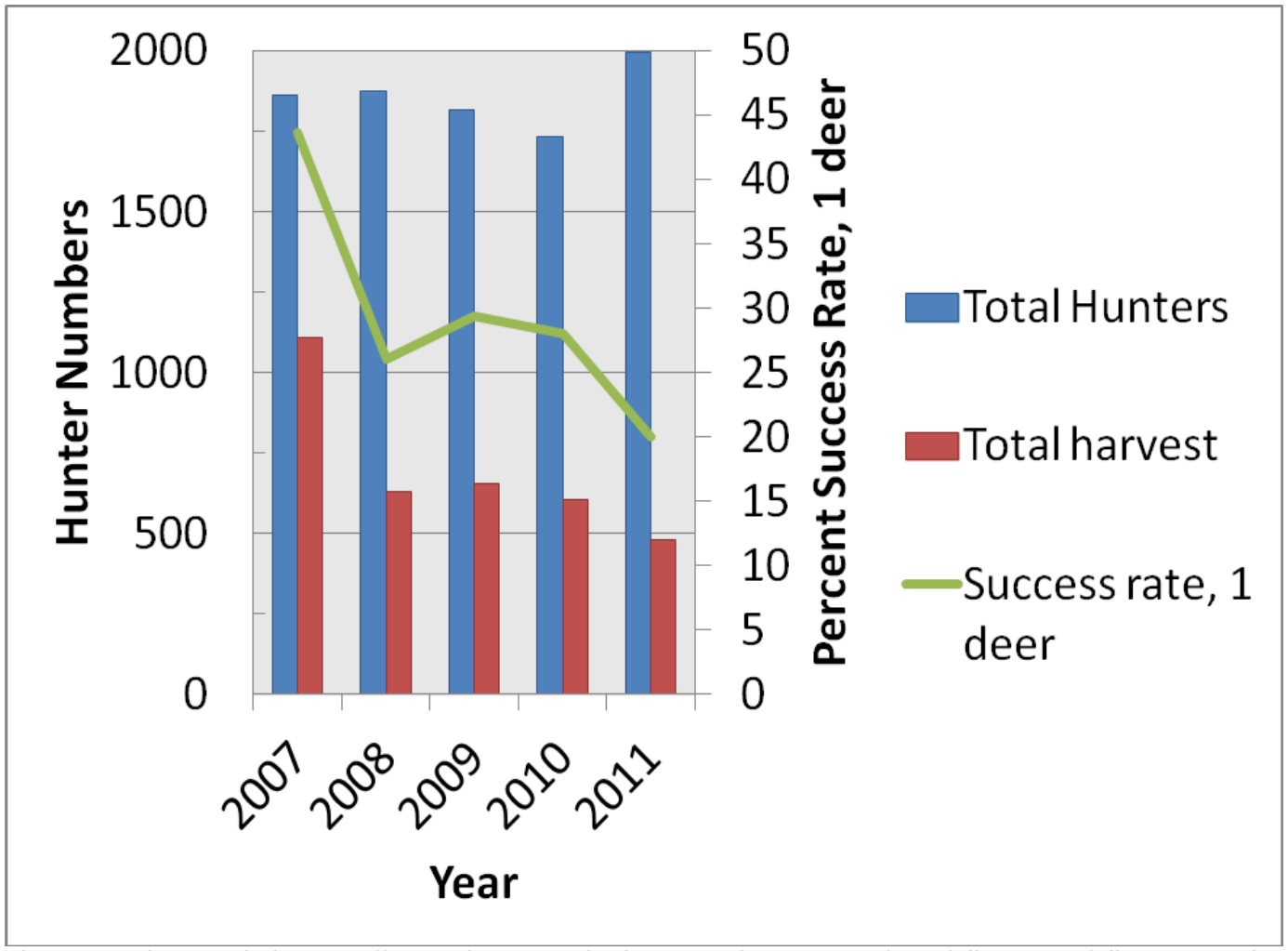


Figure 4. Changes in hunter effort and success in deer permit area 101, from fall 2007 to fall 2011, northwest Minnesota.

NORTHEAST MINNESOTA MOOSE HERD HEALTH ASSESSMENT 2007–2012

Erika A. Butler, Michelle Carstensen, Erik C. Hildebrand, and John H. Giudice

SUMMARY OF FINDINGS

This project, which began in 2007, represents the second phase (2010–2012) of an overall health assessment of hunter-harvested moose (*Alces alces*) in northeastern Minnesota. The objectives of this project were to: (1) screen hunter-harvested (and presumably healthy) moose for select disease agents and combine results from phase 1 of the project (2007-2009) to asses if there was any spatial or temporal variation in prevalence rates, (3) assess the clinical impacts of liver fluke (*Fascioloides magna*) infection on moose, and (4) determine the frequency of histological lesions consistent with brainworm (*Parelaphostrongylus tenuis*) infection. Samples were collected from 643 moose during 2007-2012 (average annual *n* = 107; range = 63-131). Moose were screened for West Nile virus, eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, malignant catarrhal fever, borreliosis (*Borrelia burgdorferi*), anaplasmosis (*Anaplasma phagocytopila*, formerly *Ehrlichia phagocytophila*) and 6 serovars of leptospirosis. There was evidence of exposure to West Nile Virus (30.1%), eastern equine encephalitis (4.1%) malignant catarrhal fever (23.3%), borreliosis (21.9%), and leptospirosis (0.5-6.4%). Portions of brain, cerebral spinal fluid, whole blood, and serum were submitted for polymerase chain reaction (PCR) for Flavivirus ribonucleic acid (RNA) (2011 only). Whole livers and brains were examined grossly and histologically for evidence of brainworm and liver fluke infections; both parasites were documented. Full serum chemistry profiles were conducted on 211 moose and will be used to determine if a correlation exists between liver fluke damage and serum liver enzymes. Whole blood samples from 217 moose were submitted for evaluation for tick-borne illnesses; anaplasmosis and piroplasma infections were also documented.

INTRODUCTION

Aerial survey data indicate a declining moose population in northeastern MN (DelGiudice 2013) and annual survival and reproductive rates during 2002-2007 were substantially lower than documented elsewhere in North America (Lenarz et al. 2007). Estimated at 2,760, the 2013 moose population estimate is significantly lower (35%) than the 2012 estimate and time series analysis of estimates since 2005 indicate a significant downward trend (DelGiudice 2013). Previous and ongoing research has been unable to determine proximate and ultimate cause(s) of non-hunting moose mortality and the possible related impacts to the long-term viability of the northeastern MN population. In 2007, the MN Department of Natural Resources (MNDNR) began a 3-year moose health assessment project to determine which diseases moose are being exposed to in northeastern MN and to establish baseline hepatic mineral levels. We believed that hunter-harvested moose would represent “healthy” animals; thus, data from these animals could be compared to known sick moose to increase our understanding of what might be considered “normal” for northeastern moose. We found that hunter-harvested moose in northeastern MN had been exposed to a variety of disease agents such as West Nile virus (WNV), eastern equine encephalitis (EEE), malignant catarrhal fever (MCF), anaplasmosis, borreliosis, and leptospirosis (Butler et al. 2010). While these findings were illuminating, additional research was needed to determine (1) the role liver damage (due to liver flukes) plays in non-hunting mortality, 2) the impact of arboviruses and how their prevalence may vary temporally, and (3) the impact of brainworm on moose survival, due to the difficulty in interpreting brain lesions caused by this parasite. The second phase of the moose health assessment project (started in 2010) will help to address these questions.

Murray et al. (2006) concluded that moose in northwestern MN were dying from high liver fluke loads. However, assessing the extent of liver damage caused by flukes can be subjective. In order to determine if liver damage caused by flukes has clinical implications, serum liver enzymes should be evaluated. Beginning in 2009, we asked hunters to collect

whole livers for evaluation and they were ranked for liver fluke loads by a board-certified veterinary pathologist. In 2009, the first year of liver examinations, we found that 35% of livers had fluke-induced lesions with some having nearly 100% of the liver parenchyma affected (Butler et al. 2010). However, poor blood- collection techniques prevented assessment of the clinical impacts of the damage caused by the liver fluke infections. In 2010 we asked hunters to alter their blood collection strategies and began collecting the whole liver and assessing serum liver enzymes, with the goal of determining whether results of gross evaluation of the liver correlated with enzyme indicators of liver function.

Our moose health assessment during 2007–2009 indicated that moose are being exposed to a variety of arboviruses, including EEE, WNV, borreliosis, and anaplasmosis (Butler et al. 2010). As climate changes, the density and distribution of capable arthropod vectors is expected to change as well (Gould and Higgs 2009). Climate is known to play a key role in determining the geographical and temporal distribution of arthropods, characteristics of arthropod lifecycles, dispersal patterns of associated arboviruses, evolution of arboviruses, and the efficiency with which they are transmitted from arthropods to vertebrate hosts (Gould and Higgs 2009). For example, there has been a substantial increase in tick-borne encephalitis in Sweden since the mid-1980s related to milder winters and earlier arrival of spring (Lindgren and Gustafson 2001). In Phase 2 of the moose health assessment study, serum was screened for these arboviruses and a few additional disease agents. Combined with results from our 2007–2009 sampling, we have 6 years of data on the incidence of arbovirus exposure in our moose herd to evaluate temporal variation in prevalence. Additionally, in 2011 only, we screened moose for western equine encephalitis (WEE) and St. Louis encephalitis (SLE).

