MANAGING BOVINE TUBERCULOSIS IN WHITE-TAILED DEER IN NORTHWESTERN MINNESOTA: A 2008 PROGRESS REPORT

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SUMMARY OF FINDINGS

Bovine tuberculosis (TB), first discovered in 2005, has now been found in 12 cattle operations in northwestern Minnesota. To date, all of the infected cattle herds have been depopulated and the Board of Animal Health (BAH) has continued to test cattle herds in the area. The strain has been identified as one that is consistent with Bovine TB found in cattle in the southwestern United States and Mexico. In response to the disease being detected in cattle, the Minnesota Department of Natural Resources (MNDNR) began surveillance efforts in free-ranging white-tailed deer (*Odocoileus virginianus*) within a 15-mile radius of the infected farms in fall 2005. To date, 25 deer have been found infected with Bovine TB. All infected deer were sampled within a 164mi^2 area, called the Bovine TB Core, which is centered in Skime, Minnesota, and encompasses 8 of the previously infected cattle farms. In fall 2008, Minnesota was granted a Split-State Status for Bovine TB by the United States Department of Agriculture (USDA) that resulted in a lessening of testing requirements for cattle in the majority of the state (status level = "Modified Accredited advanced"), with a small area in northwestern Minnesota remaining more restrictive (status level = "Modified Accredited"). Also in 2008, the Minnesota State Legislature passed an initiative that allocated funds to buy-out cattle herds located in the Bovine TB Management Zone, spending $3 million to remove 6,200 cattle from 46 farms by January 2009; resulting in the discovery of the 12th infected cattle herd. The remaining cattle farms in the Bovine TB Management Zone (*n* = 27) were required to erect deer-exclusion fencing to protect stored forage and winter feeding areas, costing an additional $690,000 in state funds. In November 2008, the MNDNR conducted Bovine TB surveillance of hunter-harvested white-tailed deer within the newly created Modified Accredited Zone, and results indicated that none of the 1,246 deer tested were positive for the disease. This marked the first large scale surveillance effort that failed to detect the disease in hunter-harvested deer since sampling efforts began in 2005. MNDNR also conducted targeted removal operations in the Bovine TB Core Area, using both aerial and ground sharpshooting, during winters 2007, 2008 and 2009. These intensive winter deer removal operations removed a combined total of 2,163 deer and detected 13 (52%) of the TB-positive deer discovered to date. Further, a recreational feeding ban, covering 4,000mi^2 in northwestern MN, was instituted in November 2006 to help reduce the risk of deer to deer transmission of the disease and enforcement officers have been working to stop illegal feeding activities. The MNDNR will continue to conduct hunter-harvested surveillance for the next 5 years to monitor infection in the local deer population, and consider the continuation of aggressive management actions (e.g., sharpshooting deer in key locations) to address concerns of deer becoming a potential disease reservoir.

INTRODUCTION

Bovine tuberculosis is an infectious disease that is caused by the bacterium *Mycobacterium bovis (M. bovis)*. Bovine TB primarily affects cattle; however, other mammals may become infected. Bovine TB was first discovered in 5 cattle operations in northwestern Minnesota in 2005. Since that time, 2 additional herds were found infected in 2006, 4 more in 2007, and 1 in 2008; resulting in further reduction of the state's Bovine TB accreditation to Modified Accredited in early 2008. By fall 2008, Minnesota was granted a split-state status for TB accreditation that maintained only a small area (2,670mi^2) in northwestern Minnesota as "Modified Accredited," allowing the remainder of the state to advance to "Modified Accredited

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advanced." To date, 25 wild deer have been found infected with the disease in northwestern MN. Although Bovine TB 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 Bovine TB in North America. In 1995, Bovine TB was detected in wild deer in Michigan. Though deer in Michigan do serve as a reservoir of Bovine TB, conditions in northwestern Minnesota are different. Minnesota has no history of tuberculosis infection in deer or other wildlife, and the *M. bovis* strain isolated from the infected Minnesota herd does not match that found in Michigan. Also, there are much lower deer densities in the area of the infected herds than in the affected areas of Michigan. Further, unlike Michigan, Minnesota does not allow baiting (hunting deer over a food source), which artificially congregates deer and increases the likelihood of disease transmission.

Bovine TB 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 Bovine TB by ingesting the bacteria from eating contaminated feed. It can take 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 on the surface of the lungs and chest 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. In Michigan, only 42% of the Bovine TB positive deer had lesions in the chest cavity or lungs that would be recognized as unusual by most deer hunters. While it is possible to transmit Bovine TB 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

In 2008, a fall surveillance strategy was developed to meet the sampling goals established in a recent Memorandum of Understanding (MOU) with USDA, signed by both MNDNR and BAH, that required 1,500 deer to be tested for Bovine TB within the newly created Modified Accredited Zone (MAZ), and 300 deer to the immediate south and west of the MAZ boundaries (Figure 1).

At the registration stations, hunters were asked to voluntarily submit lymph node (LN) samples for Bovine TB testing. Hunter information was recorded, including the hunter's name, address, 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.

Tissue collection procedures included a visual inspection of the chest cavity of the hunter-killed deer. Six cranial LNs (parotid, submandibular, and retropharyngeal) were visually inspected for presence of lesions and extracted for further testing. Samples were submitted to the Veterinary Diagnostic Laboratory (VDL) at the University of Minnesota for histological examination and acid-fast staining. 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 confiscated 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 164mi^2 TB Core Area, through the use of sharpshooters. During winters 2006 through 2008, sharpshooting from the ground was conducted by USDA-Wildlife Services (USDA-WS) professionals; supplemental sharpshooting was conducted by aerial operations during winters 2007 and 2008. 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 that were free of any visible lesions were salvaged for venison and made available to the public.

Prior to the start of the each winter sharpshooting effort, MNDNR conducted aerial surveys of the Bovine TB Core Area to assess deer numbers and distribution (Figure 2). This information was used to guide sharpshooting activities and estimate the percentage of deer removed from the area.

RESULTS AND DISCUSSION

In fall 2008, we collected 1,246 samples from hunter-harvested deer; 805 samples from within the Modified Accredited Zone and 441 samples outsize the zone (Figure 3). We did not identify any of the deer as "suspects," meaning they did not have obvious lesions on the lungs or inside the chest cavity that were consistent with clinical signs of Bovine TB. This marks the first large-scale surveillance effort since fall 2005, in which no suspects were identified. Testing of lymph node samples at NVSL has confirmed that there were no positive cases detected during the fall 2008 surveillance. However, the fall sampling effort fell 30% short of its collection goal of 1,800 samples; thus additional deer removal efforts in winter 2009 increased the sampling total to 1,984 deer, or 10% higher than targeted. Apparent prevalence of Bovine TB in the local deer population, sampled throughout a 1,730 to 2,670mi^2 surveillance zone, indicates a significant decreasing trend from 2006–2008 (Table 1, Figure 4).