Diagnostics have shown that moose are dying from brainworm in MN. It is also known that moose are able to survive low-dose infections of brainworm and even develop immunity to subsequent infections (Lankester 2002). Researchers have hypothesized that brainworm was responsible for historic declines in moose populations (Karns 1967, Prescott 1974, Lankester 1987), but it is questionable whether brainworm represents a major threat to the northeastern MN population. In 2008, we began collecting whole brains from hunter-harvested moose to determine the frequency of brain lesions consistent with past brainworm infections in presumably healthy moose. These data would allow for better interpretation of migration tracts and could prevent pathologists from wrongly assigning brainworm as the cause of death based solely on the presence of migration tracts. We continue to collect whole brains to increase our sample size and to quantify the number of presumably healthy moose that have parasitic migration tracts.

METHODS

Hunters (tribal and state) were asked to collect whole livers, blood, hair, and a central incisor from their harvested moose. State hunters were only allowed to harvest bulls, whereas some tribal hunters were able to take either bulls or cows. Wildlife Health Program staff provided a presentation and instructions on the moose health assessment project at the mandatory MNDNR Moose Hunt Orientation sessions and at tribal natural resource offices. Hunters were given a sampling kit at the sessions and were asked to drop off completed sampling kits at official registration stations at the time of moose registration. Sampling kits included a cooler, 1-60-cc syringe for blood collection, 6-15-cc serum separator tubes, 2-5-cc ethylenediaminetetraacetic acid (EDTA) blood tubes for whole blood collection, 1 heavy-duty bag for liver storage, 2 coin envelopes for the tooth and hair collected, data sheet, protocol, Sharpie marker, 1 pair of large vinyl gloves, and 1 icepack. Hunters collected blood using the 60-cc syringe after incising the jugular vein as soon after death as possible and recorded time of death and blood collection. Blood was placed in serum-separator tubes and in an EDTA tube and kept cool until they were delivered to official MNDNR registration stations or tribal natural resource offices. Livers were placed in heavy-duty, pre-labeled bags.

At the registration stations or tribal offices, serum-separator tubes were centrifuged and the serum decanted. Blood spinning time was recorded. Portable refrigerators were located in

advance at the registration stations to maintain the tissue samples. One whole blood sample (EDTA tube) and 1 mL of serum were refrigerated and submitted every 2-3 days to the University of MN (UMN)-College of Veterinary Medicine-Clinical Pathology Laboratory for a full large-animal serum chemistry profile. The remaining whole blood sample was submitted every 2-3 days to the UMN-Department of Entomology for testing for tick-borne illnesses. Remaining serum and the whole livers were frozen. Cerebral spinal fluid was collected when possible (2011 only). Whole brains were removed with the hunter's permission and placed in formalin. A 1x1x1" piece of brain was removed and frozen (2011 only). The serum, whole liver, and whole brains were submitted to the UMN Veterinary Diagnostic Laboratory (UMN VDL, St. Paul, MN). The 1x1x1" piece of brain, cerebral spinal fluid, whole blood, and 1 mL of serum were submitted to the Minnesota Department of Health (MDH) for PCR for Flavivirus RNA (2011 only). Serum samples were also submitted to the National Veterinary Services Laboratory (NVSL) in Ames, Iowa.

Serum was tested for WNV, EEE, and WEE with a plaque reduction neutralization test (PRNT) and SLE with a serum neutralization test at NVSL. Serum was screened for leptospirosis (microscopic agglutination test), borreliosis (immunofluorescence assay), anaplasmosis (card test), and MCF via peroxidase-linked assay (PLA) with positive PLA tests further tested with a virus neutralization test (VN) at the UMN VDL. The livers were ranked by a board-certified veterinary pathologist based on parenchymal damage due to liver flukes; ranking included no fluke-induced lesions (no evidence of fluke migration), mild infection (<15% of liver parenchyma is affected with mild prominence/fibrosis of bile ducts and few smaller nodules characterized by peripheral fibrosis and central presence of opaque brown pasty material), moderate infection (15-50% of the liver parenchyma affected by nodules and fibrosis), and marked infection (51-100% of the liver parenchyma affected with deformation of the entire liver by larger nodules with widespread fibrosis). Brains were examined histologically with 4 complete coronary brain, cerebellum, and brain stem sections processed from each moose. An average of 25 histological slides per animal were examined, including the frontal, temporal, parietal, and occipital lobes and the basal nuclei, thalamus, mesencephalon, and brain stem. Central incisors of moose were submitted to Mattson's Laboratory (Milltown, Montana) for aging by cementum annuli (Sergeant and Pimlott 1959).

RESULTS AND DISCUSSION

We obtained samples from 643 hunter-harvested moose during 2007-2012 and the samples were well distributed throughout moose hunting zones (Figure 1.). The average age of hunter-harvested moose during 2007-2011 (results from 2012 are pending) was 4.4 years (n = 533, median = 4, range: 0.5-14; Fig. 2).

Eastern Equine Encephalitis

Evidence of exposure to EEE was detected in 23/557 (4%, 95% CI: 3-6%) moose sampled from 2007–2012 (Figure 3). Due to the small number of positive animals in each year and county, we were unable to accurately estimate the magnitude of spatial or temporal variation in EEE prevalence rates (other than to conclude that they were low).

A total of 65 moose were sampled (frozen brain, cerebral spinal fluid, serum, and whole blood) by the MDH by PCR for evidence of any Flavivirus RNA in 2011. Positive results would indicate that the moose actually had virus present in the tissues sampled. All results were negative.

Mosquitoes spread EEE, which can cause neurologic signs and often death. It poses a greater mortality threat for most species than WNV, although the effects of EEE infection have not been studied in moose. A titer that is greater than 100 is considered a very strong positive and means the serum was able to neutralize nearly 100% of the virus. Titers >100 were observed in 9% of positive samples.

West Nile Virus

Evidence of exposure to WNV was detected in 171/557 (30%, 95% CI: 27-35%) moose sampled from 2007–2012 (Figure 4). There appeared to be an increase in prevalence in adult moose from east to west (Cook County: 27%, CI: 21-35%; Lake County: 39%, 95% CI: 32-48%; St. Louis County: 53.1%, CI: 41.3-68.2%). There also was some evidence (z = -2.099, p =0.035) that the log odds of exposure in adult moose was lower in 2011 than in 2007-2010 ($\widehat{\boldsymbol{\beta}}$ = -0.563, 95% CI: -1.177 to -0.084), although the biological significance of estimated differences in exposure rates is unknown (e.g., $\widehat{\boldsymbol{p}}$ = 0.156 vs. 0.274 in Cook county). Furthermore, this model was fit after exploring the data and, therefore, estimates of effect sizes are likely optimistic (i.e., exploratory models tend to overfit the data and predict poorly on new data). Finally, positive serological results indicate that animals were exposed to the WNV, but it does not necessarily indicate illness. Similar to EEE, a titer that is greater than 100 is considered a very strong positive. Titers of 100 or greater were found in 5% of positive samples.

Western Equine Encephalitis and St. Louis Encephalitis

Of the 64 sera samples submitted for WEE and SLE testing (2011 only), none tested positive. Both of these diseases are mosquito-borne. Western equine encephalitis is known to occur infrequently in MN, although when it does, it is often part of a regional outbreak. Testing was performed at the suggestion of the Minnesota Department of Health as part of our collaborative project investigating arboviral prevalence rates in Minnesota's wildlife.

Malignant Catarrhal Fever Virus

Evidence of exposure to MCF was detected in 129/553 (23.3%, CI: 19.9-27.1%) moose sampled from 2007–2012. Follow-up testing with VN was negative for 110/129, 18 were unsuitable for testing, and one was weakly positive (likely a false positive). The PLA test is more sensitive than VN, meaning it is much better at identifying true positives, whereas VN is more specific and thus better at identifying true negatives. Malignant Catarrhal Fever is a gammaherpes virus, of which there are multiple strains (e.g., wildebeest strain of MCF, sheep strain of MCF, deer strain of MCF). The PLA reacts with multiple gammaherpes viruses. A PLA positive does not indicate the strain of exposure. The VN test only screens for the wildebeest strain (which is exotic to the U.S.) and would be negative if other strains are present. This means a sample that was positive on PLA and negative on VN was likely exposed to MCF, but not the wildebeest strain. There were some large differences in estimated probability of MCF exposure in adults by year (e.g., $\widehat{\boldsymbol{p}}$ 4% in 2007 vs. 72%in 2008), but we found no evidence that probability of exposure varied significantly by location or age.

Gammaherpes viruses have been documented to cause serious illness and death in moose and other ruminants. The clinical symptoms can mimic brainworm infection, including neurological deficits, blindness, and thrashing on the ground prior to death. While infection with MCF frequently results in death, carrier status can occur and is identified with serology. Zarnke et al. (2002) found serologic evidence of exposure in numerous species across Alaska and reported 1% prevalence in moose.

Anaplasmosis

Evidence of exposure to anaplasmosis was detected in 1/426 (0.2%, CI: 0-2%) moose sampled from 2007–2010 (testing was no longer available in 2011). Results indicate that exposure to this bacterium is likely occurring, albeit at a very low rate.

Moose are thought to be susceptible to infection with *A. phagocytophilum*. In Norway, anaplasmosis was diagnosed in a moose calf, which displayed apathy and paralysis of the hind-quarters (Jenkins et al. 2001). This moose was concurrently infected with *Klebseilla* pneumonia, to which the calf's death was attributed, though the *Klebseilla* infection was most likely secondary to and facilitated by the primary infection with *A. phagocytophilum*. In sheep, this disease produces significant effects on the immunological defense system, increasing their susceptibility to disease and secondary infections (Larsen et al. 1994).