To supplement the number of samples collected through fall hunter-harvested surveillance and to further reduce deer density in the area where TB-positive deer had been confirmed, MNDNR used both ground and aerial sharpshooting in the Bovine TB Core Area during winters 2007–2009. In total, these operations removed 2,163 deer from the TB Core Area, included 13 TB-positive individuals. The most recent case was a 5.5 year old male found positive during the winter 2009 sharpshooting effort, which removed a total of 738 deer (Figure 5). Disease prevalence in the TB Core Area has decreased dramatically from 2007 to 2009 (Table 1, Figure 4). Although disease prevalence estimates in the TB Core Area are biased due to the limited geographic distribution of TB-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 populations in the fall.

Aerial survey results from February 2009 estimated that the deer population in the Bovine TB Core Area was a minimum of 664 ± 87 deer (Figure 2). This is not significantly different than the January 2008 population estimate of 806 ± 133, but less than the February 2007 estimate of 935 ± 150. It is apparent that aggressive deer removal in the TB Core Area through liberalized hunting, disease management permits, landowner shooting permits, and targeted sharpshooting has not been able to dramatically reduce the deer population in this 164mi^2 area. It is likely that that the TB 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 TB Core Area as deer numbers are reduced and habitat availability increases. The lack of severe winter weather condition in recent years has also allowed for good overwinter survival, increased reproduction, and recruitment into the local deer population.

The proximity of the TB-infected deer to infected cattle herds, the strain type, and the fact that disease prevalence (<0.2%) is low, supports our theory that this disease spilled-over from cattle to wild deer in this area of the state. To date, we have sampled 6,206 deer in the northwest since 2005, and a total of 25 confirmed culture-positive deer (Figure 6). Further, all deer found infected to date would have been alive in 2005, when the initial detection of Bovine TB in cattle occurred. The lack of infected yearlings or fawns and limited geographic distribution of infected adults further supports that this disease is not being spread efficiently in the local deer population.

In November 2006, a ban on recreational feeding of deer and elk was instituted over a 4,000mi^2 area to help reduce the risk of disease transmission among deer and between deer

and livestock (Figure 7). Enforcement officers continue to enforce this rule and compliance is thought to be very high within the Bovine TB Management Zone.

Further, the Minnesota State Legislature passed an initiative in 2008 that allocated funds to buy-out cattle herds located in the Bovine TB Management Zone, spending $3 million to remove 6,200 cattle from 46 farms by January 2009; resulting in the discovery of the 12th infected cattle herd. The remaining cattle farms in the TB-endemic area (*n* = 27) were required to erect deer-exclusion fencing to protect stored forage and winter feeding areas, costing an additional $690,000 in state funds.

As part of the requirements to regain TB-Free accreditation, USDA has required BAH to test all cattle herds within the Modified Accredited Zone annually, with additional movement restrictions for farms located within the Bovine TB Management Zone. The MNDNR is committed to assisting BAH in regaining Minnesota's TB-Free status as soon as possible. To accomplish this, the MNDNR will continue to conduct fall surveillance annually until 5 consecutive years with no TB-positive deer can be achieved, which would indicate that the disease was either eradicated or present in undetectable levels in the local deer population.

ACKNOWLEDGMENTS

There is no way to complete a project of this scale without the assistance and leadership from St. Paul and regional staff, including Ed Boggess, Dennis Simon, Dave Schad, Paul Telander, John Williams, and Mike Carroll. For all the help with field collections, we'd like to thank area staff from Thief Lake, Red Lake, Norris Camp, and Thief River Falls, Erik Hildebrand (Wildlife Health Specialist), Margaret Dexter (Fish and Wildlife Specialist), as well as students and faculty from the University of Minnesota, College of Veterinary Medicine. Randy Prachar provided excellent leadership through the aerial operations, and we appreciate his efforts to support our carcass examination crews. Special thanks to Mary Reiswig for coordinating the venison donation program and Tammi Jalowiec for assisting with communication needs during the project. Also thanks to Bob Wright, Eric Nelson, and the enforcement pilots (Tom Pfingsten and John Heineman) for conducting a deer survey within the Bovine TB Management Zone, as well as identifying illegal deer feeding activities. Also thanks to John Giudice for analyzing the survey data. We had an excellent team of GIS support including Steve Benson, Julie Adams, Bob Wright, and Chris Scharenbroich. We also want to recognize the tremendous amount of work conducted by USDA-Wildlife Services staff, led by John Hart (Grand Rapids) and disease biologist Paul Wolf (St. Paul). USDA-WS also loaned us disease biologists to assist with sample collections, including Randy Mickley (Mass), Dallas Virchow (NE), Kirk Shively (MA), Tom Hutton (MO), Jason Kloft (KS), Mark Lutman (CO), Tony Musante (NJ), and Brian Thomas (OR).

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Table 1. Number of deer sampled for bovine TB and testing results listed by sampling strategy, fall 2005 to spring 2009, northwestern Minnesota.

Sampling Strategy	**2005**	**2006**	**2007**	**2008**	**2009**	**Totals**
Hunter-harvested (Oct-Jan)	474	942	1,166	1,246	n/a	3,828
# TB-positive	1	5	5	0		
Apparent Prevalence	0.21%	0.53%	0.43%	0.0%		
Sharpshooting (Feb-May)	0	0	488	937	738	2,163
# TB-positive			6	6	1	
Apparent Prevalence			1.23%	0.64%	*0.14%	
Landowner/Tenant	0	90	0	125	0	215
# TB-positive		1		0		
Total Deer Tested	474	1,032	1,654	2,308	738	6,206
Total # TB-positive	1	6	11	6	1	25

*Final culture results from winter 2009 sampling are still pending at NVSL.