Borreliosis

Evidence of exposure to borreliosis was detected in 120/546 (22%, CI: 19-26%) moose sampled from 2007–2012. There were some large differences in estimated probability of borreliosis exposure in adults by year (e.g., 2% in 2008 vs. 30% in 2010), but again we found no evidence that probability of exposure varied significantly by location or age.

Borreliosis is a tick-borne bacterial disease that is maintained in a wildlife/tick cycle involving a variety of species, including mammals and birds. While evidence of natural infection in wildlife exists, there has been no documentation of clinical disease or lesions reported in wildlife species.

Leptospirosis

A total of 559 samples were screened for 6 serovars of *Leptospira interrogans*. Results per serovar are as follows:

- *L. interrogans bratislava*:
  - 7/559 (1.3%, CI: 0.5-2.6%)
- *L. interrogans canicola*:
  - 3/559 (0.5%, CI 0.1-1.7%)
- *L. interrogans grippothyphosa*:
  - 9/559 (1.6%, CI: 0.8-3.1%)
- *L. interrogans hardjo*:
  - 3/559 (0.5%, CI: 0.1-1.7%)
- *L. interrogans icterohaemorrhagicae*:
  - 24/559 (4.3%, CI: 2.8-6.4%)
- *L. interrogans pomona*:
  - 36/559 (6.4%, CI: 4.7-8.8%)

We found no evidence of higher exposure rates among adults vs. juveniles and no evidence of a year effect on prevalence rates. We did find a significant ($p < 0.05$) positive correlation between infection with *L. interrogans pomona* and *L. interrogans grippothyphosa* ($\varphi_2 = 0.37$, 95% CI: 0.18-0.53) and *L. interrogans hardjo* and *L. interrogans icterohaemorrhagicae* ($\varphi_2 = 0.34$, 95% CI: 0.19-.051).

Leptospirosis is a bacterial disease that can infect a wide variety of mammals, both domestic and wild. Moose could be at an increased risk for leptospirosis, as it is often propagated by mud and water contaminated with urine, not uncommon in moose habitat.

General Tick-Borne Illness Screening

Whole blood samples from 217 (*n* = 109, 59, 49 in 2010, 2011, and 2012, respectively) moose were submitted to the UMN Department of Entomology, where we are collaborating with Dr. Ulrike Munderloh to determine if hunter-harvested moose are infected with tick-borne illnesses. Samples were screened with a variety of PCR techniques. Results, only available for the 2010 samples, indicate that 10% of the moose were infected with anaplasmosis and 32% were positive for prioplasma primers. A hemolytic *Mycoplasma* was also identified in 19 of the samples. Further analysis is pending.

Brain Histopathology

Whole brains from 151 moose were collected since 2008 (*n* =23, 24, 40, 31, and 33 in 2008, 2009, 2010, 2011, and 2012, respectively) and 118 have been examined (results from 2012 are pending). No lesions were found in 101 (85.6%) of the brains, 12 (10.1%) had lymphocytic infiltration (unspecific chronic inflammatory lesion), and 5 (4.2%) had lesions consistent with larval migration tracts (mild to moderate meningitis, axonal degeneration, and secondary demyelination).

Whole Liver Evaluation

Whole livers were collected from 271 (*n* = 57, 108, 61, and 45 in 2009, 2010, 2011, and 2012, respectively) and have been submitted for gross examination. Of these livers, 192 (70.8%) had no fluke-induced lesions, 42 (15.5%) had mild infection, 28 (10.3%) had moderate infection, and 9 (3.3%) had marked infection. Additionally, beginning in 2010, serum was submitted for a serum chemistry profile in an attempt to correlate serum liver enzyme levels with the level of fluke-induced damage. Analyses of these results are pending.

Serum Chemistries

A total of 211 (*n* = 95, 63, and 53 in 2010, 2011 and 2012, respectively) serum samples were submitted for a full large animal serum chemistry profile. Analysis of these results is pending. The purpose of collecting these data is to determine if there is a correlation between the liver ranking and serum liver enzymes, as well as to establish baseline “normals” for animals in this population.

ACKNOWLEDGMENTS

This project would not have been possible without assistance from a number of MNDNR personnel, tribal biologists, and volunteers. We would like to especially thank our crews that worked moose registrations stations: T. Rusch, J. Hines, D. Ingebrigtsen, B. Kirsch, N. Gellerman, W. Gessler, D. Litchfield, D. Pauly, M. Minchak, P. Backman, K. Carlisle, M. Dexter, and J. Hines. J. Hines also made our area maps. We also would like to thank tribal biologists M. Schrage, A. Edwards, L. Overland, and S. Moore for their help with the project.

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Figure 1. Harvest locations of hunter-harvested moose (*n*=628) included in the 2007–2012 moose health assessment project, northeastern Minnesota.

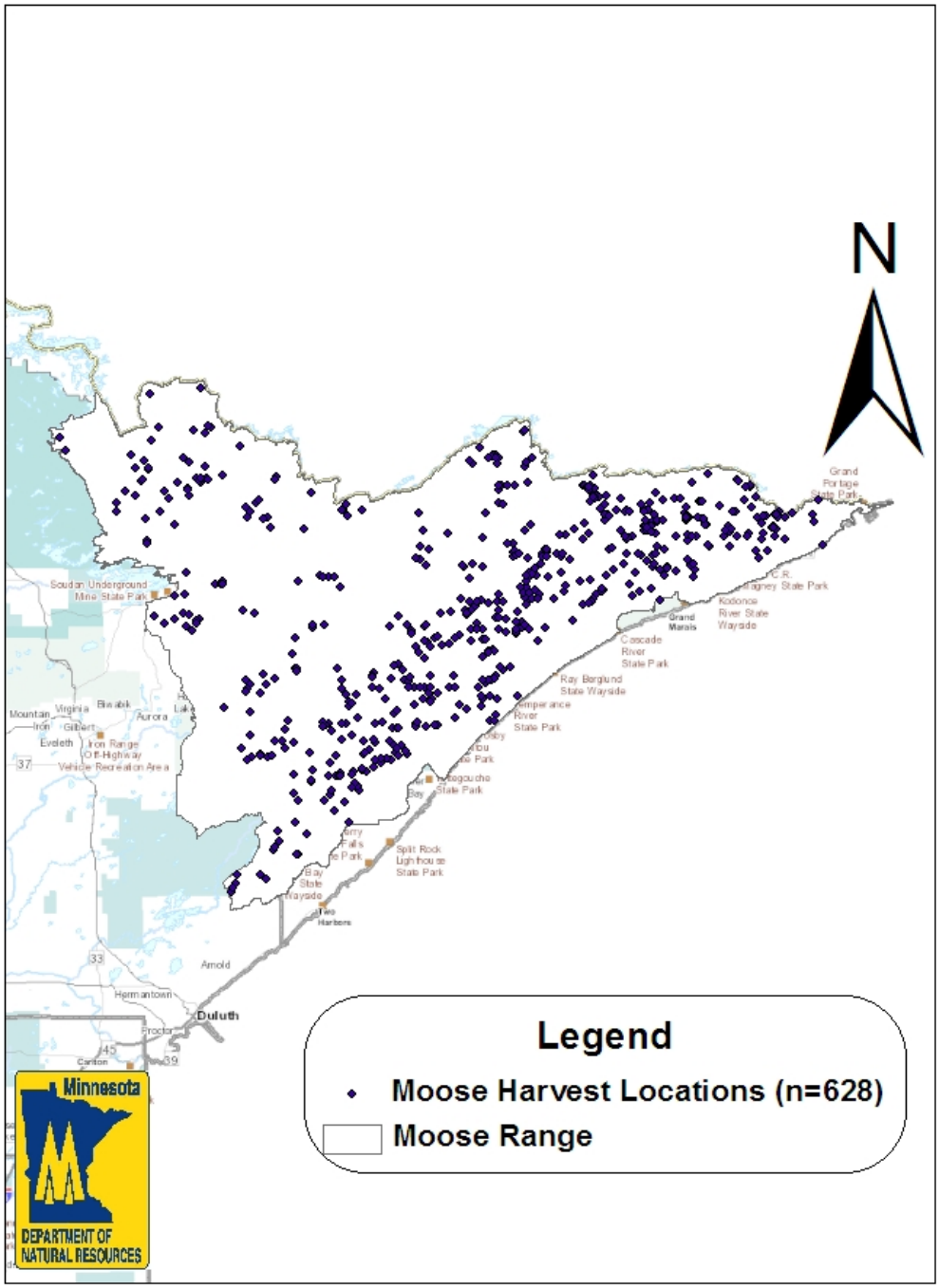


Figure 2. Distribution of ages of hunter-harvested moose (*n*=533) included in the 2007–2012 moose health assessment project, northeastern Minnesota; 2012 results pending.

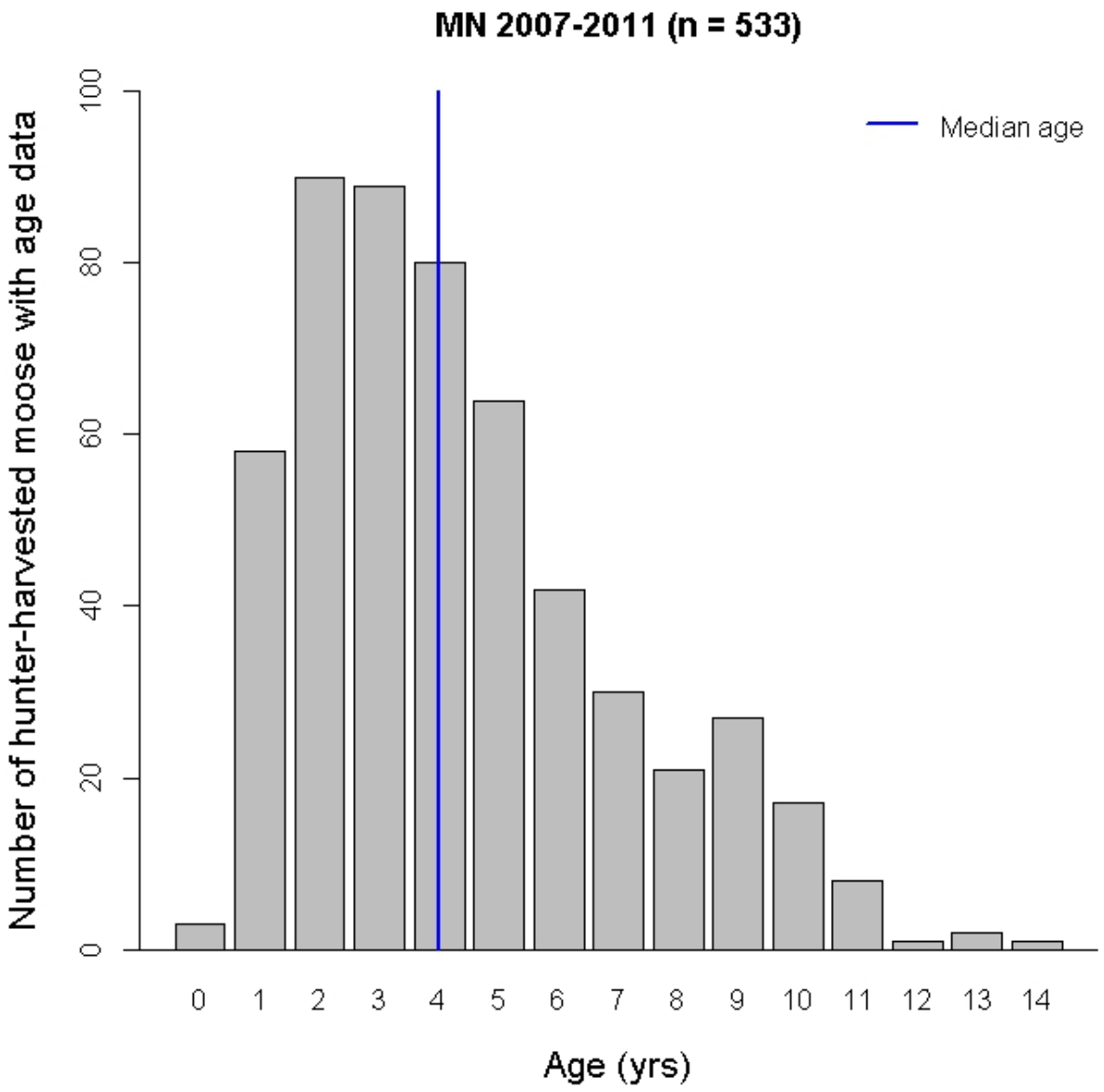


Figure 3. Locations of hunter-harvested moose (*n*=554) tested for eastern equine encephalitis included in the 2007–2012 moose health assessment project, northeastern Minnesota. Three moose were not included due to insufficient location data.

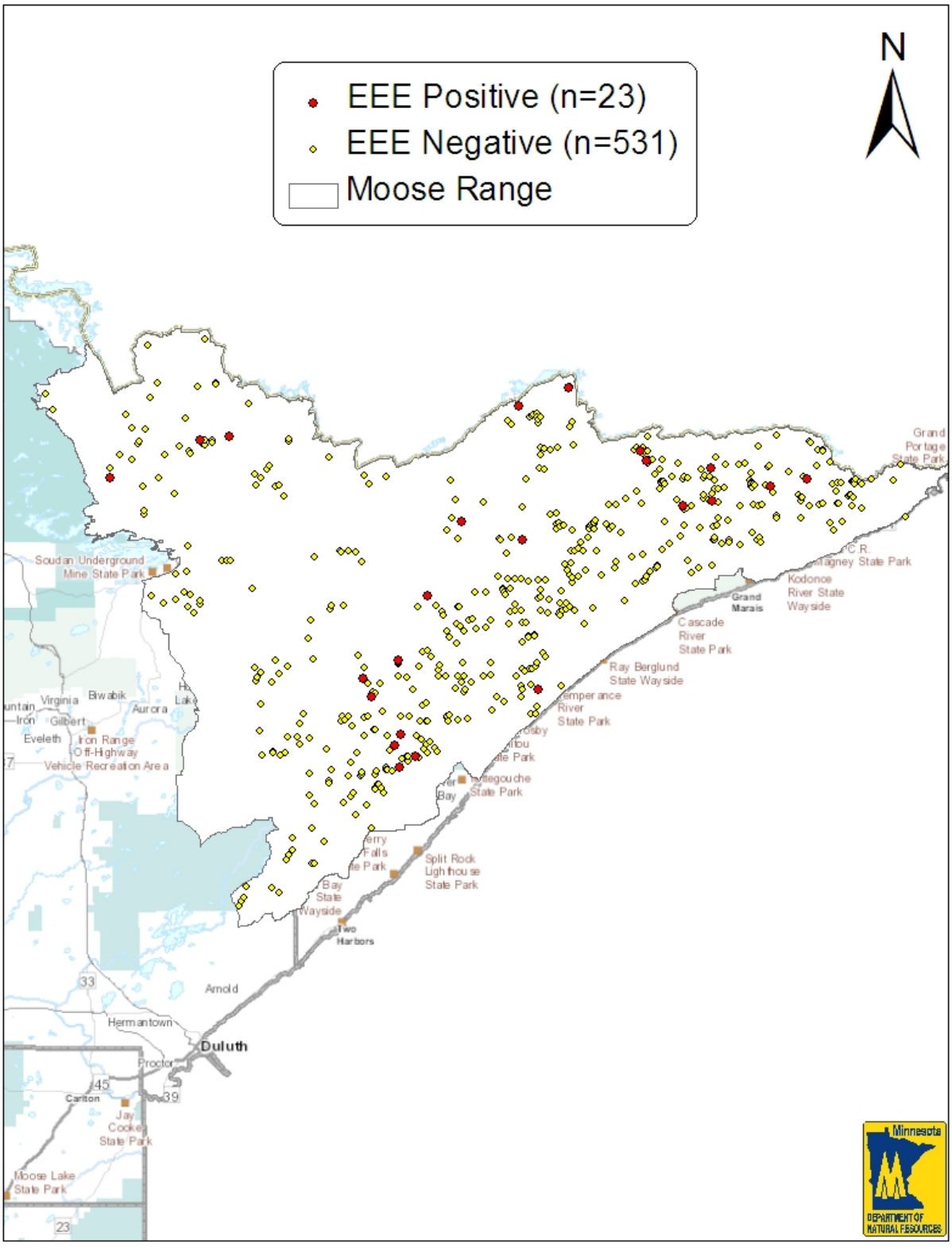
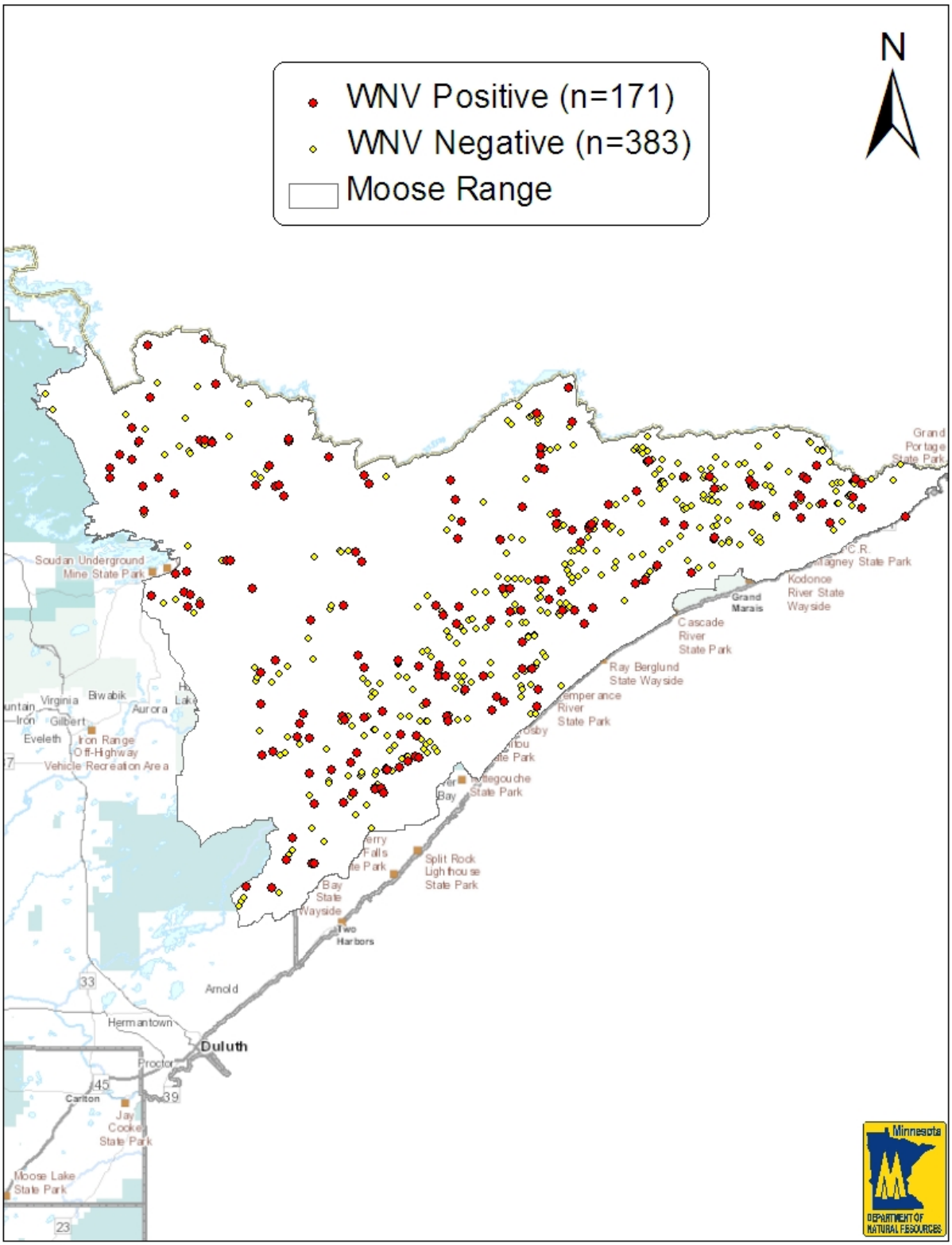


Figure 4. Locations of hunter-harvested moose (*n*=554) tested for West Nile Virus included in the 2007–2012 moose health assessment project, northeastern Minnesota. Three moose were not included due to insufficient location data.