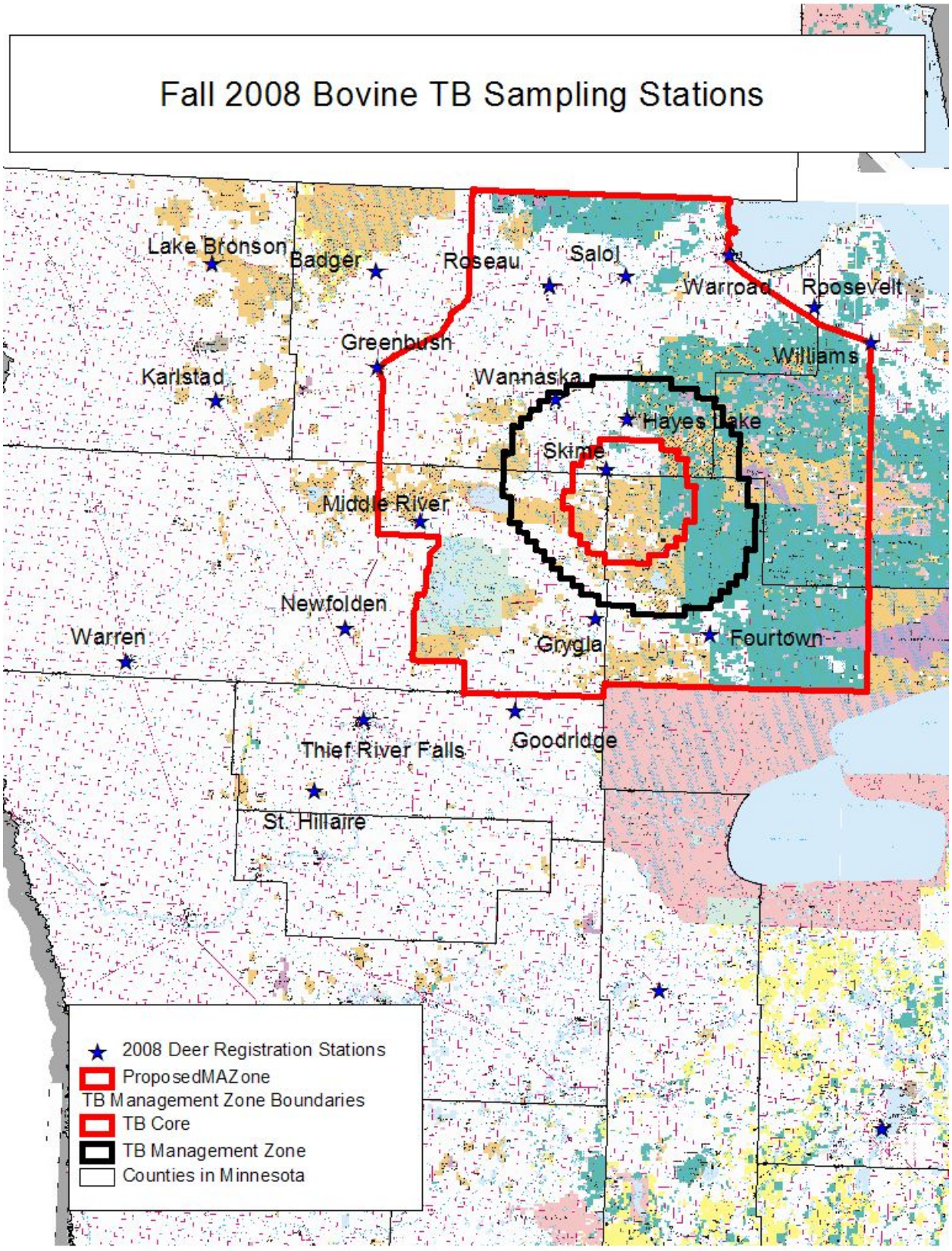


Figure 1. Locations of deer registration stations for sampling hunter-harvested deer for bovine tuberculosis during fall 2008, northwestern Minnesota.

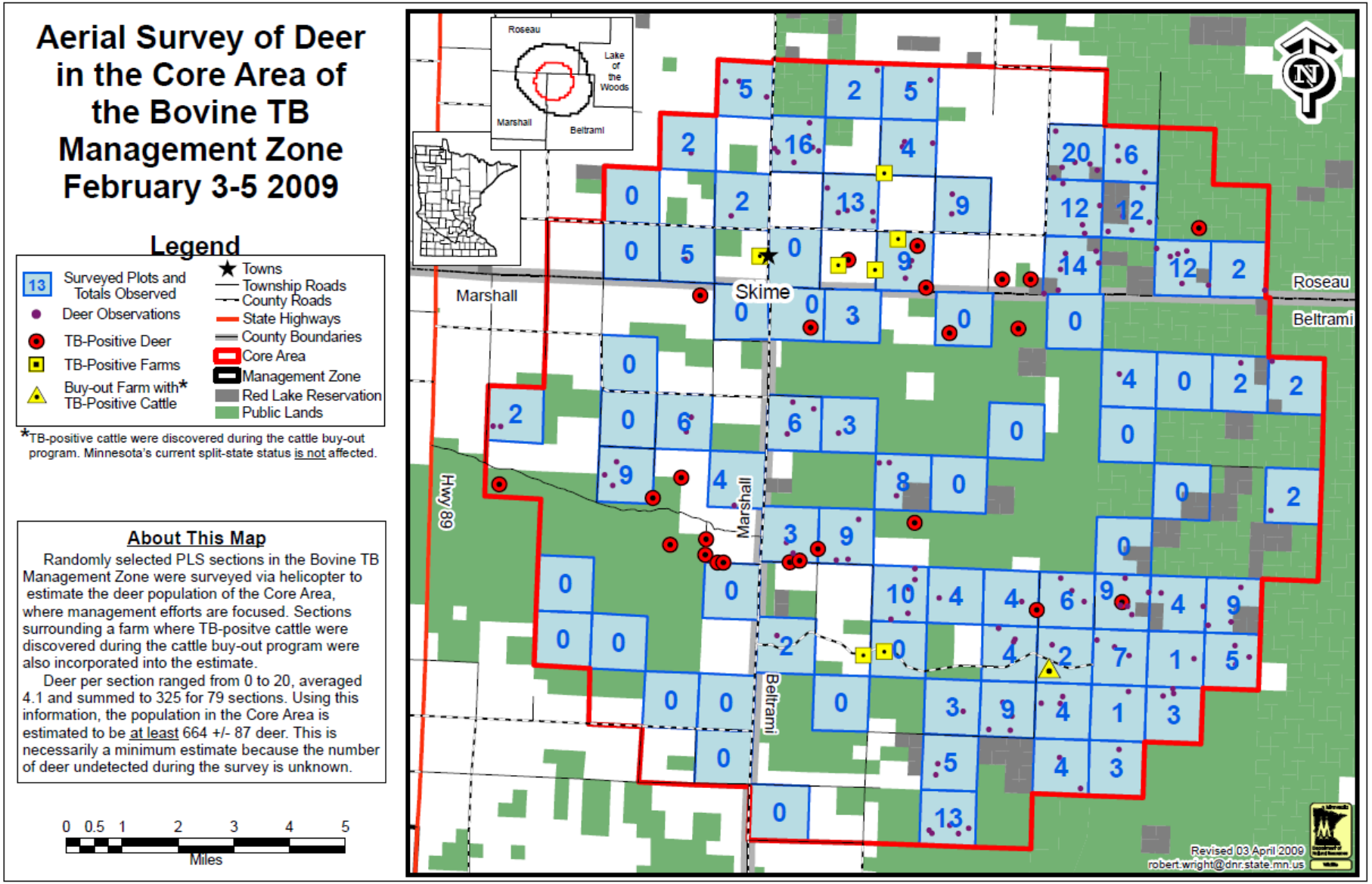


Figure 2. Results of aerial white-tailed deer survey of the Bovine TB Core Area in February 2009, northwestern Minnesota.