DETERMINING CAUSES OF DEATH IN MINNESOTA'S DECLINING MOOSE POPULATION: A PROGRESS REPORT

Erika A. Butler, Michelle Carstensen, Erik C. Hildebrand, and David C. Pauly

SUMMARY OF FINDINGS

The primary goal of this project is to improve our understanding of non-anthropogenic (i.e., health-related) mortality of the northeastern Minnesota moose population. Our objectives are to determine causes of non-hunting mortality (i.e., identify specific disease and parasite agents), and assess the role nutrition plays as a potential contributing factor. To accomplish this, it is crucial that mortalities be investigated within 24 hours of death notification. From January 20-February 7, 2013, 111 moose were captured, radio-collared and released in northeastern MN. Serologic evidence of exposure to West Nile Virus, malignant catarrhal fever, various serovars of *Leptospira interrogans*, and *Borreilia* was documented. Nearly 1/3 of the 103 moose where body condition was assessed at capture were classified as thin or very thin. Serum progesterone levels indicated a 75% pregnancy rate. Mortalities investigated as of 20 June, 2013 (*n*=15) included 5 wolf kills, 2 wolf-related injuries with secondary lethal infections, one brainworm (*Parelaphostrongylus tenuis*), 3 winter ticks (*Dermacentor albopictus*), and 4 health-related causes with results pending.

INTRODUCTION

Historically, moose were found throughout the forested zone of northern Minnesota. By the 1960's there were two distinct populations, the northwest (NW) population of the aspen parklands and northeast (NE) population of the boreal forest (Fuller 1986). In the mid-1980's the NW population began a precipitous decline, falling from 4,000 to <100 animals (Murray et al. 2006, Lenarz 2007). Murray et al. (2006) identified pathogens, including liver flukes (*Fascioloides magna*) and brainworm (*Paralaphostrongylus tenius*), as the principal cause of death for 37-62% of radio-collared animals; 25% of additional mortalities were likely pathogen-induced, but limited necropsy evidence was inconclusive. They also observed that many moose in NW MN dying of natural causes were malnourished, as evidenced by 51.4% of carcasses having bone marrow fat (BMF) contents below a critical threshold (< 30%) and trace mineral deficiencies (i.e., copper and selenium). No age or sex affects were identified.

Subsequently, in NE MN, Lenarz et al. (2009) reported a 21% average non-hunting mortality rate for radiocollared males and females, which was much higher than the 8-12% reported for moose elsewhere in North America (Larsen et al. 1989, Ballard 1991, Kufeld and Bowden 1996). Specific causes of most of the non-anthropogenic mortality (89%) could not be determined, as assessing cause-specific mortality was not the primary objective of the study (Lenarz et al. 2009). Many of the deaths appeared health-related, with prime age animals dying during unusual times of the year or carcasses found intact with little evidence of scavenging.

Aerial surveys also indicate the NE population is declining. Since the estimated peak at 8,840 moose in 2006, the 2013 estimated moose population (2,760) is significantly lower (35%) than the 2012 estimate and time series analysis of estimates since 2005 indicate a significant

downward trend (DelGiudice 2013). Butler et al. (2010) documented evidence of exposure of NE MN moose to a variety of disease agents (e.g., West Nile Virus, eastern equine encephalitis, malignant catarrhal fever), which could be potential mortality factors. Additionally, sick moose reported by the public have been found to be infected with a variety of disease agents, including brainworm (*Parelaphostrongylus tenuis*), liver flukes (*Fascioloides magna*), arterial worm (*Elaeophora schneiderei*), and *Setaria* sp. (Minnesota Department of Natural Resources [MNDNR], unpublished data). Brainworm and arterial worm are known mortality factors of moose elsewhere in the U.S. (Anderson 1964, Worley et al. 1972, Pessier et al. 1998). Researchers have hypothesized that brainworm was responsible for historic declines in moose populations (Karns 1967, Prescott 1974, Lankester 1987), but it is questionable whether brainworm currently represents a major threat to the NE MN population; clinical signs consistent with brainworm infection were first reported in MN moose in 1912 (Fenstermacher and Olson 1942). Lenarz et al. (unpublished data) found that brainworm may have caused an average 19% (0-32%) of the population's total annual mortality.

The relationship between diseases, parasites, and nutritional restriction of ungulates can be very complex, and moose numbers may be influenced by interactions among these factors (DelGiudice et al. 1997). All can act either singularly or in concert to negatively affect survival. Poor body condition, potentially related to nutritional deficiencies, was reported in some NE MN moose (Lenarz et al. 2009). Using ultrasonographic measurements of rump fat and body condition scoring, DelGiudice et al. (2011) found that 21.1% of radiocollared adult females may have been seriously challenged by poor condition in 2003. A strong relationship exists between maximum depth of rump fat (Maxfat) and ingesta-free body fat (IFBF) of moose; when rump fat is depleted, IFBF is no more than 5.6% (Stephenson et al. 1998). Cook et al. (2004) reported that the probability of winter survival for northern Yellowstone elk with >5.0% IFBF during February-March was good to excellent. Urine collected from snow (snow-urine) can be chemically analyzed for urea nitrogen (UN), potassium (K), and creatinine (C). Urinary UN:C and K:C ratios have been used to assess the degree of nutritional restriction and endogenous protein catabolism in Isle Royale moose (DelGiudice et al. 1997) and Yellowstone elk (*Cervus elaphus*) and bison (*Bison bison*) (DelGiudice et al. 2001). DelGiudice et al. (1997) showed that UN:C data indicated abnormally severe nutritional restriction in a high proportion of moose from 1988 to 1990, coinciding with a 26% decline in the population, and they reported a negative correlation between population rate-of-increase and UN:C ratios, suggesting that nutritional restriction and an associated winter tick (*Dermacentor albpictus*) infestation may have contributed to the population decline on Isle Royale during 1988-1990. Assessment of body condition at the individual and population levels is essential to better understand relations of seasonal heat stress, body condition, habitat use, demographic parameters and performance of this population (DelGiudice et al. 2011).

The primary goal of this project is to improve our understanding of non-anthropogenic (i.e., health-related) mortality of the NE MN moose population. Our objectives are to determine causes of non-hunting mortality (i.e., identify specific disease and parasite agents), and assess the role nutrition plays as a potential contributing factor. To accomplish this, it is crucial that mortalities be investigated in within 24 hours of death notification. The technology being utilized in this study to facilitate rapid responses to mortalities is the first of its kind.

METHODS

The study area (Figure 1) is classified as the Northern Superior Upland region (MNDNR 2007), and includes a variety of wetlands (e.g., bogs, swamps, lakes, streams) and multiple species of conifers, such as northern white ceder (*Thuja occidentalis*), black spruce (*Picea mariana*), and tamarack (*Larix laricina*) in the lowlands and balsam fir (*Abies balsamea*) and jack (*Pinus banksiana*), white (*P. strobes*), and red pines (*P. resinosa*) in the uplands. Deciduous trees, including quaking aspen (*Populus tremuloides*) and white birch (*Betula papyrifera*) are intermixed with conifers on uplands. Potential predators of moose include wolves (*Canis lupus*) and black bears (*Ursus americanus*).

Moose were captured by aerial darting with carfentanil (4.5 mg or 6.0 mg) and xylazine (100 mg or 150 mg) from a helicopter; immobilizations were reversed with naltrexone and tolazoline. Blood (serum and whole blood) was collected at capture by venipuncture of the jugular vein. Serum was screened for evidence of exposure to 10 disease agents following the same protocol as described by Butler et al. (2010). Additionally, serum was submitted for a full large animal serum chemistry profile for chemistries and reproductive hormones to assess physiological status, overall health, and pregnancy status (Franzmann and LeResche 1978, Haig et al. 1982, Duncan et al. 1994). Serum progesterone levels were determined by the Smithsonian Institute; levels >2.0 ng/mL were considered pregnant. Whole blood in Ethylenediaminetetraacetic acid (EDTA) was used to make blood smears and complete and differential blood cell counts were performed, which may be indicative of condition and health status (Duncan et al. 1994), presence of tick-borne illnesses, and evaluation for the presence of microfilaria. An incisor (I4) was removed for aging by cementum annuli (Sergeant and Pimlott 1959). A general fecal floatation examination for parasites was performed. Rump Maxfat (cm) was measured by ultrasound to assess body condition and nutritional status (Cook et al. 2010, DelGiudice et al. 2011). A thorough physical examination was performed, including assessment of body condition score (very thin, thin, normal, fat), winter tick load, and hair loss. Total body length, girth, and hind leg length (cm) were measured (Franzman et al. 1978) and used to estimate body weight of moose and to standardize estimates of IFBF from Maxfat (Stephenson et al. 1998, Cook et al. 2010).