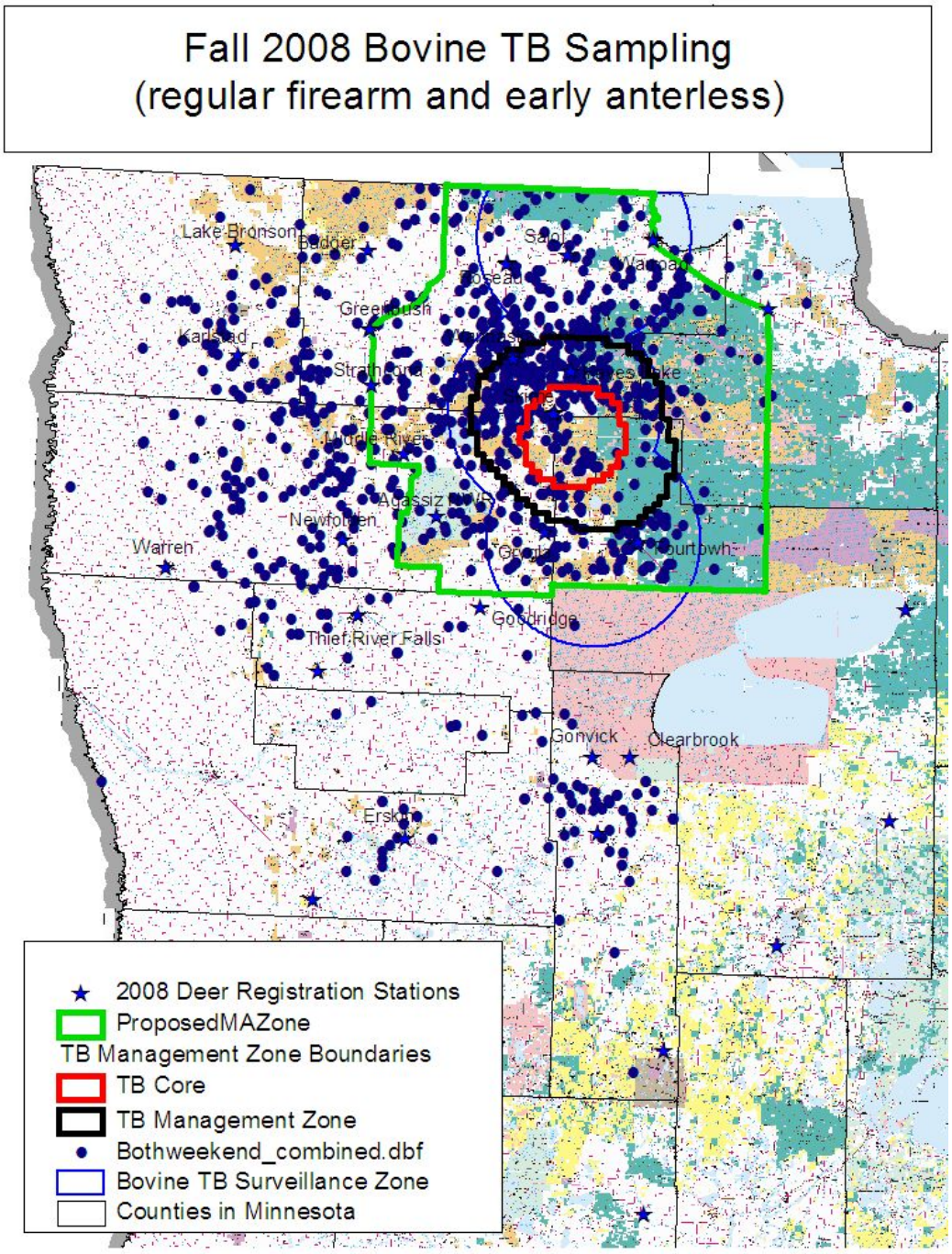


Figure 3. Locations of hunter-harvested deer (*n*=1,246) sampled for Bovine tuberculosis (TB) during fall 2008 in northwestern Minnesota.