Moose were fitted with Irridium Global Positioning System (GPS) radiocollars manufactured by Vectronic Aerospace (Berlin, Germany). The DNR purchased 100 collars, and 10 collars were purchased by a researcher at the University of Minnesota, James Forrester, to be incorporated into our adult mortality project. Collars were programmed to obtain a location approximately once every 4 hours with one transmission per day. The Irridium component allows remote programming of these collars. The location fix schedule and transmission schedule were changed in the month of May (1 fix every 1 hour, 3 minutes, and 44 seconds, with 3-4 transmissions/day) to assist with the calf study (a corresponding research project assessing the proximate and ultimate causes of mortality in calves born to cows collared as part of this project). Battery life of 3-4 years is expected. Collars include a mortality signal triggered by a motion-sensitive switch. In turn, the mortality signal triggers a text message to be sent to the moose mortality response team, alerting us that the moose has died. A program was also developed to analyze locations, and if they are within a 20 m radius, a “localization notification” is generated. This is useful in detecting sick animals that are potentially moribund. Mortality implant transmitters (MITs), which are manufactured by Vectronics, were placed orally into the

reticulum of a subset of the captured moose. These devices are similar to a cow magnet in size, log internal temperatures every 15 minutes, and transmit a subset of this data to the collar. Additionally, MITs are meant to provide immediate notification of mortality via detection of minimal internal activity (e.g., lack of a heart beat) and this notification is also made via text message to the moose mortality response team. Temperature loggers (Hobo TibdbiTv2) were affixed to the GPS collar and were programmed to collect ambient temperature every 60 minutes.

Any mortalities that occurred within two weeks of the capture date were censored from the study.

Necropsy response teams were organized and have undergone extensive necropsy training. Responders were on-call and were ready to respond quickly in the event of a notification. If a moose is found to be alive, but obviously ill, it was euthanized and necropsied. Every effort will be made to remove carcasses intact and deliver them to the University of Minnesota Veterinary Diagnostic Laboratory (UM VDL) for a complete necropsy by a board-certified pathologist. If carcass extraction is not possible a thorough and complete field necropsy was performed, guided by an established protocol, and samples were submitted to the UM VDL for diagnostics.

RESULTS AND DISCUSSION

Capture Summary

From Jan 20 – Feb 7, 2013, 111 moose (84 females, 27 males) were captured and fitted with GPS collars (Figure 1). Four moose (2 females, 2 males) died within 2 weeks of capture and were censored; this corresponds to a 3.6% capture-related mortality rate. Though mortality rates < 2% are the goal for any wild ungulate capture, this assumes a routine capture within a healthy population (Arnemo et al., 2003). There are indications that MN's moose herd was not healthy; thus the observed capture-related mortality rate was not higher than expected. In fact, Roffe et al. (2001) reported mortality rates in moose captured with Carfentanil combinations range from 6-19%. At necropsy, one of the capture-related mortalities was found to have parasitic tracts in its brain (likely due to *P. tenuis* infection); thus suggesting that other contributing factors may have been involved in these deaths.

Collar and MIT Functioning

Of the 100 collars purchased by the MN DNR, 26 have had mortality switch malfunctions resulting in the collars being locked in mortality mode. To address this, we worked with the collar manufacturer to develop a localization program, which evaluates all the locations and generates a text message notification if the GPS fixes are within a 20 m radius. This program is currently functioning on both our locked-in-mortality collars (notification generated if fixes from the past 12 hours are within a 20 m area) and our properly functioning collars (notification generated if the fixes from the past 24 hours are within a 20 m area). This program has actually increased our ability to recognize animals that are moribund, but are not actually dead, allowing us to euthanize the animal. This allows never before documented clinical signs to be observed and key samples (e.g., fresh blood) to be collected, which is vital when trying to determine cause of death. This program has also helped us identify wolf-kills faster, as wolves will feed under the collar and prevent it from going into mortality until they have left the carcass.

Initially, we deployed 9 MITS; however, Vectronics discovered that the acceleration function malfunctioned (though the temperature sensor was still functioning) and requested that all remaining MITs be returned to them for reprogramming. While replacements were returned to DNR, we did not receive all of them in time for deployment prior to the end of the capture operation. In total, we deployed 28 MITs (9 of the first generation, 19 of the second generation). The first generation MITs are only collecting temperature data, while the other 19 should be functioning as designed. Unfortunately, 6 MITs were regurgitated by the animals soon after deployment and are no longer functioning. Three moose with MITs have died; 2 of which functioned as designed and generated an immediate notification of mortality. The other was a second generation and did not notify us of the mortality but recorded internal temperatures. Currently, there are currently 11-2nd generation and 8-1st generation MITs remaining in moose.

Body Condition Score and Rump Fat Measurement at Capture

The body condition score of each animal was evaluated and recorded whenever possible. Nearly 1/3 of the 103 moose assessed were classified as either very thin (4, 3.8%) or thin (30, 29.1%). Sixty-seven were categorized as normal (65%) and 2 (1.9%) were identified as being fat.

Serum Progesterone Results

Serum from 75 females were screened for progesterone levels. Fifty-six (74.6%) cows were identified as pregnant.

Disease and Parasite Screening at Capture

Evidence of exposure to West Nile Virus (WNV) was detected in 15/96 (15.6%) moose at capture. These results were lower than those reported during the MN hunter harvested moose surveillance project (34.8%; Butler et al. 2010). Any titer ≥10 was considered positive and indicates that animals were exposed to the WNV, but does not necessarily indicate illness. A titer that is greater than 100 is considered a very strong positive and means that the serum was able to neutralize nearly 100% of the virus. Multiple hunter-harvested animals had titers ≥100; however, at capture the only positive titers were 10. This could indicate that the antibody response to WNV in moose is not very long lived.

Evidence of exposure to eastern equine encephalitis (EEE) was not detected in any of the 96 moose tested at capture. The lack of detection was unexpected as an average exposure rate of 6.1% was documented during the MN hunter-harvested moose surveillance project (Butler et al. 2010). This could indicate that the antibody response to WNV in moose is not very long lived. Like WNV, mosquitoes spread EEE, which can cause neurologic signs and often death. It poses a greater mortality threat for most species than WNV, although the effects of EEE infection have not been studied in moose.

Evidence of exposure to malignant catarrhal fever (MCF) was detected in 48/96 (50%) moose sampled at capture. Follow-up testing with virus neutralization was negative for all moose, indicating that they were not exposed to the wildebeest strain of MCF. These results are higher than what we reported from 2007 to 2009 during the MN hunter-harvested moose surveillance project (35%; Butler et al. 2010). Malignant catarrhal fever is a gammaherpes virus, of which there are multiple strains (e.g., wildebeest strain of MCF, sheep strain of MCF, deer strain of MCF). Gammaherpes viruses have been documented to cause serious illness and death in moose and other ruminants (Neimanis et al. 2009). Clinical symptoms mimic brainworm

infection, including neurological deficits, blindness, and thrashing on the ground prior to death. While infection with MCF frequently results in death, carrier status can occur and is identified with serology.

Evidence of exposure to *Borreilia* was detected in 22/96 (22.9%) moose sampled at capture. These results are similar to results from 2007 to 2009 during the MN hunter-harvested moose surveillance project (23%, Butler et al. 2010). Borreliosis is a tick-borne bacterial disease that is maintained in a wildlife/tick cycle involving a variety of species, including mammals and birds. While evidence of natural infection in wildlife exists, there has been no documentation of clinical disease or lesions reported in wildlife species.

BA total of 96 moose were screened for 6 serovars of *Leptospira interrogans*. Results per serovar are as follows:

- *L. interrogans bratislava*:
  - 0/96 (0%)
- *L. interrogans canicola*:
  - 0/96 (0%)
- *L. interrogans grippothyphosa*:
  - 1/96 (1.0%)
- *L. interrogans hardjo*:
  - 0/96 (0%)
- *L. interrogans icterohaemorrhagicae*:
  - 3/96 (3.1%)
- *L. interrogans pomona*:
  - 8/96 (8.3%)

While the prevalences are lower for most of the serovars compared with data from the MN hunter-harvested moose surveillance project, the prevalence of *L. pomona* was slightly higher (Butler et al. 2010). Leptospirosis is a bacterial disease that can infect a wide variety of mammals, both domestic and wild. Moose could be at an increased risk for leptospirosis, as it is often propagated by mud and water contaminated with urine, not uncommon in moose habitat.

Fecal floatation was used to screen moose at capture for parasites. A total of 84 animals were sampled, with 31 (36.9%) infections identified. *Nematodirus* sp. was found in 20 moose, Strongyle-type ova were identified in 6 moose, *Moniezia* sp. was found in one moose, two moose were coinfected with *Nematodirus* sp and and Strongyle-type ova, one had *Moniezia* and *Nematodirus*, and one had *Moniezia* and Strongyle-type ova. This is a higher rate than found during the MN DNR hunter-harvested moose health assessment project (12.9%, Butler et al. 2010) and is likely due to a change in methods (feces were no longer frozen, but submitted chilled) as freezing can cause ova to lyse and make identification difficult or impossible.

Analysis of the CBC and serum chemistry results is pending. Central incisors have been submitted for aging, but results aren't expected until mid-summer.

Moose Mortalities to Date

As of 25 June, 2013, 15 of 107 (14%; 11 females, four males) collared moose have died. Causes of death are as follows: 5 wolf kills, 2 wolf-caused injuries with secondary lethal

infections, one *P. tenuis* (with a worm in both the brain and an eye, likely with partial blindness), 3 winter ticks, and 4 health-related mortalities with results pending. Two of the moose were found alive and euthanized (the *P. tenuis* case and one of the wolf-caused injuries with a secondary bacterial infection), 4 carcasses were extracted intact and delivered to the UM VDL for a complete necropsy, and 11 were necropsied in the field. The causes of mortalities determined to-date have not been unexpected. Spring is the most stressful time of year for moose (i.e., lack of adequate forage, very little remaining fat reserves) and coupled with snow conditions that favored wolves and winter ticks, these types of mortalities are not unusual. As this study continues, we expect mortalities to occur in late summer and early fall, as was documented by previous studies (Mike Schrage, Fond du Lac Resource Management Division, personal communication), and these cases may be more illuminating as to what health-related pathogens may be driving this moose decline.

Mortality Response Times

Response time has varied, though the majority of responses (53%) have occurred within 24 hours of mortality notification, 4 (27%) were within 32 hours and 3 (20%) ranged from 33-96 hours. Three of the wolf-killed moose had a delayed response time (31, 46, and 60 hours) because the wolf activity at the death site prevented the collar from going into mortality mode (thereby, no notification was generated) until they left the carcass remains. The new localization program will hopefully minimize this lag affect in the future. The moose with a 96 hour response time had sent a localization notification, but no mortality notification. While viewing the GPS location data for this moose, the moose response team believed it was alive and making small- movements; clearly, this was not the case and we have altered our response strategy accordingly.

ACKNOWLEDGEMENTS

This project is very demanding and would not be possible without the assistance of the following groups and individuals: the Environmental and Natural Resources Trust Fund for funding the majority of this project, Glenn DelGiudice for his input in study design and methodology and assistance at captures, Arno Wuenshmann and Anibal Armien (UM VDL) for their diagnostic investigations of the mortalities, Mike Schrage (Fond du Lac Resource Management Division) and Andy Edwards (1854 Treaty Authority) for their assistance in the field and purchase of MITs, Richard Gerhold and Caroline Grunenwald (University of Tennessee) for assisting with the identification of microfilaria, Ulrike Munderloh (University of MN, Department of Entomology) for testing samples for tick-borne illness, J. P. Dubey (USDA, ARS) for neospora testing, Robert Wright (MNIT) for his GIS and technical assistance, our team of primary responders (Nancy Gellerman, Dave Ingebrigtsen, Dawn Plattner, and Margaret Dexter; MN DNR), our team of secondary responders (Tom Rusch, John Giudice, Bob Kirsch, John Giudice, and Jeff Hines; MN DNR), Dan Ryan (US Forest Service), Brandon Seitz (Grand Portage National Monument) and Katie Foshey (MNDNR volunteer) for their assistance in the field, Larissa Minicucci (University of Minnesota, College of Veterinary Medicine) for training us how to place MITs, the MN DNR enforcement pilots (Jason Jenson, John Heineman, and Tom Bucker) for their assistance during captures, and Amanda McGraw (University of MN) for her assistance with data management and gearing-up for captures.

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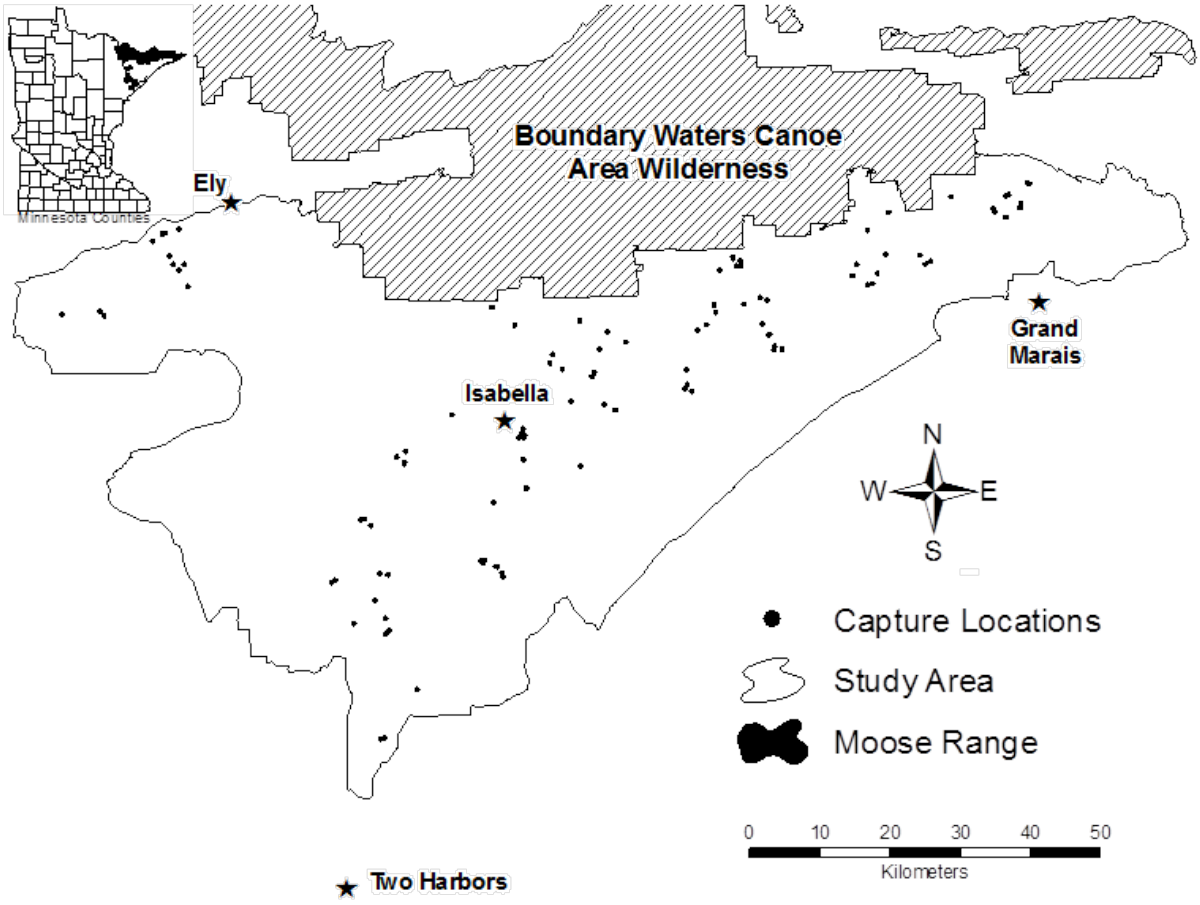
Figure 1. Locations of moose captured (*n*=111) during Jan 20- Feb 7, 2013 in northeast Minnesota.



The ecology of eastern equine encephalitis virus in wildlife and mosquitoes in Minnesota

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INTRODUCTION

Eastern equine encephalitis virus (EEEV) is a reemerging arbovirus endemic in North America. In the United States, EEEV cases have been reported in and around freshwater hardwood swamps in Eastern and Midwestern states. Wild birds serve as the primary reservoir host; they provide long-term maintenance of the virus and exchange infection between competent mosquitoes during blood meals. The highly ornithophilic mosquito species, *Culiseta melanura*, acts as the primary enzootic vector, providing stable transmission of the virus between the reservoir hosts. Species of the genera *Culex, Aedes and Coquilletidia* are thought to act as bridge vectors, leading to incidental infections in dead-end hosts, which move the virus beyond the endemic hardwood swamp habitat. EEEV is considered to be one of the most severe arbovirus infections with estimated human and equine mortality rates of 50-75% and 70-90%, respectively.

In this study, elk and moose serological surveys were used to calculate EEEV seroprevalence in the northwestern and northeastern portions of Minnesota. The serological surveys were conducted as part of the annual moose and elk herd health assessment plans conducted by the Minnesota Department of Natural Resources (MNDNR) from 2007-2011. Specific trapping sites were selected based on elk and moose seroprevalence, proximity to bog habitat and vehicular accessibility. This resulted in 2 trapping sites in the northwest and 10 trapping sites in the northeast.

METHODS

Mosquitoes were collected weekly at each of the trapping sites using Clarke® CO_2 baited ABC basic light traps that operated overnight. The trapping began in June 2012 and continued to September 2012. At each trapping site, a trap was placed at ground level and another at the canopy to maximize the amount of mosquitoes caught. The mosquitoes were transferred from the field to a freezer to be preserved until identification by taxonomic key. Samples larger than 100 mosquitoes were volumetrically subsampled and rare species in the larger samples were individually identified. Species were separated by location and date into pools of ≤ 50 mosquitoes. The mosquito pools were tested for EEEV at the Minnesota Department of Health Public Health Laboratory by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

RESULTS AND DISCUSSION

In total 54,319 adult female mosquitoes, comprised of 5 genera and 29 species were trapped during the summer of 2012. An average of 315 mosquitoes were trapped per trap-night. A total of 79 *Culiseta melanura* were found across 11 of the 12 trapping locations. *Cs. melanura* was trapped in all of the locations except moose hunting zone 30. *Coquillletidia perturbans* was the most abundant species trapped and was present through the entire trapping period. An average

of 241 *Cq. perturbans* were trapped per trap night. Other abundant mosquitoes trapped were *Aedes abserratus*, *Aedes canadensis*, *Aedes intrudens*, *Aedes sticticus*, *Aedes vexans*, *Culex restuans/pipiens* and *Culiseta morstians*. Of these abundant species, *Aedes canadensis, Aedes sticticus, Aedes vexans, Culex restuans/pipiens* and *Culiseta morstians* have tested positive for EEEV in previous studies.