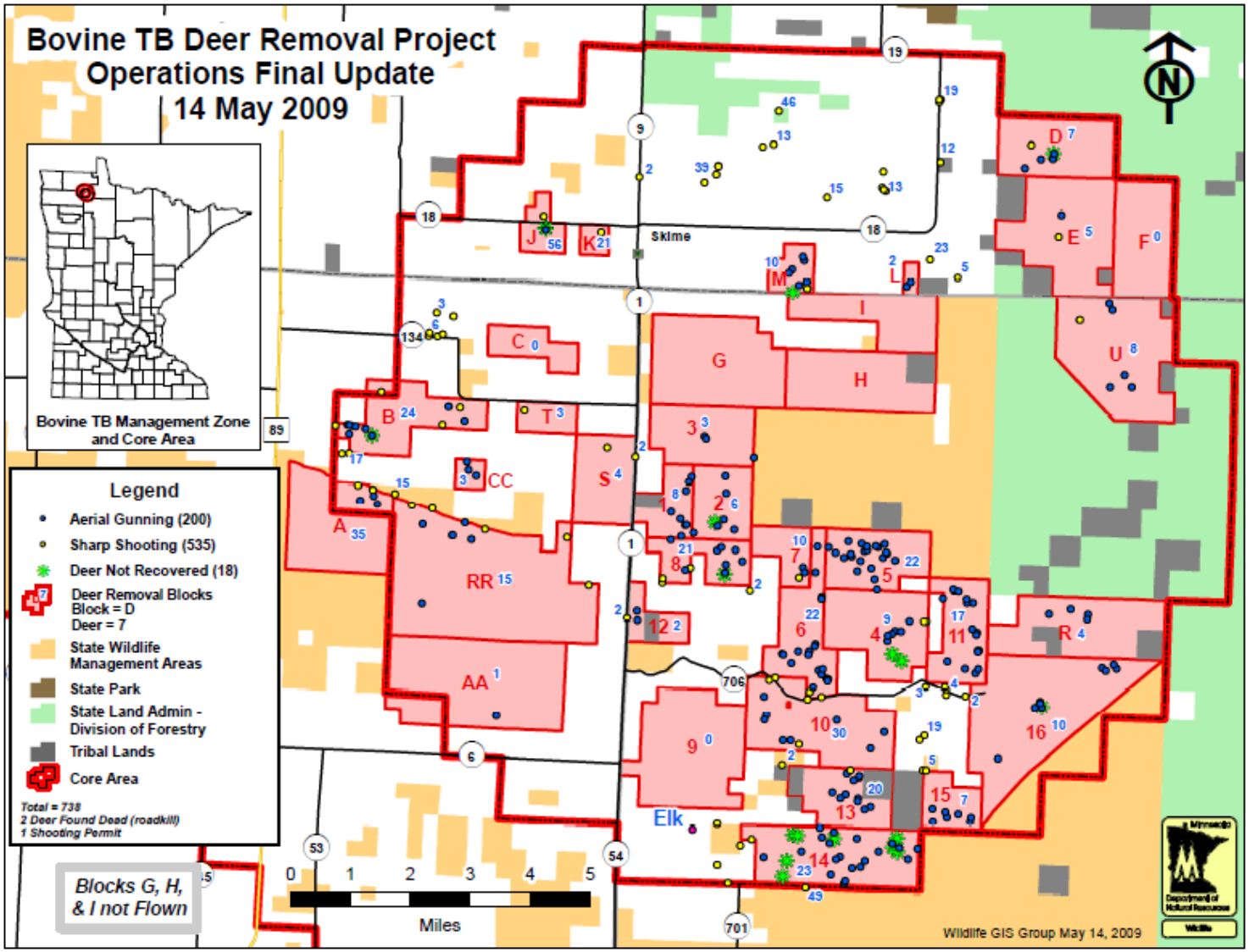
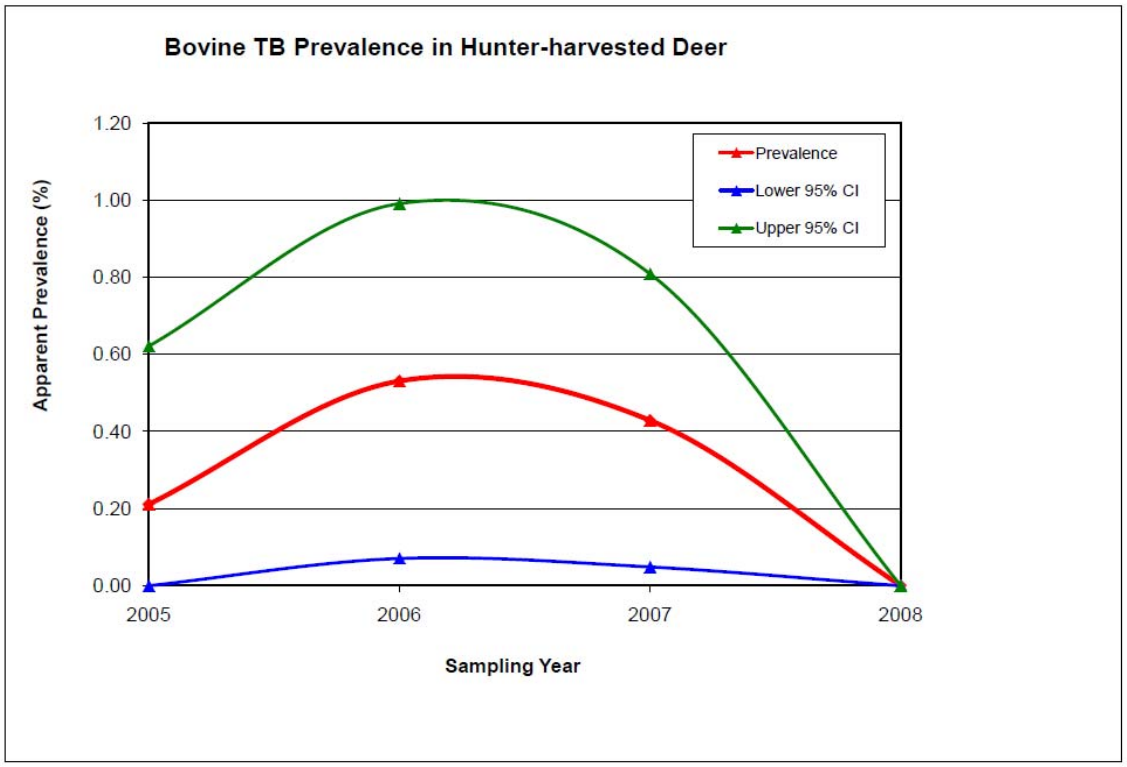


Figure 4. Locations of deer removed (*n*=738) by USDA ground sharpshooters and aerial gunning during February-April, 2009 within the Bovine TB Core Area, a 164mi^2 area within Bovine tuberculosis Management Zone in northwestern Minnesota.