To date, 240 mosquito pools have tested negative for EEEV. There are 300 samples in the process of testing and we anticipate those results to arrive in the next month. By understanding the mosquito populations in northwestern and northeastern Minnesota, we can begin to unravel the multifarious ecological conditions that have lead to elk and moose EEEV exposure.

Transmission of Newcastle Disease Virus in Double-crested Cormorants

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INTRODUCTION

Newcastle Disease, a reportable disease in poultry, was last detected in US poultry flocks in California in 2002, but continues to cause mortality events in wild birds, particularly double-crested cormorants (DCCO). The frequency of DCCO mortality events caused by the Newcastle Disease Virus (NDV) appears to be increasing in the Midwest with at least one epizootic of NDV occurring from 2006-2010 (NWHC, unpub data) compared to the 11 year period between the first documented event in 1992 and second event in 2003. In this study we determined age-specific immune and infection status for adults and two juvenile age groups (1–3 week olds and 4–6 week olds) of DCCOs at study sites in Minnesota which have a history of ND outbreaks. The age-specific prevalence data will be used to evaluate the hypothesis that prevalence of maternal antibodies drives episodic ND outbreaks in DCCOs. This project involved collaboration between US Geological Survey-National Wildlife Health Center, US Department of Agriculture-Wildlife Services, Minnesota Department of Natural Resources, and Leech Lake Band of Ojibwe, all of which are involved with the monitoring and/or management of cormorant populations and have identified understanding the transmission dynamics of NDV as important for developing management strategies for this wildlife disease.

Clinical signs and mortality are most often reported in the 4–6 week old age group (Meteyer et al., 1997. Avian Dis 41:171-180). By this stage, maternal antibodies is thought to have waned leaving this age group susceptible to circulating or introduced virus. Juveniles in this age class also become mobile and regularly leave the nest to form crèches (congregate), thereby increasing contact rates and possibly virus transmission. The accumulation of a sufficiently large population of susceptible juveniles at a breeding colony may be a driving factor in NDV episodic events. It is possible that when a larger portion of the adult population is naïve to NDV or has low antibody levels, the passive transfer of antibodies to the juvenile population is insufficient for protection. Understanding the role of maternal antibodies in the transmission dynamics of Newcastle Disease virus was the primary objective of this study. Adults were examined as a potential source of NDV because although mortality and clinical signs in adult cormorants have not been reported during ND outbreaks, they may still be capable of being infected and transmitting virus.

Since 2011 was a non-epizootic year and based on the 2-3 year pattern of large-scale epizootic events observed during the 2000's in the Midwest, we predicted 2012 to be an outbreak year. This was indeed the case, and a NDV outbreak occurred on one (Leech Lake) of the three Minnesota study sites. The data in this report pertains to the work that occurred on Marsh Lake and Wells Lake with some general discussion of findings from Leech Lake for comparative purposes. This study was planned as a multi-year longitudinal study to better understand the prevalence of NDV in various age classes during both epizootic and non-epizootic years. Nevertheless, the 2012 data provided important information that is being used to examine and further refine our hypotheses and design field and laboratory experiments to test these hypotheses. The overall goal of this research is to develop a sufficient understanding of NDV dynamics to predict epizootic events, and provide managers with a scientific basis to take management actions to prevent or reduce epizootic events in DCCO.

METHODS

The study sites were cormorant breeding colonies at Wells Lake (Rice County, MN) and Marsh Lake (Lac Qui Parle County, MN). During 2012 we also sampled Leech Lake (Cass County, MN) and Pilot Island (Door County, Wisconsin) and these data are presented in reports to Leech Lake Band of Ojibwe and USFWS.

Juvenile cormorants were captured by hand and adult cormorants were captured using reduced tension foot-hold traps or were culled during management activities. Lethal sampling was also used to sample adults at the end of the summer when they were no longer tied to nests. Live captured birds were banded to allow for data collection on disease and other parameters through time if the individuals were recaptured during additional sampling periods or years. Capture locations were recorded with a handheld GPS when possible.

Sex was determined on adult-lethal-sampled birds by gonadal inspection. Morphological characteristics (wingchord, culmen depth, culmen length, weight, and tarsus length) were recorded on all adult birds and will be used to develop morphometric predictor of sex in live sampled adult birds. Morphological characteristics (wingchord, ulna, weight, and feather measurements) were measured on all juvenile birds to assist with age class stratification. Blood volume not exceeding 1% of body weight (~ 2-3 mL in adults, < 1 mL in juveniles) was taken. When lethal sampling was used blood was collected from the heart and/or body cavity. Oral-pharyngeal and cloacal swabs were placed together in a single cryovial containing viral transport media.

Blood was centrifuged within 24 hours and serum was stored in cryovials and frozen until testing was performed. Serum was tested at NWHC with the ID Screen® Newcastle Disease Competition ELISA (IDVET Innovative Diagnostics, Montpellier, France) which can detect all APMV-1, including NDV. Oral-pharyngeal and cloacal swabs from each bird were combined and screened with a NDV matrix gene RT-PCR assay. Virus isolation in embryonating chicken eggs was performed on RT-PCR-positive samples, and hemagglutination positive isolates were sequenced using primers that cover the region containing the NDV fusion protein gene protease cleavage site to determine virus virulence.

RESULTS AND DISCUSSION

A total of 643 blood and swab samples were collected from double-crested cormorants from Marsh Lake and Wells Lake. Adults were difficult to sample during the late season (August) since they were no longer tied to nests. We attempted both nighttime dip netting of birds on the water, noose carpet trapping, and lethal sampling of adults at Marsh Lake but was unable to obtain the goal of 125 adult birds during this sample period. We therefore sampled young of the year birds (>10 weeks old) at Marsh Lake during August as well as adults.

We predicted that lower maternal antibody levels in a population could result in a smaller portion of the juvenile population protected (i.e., a larger susceptible juvenile class) thereby allowing an epizootic in juveniles to occur in a population. According to our hypothesis an outbreak would have been more likely at Marsh Lake where early season seroprevalence in adults was the lowest among the sampled sites. Instead, an outbreak occurred at the Leech Lake site where early season adult seroprevalence was higher. High adult seroprevalence did not seem to be reflected in the 1–3 week olds as seroprevalence in this age group was <15% at Marsh Lake, and was not significantly different between the outbreak (Leech Lake) and non-outbreak site (Marsh Lake). Furthermore, we found no difference in seroprevalence in the 4–6 week old age class between the outbreak and non-outbreak sites, although the seroprevalence was lower as expected between in the 4-6 week old compared to the 1–3 week olds at each site.

The seroprevalence data based on the ELISA test do not currently support the proposed hypothesis that maternal antibodies drive episodic events of NDV in cormorants; however, the ELISA used, and whose results are reported herein, is a general, non-species specific test for AMPV-1. APMV-1 strains other than NDV also belong to this group of viruses, and occur in DCCOs. Thus, the ELISA provides only a measure of seroprevalence to this entire group of viruses. Therefore, additional serology work using more specific tests is underway to further characterize the ELISA positive cormorants ithat had antibodies to NDV. This will provide a clearer understanding of NDV seroprevalence results and maternal antibody protection. We are continuing data collection at these sites in 2013 to help determine if the differences in early season adult seroprevalence at Leech Lake (2012 outbreak site) compared to Marsh and Wells Lakes (both 2012 non-outbreak sites) were due to NDV transmission dynamics of the disease. There may exist site-specific characteristics (e.g., spatial structure of the colony, location of wintering sites, etc.) that play a critical role in NDV transmission dynamics, and require within colony comparisons between epizootic and non-epizootic years to adequately assess the role of maternal antibodies in NDV epizootics. The 2013 field study will contribute to a better assessment of the validity of between colony comparisons. Additionally, experimental work using captive DCCOs is on-going to test whether adults are functional carriers of NDV. Together this project is the most detailed study of NDV transmission in double crested cormorants to date and our results will help to improve the understand of NDV transmission dynamics in cormorants.

CONGENITAL TRANSMISSION OF NEOSPORA CANINUM IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

J. P Dubey, M. C. Jenkins, O.C.H Kwok, L. R. Ferreira, S. Choudhary, S. K. Verma, I. Villena, E. Butler, and M. Carstensen

ABSTRACT

Neosporosis is an important cause of bovine abortion worldwide. Many aspects of transmission of *Neospora caninum* in nature are unknown. The white-tailed deer (*Odocoileus virginianus*) is considered one of the most important wildlife reservoirs of *N. caninum* in the USA. During the hunting seasons of 2008, 2009, and 2010, brains of 155 white-tailed deer fetuses were bioassayed in mice for protozoal isolation. Viable *N. caninum* (NcWTDMn1, NcWTDMn2) was isolated from the brains of two fetuses by bioassays in mice, and subsequent propagation in cell culture. Dams of these two infected fetuses had antibodies to *N. caninum* by Neospora agglutination test at 1:100 serum dilution. DNA obtained from culture-derived *N. caninum* tachyzoites of the two isolates with Nc5 PCR confirmed diagnosis. Results prove congenital transmission of *N. caninum* in the white tailed deer for the first time.

[1]Veterinary Parasitology, Volume 2013, http://dx.doi.org/10.1016/j.vetpar.2013.03.004, *in press*