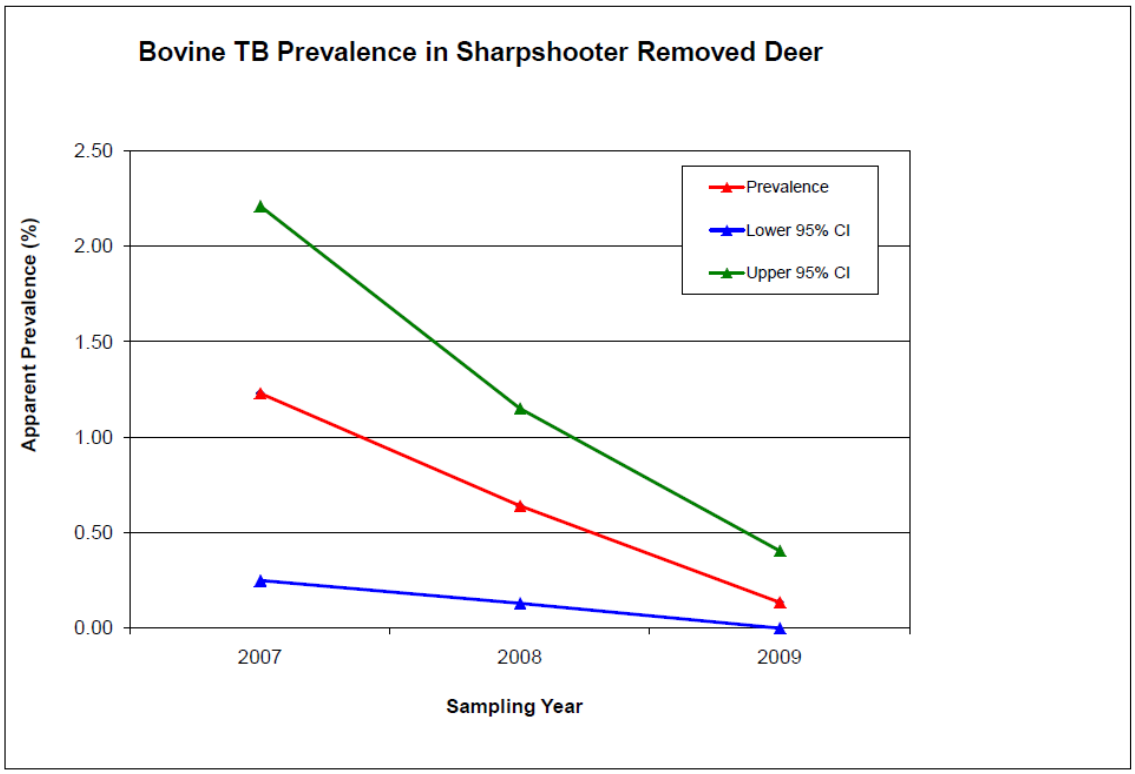


Figure 5. Prevalence of Bovine TB in hunter-harvested deer from 2005–2008 in the Bovine TB Surveillance Zone and disease prevalence from sharpshooter removed deer from 2007–2009 in the Bovine TB Core Area, northwestern Minnesota.

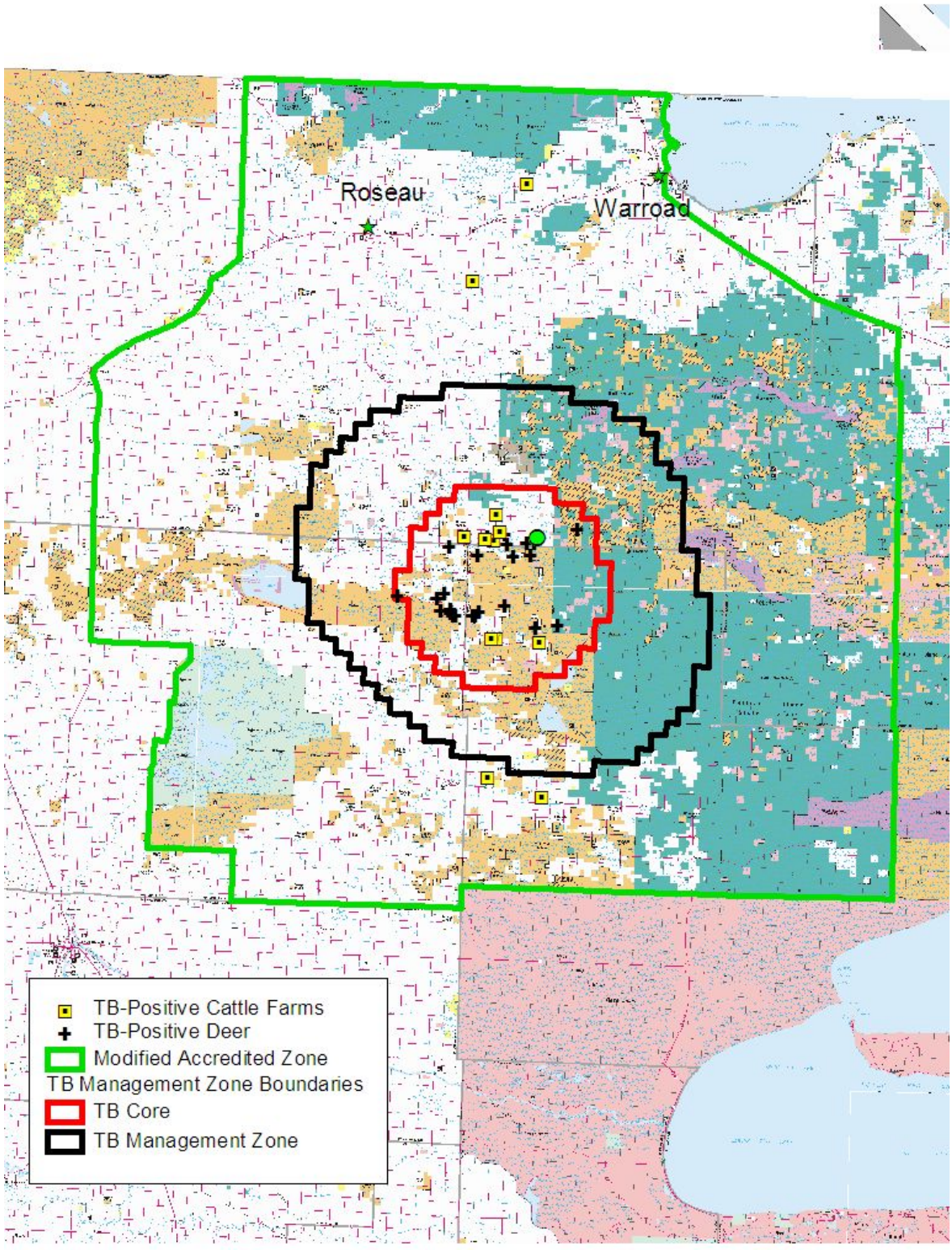
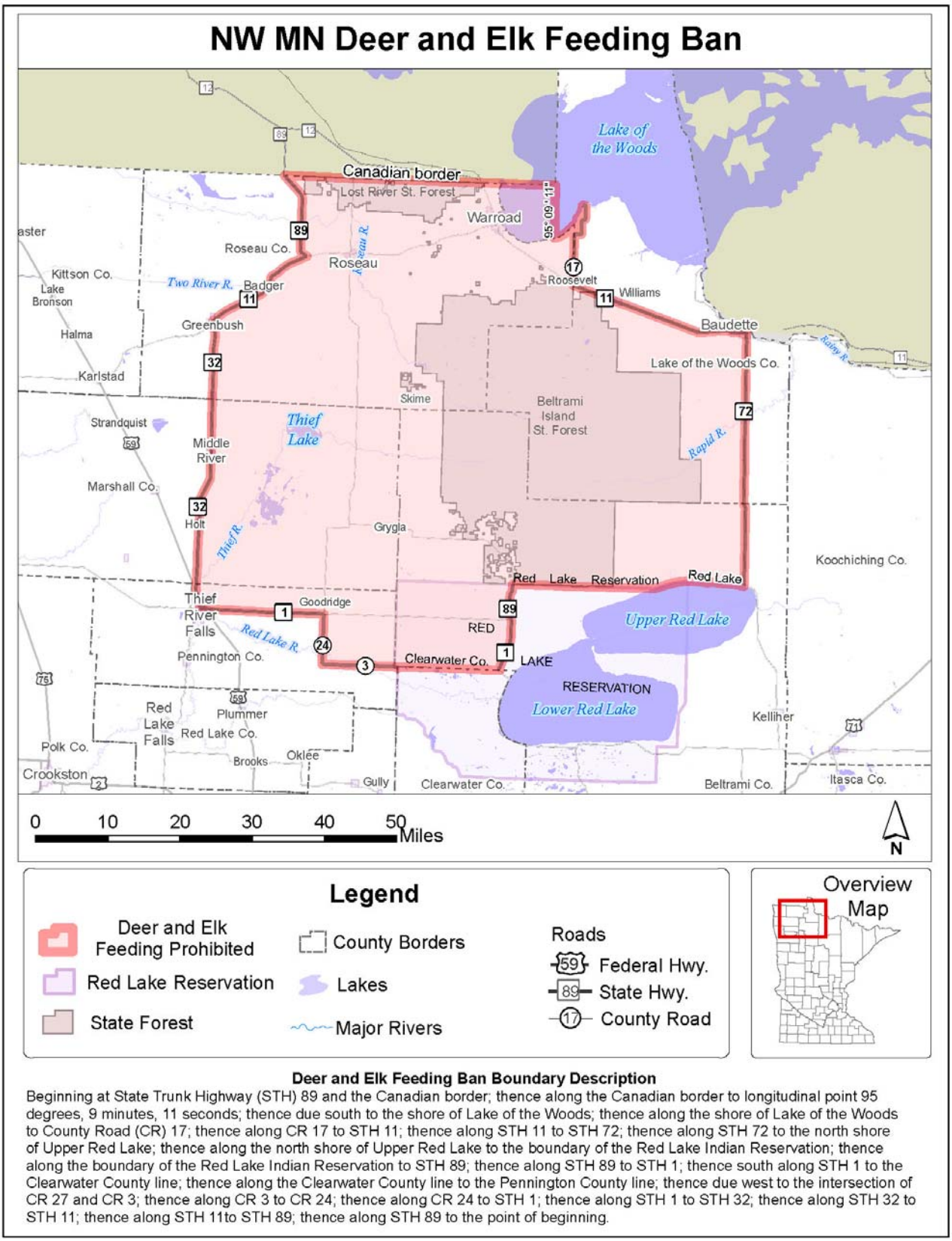


Figure 6. Locations of white-tailed deer found infected (*n*=25) with Bovine TB since fall 2005 in northwestern Minnesota, with the most recent case detected in March 2009 indicated in green. The 12 previously-infected cattle operations are also included.



Deer and Elk Feeding Ban Boundary Description

Beginning at State Trunk Highway (STH) 89 and the Canadian border; thence along the Canadian border to longitudinal point 95 degrees, 9 minutes, 11 seconds; thence due south to the shore of Lake of the Woods; thence along the shore of Lake of the Woods to County Road (CR) 17; thence along CR 17 to STH 11; thence along STH 11 to STH 72; thence along STH 72 to the north shore of Upper Red Lake; thence along the north shore of Upper Red Lake to the boundary of the Red Lake Indian Reservation; thence along the boundary of the Red Lake Indian Reservation to STH 89; thence along STH 89 to STH 1; thence south along STH 1 to the Clearwater County line; thence along the Clearwater County line to the Pennington County line; thence due west to the intersection of CR 27 and CR 3; thence along CR 3 to CR 24; thence along CR 24 to STH 1; thence along STH 1 to STH 32; thence along STH 32 to STH 11; thence along STH 11to STH 89; thence along STH 89 to the point of beginning.

Figure 7. Area in northwestern Minnesota where recreational feeding of deer and elk was banned in November 2006, as a preventative measure to reduce risk of disease transmission.

PRELIMINARY RESULTS FROM THE 2007-2008 MOOSE HERD HEALTH ASSESSMENT PROJECT

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SUMMARY OF FINDINGS

The purpose of this project is to screen 2007-2009 hunter-harvested (and presumably healthy) moose (*Alces alces*) for a variety of disease agents. The results are intended to indicate which diseases the northeast Minnesota (NE MN) moose population is being exposed to as well allowing for comparisons between similar testing completed on non-hunting moose mortalities from the same population. Positive results confirm moose were exposed to, though not necessarily ill from, eastern equine encephalitis, West Nile Virus, malignant catarrhal fever, *Neospora*, anaplasmosis, bovine herpes virus 1, bovine viral diarrhea virus 1 and 2, *Leptospira sp,* and parainfluenza virus 3. A variety of fecal parasites were identified on fecal examination and multiple organisms were cultured from lung and liver samples. Histological examination was performed on all submitted tissues. All results were negative for *Mycobacterium paratuberculosis*, brucellosis, blue tongue virus, epizootic hemorrhagic disease, chronic wasting disease, and bovine tuberculosis.

INTRODUCTION

Several lines of evidence suggest that the moose population in northeastern Minnesota is declining. Since 2002, annual survival and reproductive rates were substantially lower than documented elsewhere in North America (Lenarz et al. 2007) and population modeling based on these vital rates indicated that the population has declined since at least 2002 (Lenarz unpublished). Recruitment rates and the percent twins observed during aerial surveys have steadily declined since 2002 (Lenarz 2009). In addition, hunter success rates have steadily declined over the past 8 years (Lenarz 2009). Finally, anecdotal reports from local residents have reported a noticeable decline in moose numbers.. Parasites have been documented, including *Parelaphostrongylus tenuis, Echinococcus granulosus, Elaeophora schneideri, Sarcocystis spp*., *Fascloides magna,* and *Dermacenter albipictus*. Copper deficiency has been documented in some moose. Many causes of mortality remain unknown with numerous prime-age animals dying, often during low stress periods of the year. Poor antler development has also been noted in some bull mortalities.

The purpose of this project is to screen 2007-2009 hunter-harvested (and presumably healthy) moose for a variety of disease agents. The results are intended to indicate which diseases the NE MN moose population is being exposed to as well as allowing for comparisons between similar testing completed on non-hunting moose mortalities from the same population. Positive results only indicate that the animal was exposed to the disease agent and are not diagnostic of clinical disease. While some of the test results may be all negative, this does not necessarily mean that the disease is not present or impacting the population. Some diseases cause death so quickly, or without an immune response, that finding a positive in a seemingly healthy animal would be extremely rare.

METHODS

In order to conduct this herd health assessment, hunters (both tribal and state) were asked to collect samples of lung, liver, blood, feces, hair, ticks, and an incisor for aging. We

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provided a presentation and instructions relative to the moose health survey at the mandatory Minnesota Department of Natural Resources (MNDNR) Moose Hunt Orientation Sessions and tribal natural resource offices. Hunters were given a sampling kit with instructions at the orientation sessions. Post-harvest, these samples were dropped off at official registration stations by the hunters when they registered their moose. At the time of registration, hunters were asked to locate their kill site on appropriate maps.

We provided hunters with all equipment needed for sample collection/preservation. Sample kits included the following items: styrofoam cooler; 1-60cc syringe for blood collection; 6-15cc serum tubes for blood storage; 3 whirlpaks for a sample of liver, lung and feces; 2 specimen jars with formalin for liver and lung samples; 2 coin envelopes for tooth and hair; datasheet; protocol; Sharpie marker; 1 pair of large vinyl gloves; and 1 icepack. In 2008, 1 15-cc EDTA blood tube was added to the kit.

The hunter collected blood from the chest cavity as soon after death as possible, using a 60 cc syringe. The blood was placed in serum tubes and kept cool until they were delivered to official MNDNR registration stations or tribal natural resource offices. Liver and lung samples were collected and split, with half placed in a formalin jar, while the other half was frozen in whirlpak bags. If the hunter found anything unusual, such as a large abscess or tumor, those samples were collected and split between the preservative methods (formalin fixation and freezing). Blood was centrifuged at the registration stations or tribal natural resource offices and serum was extracted and frozen. In 2008, whole blood was also collected. Blood smears were made and the whole blood was frozen. Also, retropharyngeal lymph nodes, obexes, and brains (2008 only) were removed by trained MNDNR staff, tribal staff, and volunteers at the registration stations with permission of the hunter. Portable refrigerators were located in advance at the registration stations to maintain the tissue samples. Samples were submitted to the University of Minnesota Veterinary Diagnostic Laboratory (U of M VDL), where much of the testing occurred. A few of the tests were outsourced to the National Veterinary Services Laboratories (NVSL) in Ames, IA.

RESULTS AND DISCUSSION

In 2007, moose hunters at MNDNR registration stations and tribal natural resource offices throughout moose range turned in a total of 135 sampling kits in northeastern Minnesota (Figure 1). Of the kits submitted, 118 were complete, with the reminder being partial submissions.

In 2008, moose hunters turned in 123 sampling kits (Figure 1). Of the kits submitted, 111 were complete. The quality of the samples collected both years was quite good, with very few errors in tissue identification or insufficient quantities. The following is a brief overview of the major findings:

Eastern Equine Encephalitis (EEE)

A combined total of 228 serum samples were submitted to NVSL for Virus Neutralization (VN) testing in 2007 and 2008. Positive results were reported for 14 of the moose (14/228 = 6.1%). Multiple animals had titers ≥ 100. See Table 1 for a breakdown of results by year and titer levels.

The positive results indicate that these animals were exposed to the EEE virus as the VN test prevents cross-reactivity with other viruses. 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.

EEE is spread by mosquitoes and causes neurologic signs and often death. It poses a greater mortality threat for most species than West Nile Virus does (though the effects of EEE infection have not been studied in moose).

West Nile Virus (WNV)

A combined total of 229 samples were submitted to NVSL for VN testing in 2007 and 2008. Positive results were reported for 87 of the moose (87/229 = 37.9%). Multiple animals had titers ≥ 100. See Table 1 for a breakdown of results by year and titer levels.

The positive results indicate that these animals were exposed to the WNV virus as the VN test prevents cross-reactivity with other viruses. 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.

Little is known about the effects of WNV in moose. In white-tailed deer (*Odocoileus virginianus*) it has been found that they often have a low titer and no clinical signs. However, the USDA has found that reindeer (*Rangifer tarandus*) infected with WNV have high mortality rates and high titers, indicating that the virus is more serious for some species than others.

Malignant Catarrhal Fever (MCF)

A combined total of 229 samples were submitted to NVSL for peroxidase-linked assay (PLA) testing in 2007 and 2008. If the PLA test came back positive, the samples were screened with a VN test. A total of 90 samples tested positive on the PLA test (90/229 = 39.3%). All the VN testing results were negative.

The PLA test is more sensitive than the virus isolation, meaning it is much better at identifying true positives. VN is more specific, which means it is better at identifying true negatives. There are a couple of issues with this testing. The PLA reacts with multiple Gammaherpes Viruses (such as the wildebeest strain, the sheep strain, the deer strain, etc). A PLA positive does not indicate what strain has been found, only that one has. The higher the positive value with the PLA test, the stronger the positive in the sample. 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 a gammaherpes virus, but not the wildebeest strain.

Gammaherpes viruses have been documented to cause serious illness and death in moose and other ruminants. The clinical symptoms can mimic *P. tenuis* infection as the animals often exhibit neurological deficits, go blind, and thrash 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. found serologic evidence of exposure in numerous species across Alaska and reported 1% prevalence in moose (2002).

Fecal Examination for Parasites

A combined total of 225 fecal samples were screened for evidence of parasites in 2007 and 2008. Parasites were identified in 28 samples (28/225 = 12.4%). In 2007, evidence of parasitism was found in 18 of the samples (18/123 = 14.6%). Five of the samples contained *Nematodirus*, 5 contained *Moniezia*, 6 contained Strongyle type ova, 1 contained *Nematodirus*/*Moniezia*, and 1 contained *Dictyocaulus.* In 2008, evidence of parasitism was found in 10 of the samples (10/102 = 9.8%). Three of the samples contained *Nematodirus*, 5 contained *Moniezia*, and 2 contained Strongyle type ova.

Negative results do not necessarily mean the animal was parasite free, only that it was not actively shedding at the time the feces were collected.

Fecal Sedimentation

In 2007, a total of 12 fecal samples underwent fecal sedimentation. Sedimentation is used to identify patent liver fluke infection. None of the samples were positive for liver fluke ova**.** This screening was not repeated in 2008.

Moose are considered dead-end hosts for liver fluke, though reports of moose passing fluke ova in their feces exist. Negative results do not mean that the animals weren't infected with liver flukes, only that they were not actively shedding ova in their feces.

Liver and Lung Culture

In 2007, a total of 121 livers were cultured for bacteria. No significant growth was found in 119 samples, *E. coli* was isolated from 1 sample, and *Pantoea sp*. was isolated from 1 sample. A total of 125 lung samples were submitted for bacterial culture. No significant growth was found in 124 of the samples and *E. coli* was isolated from 1 sample. The *E. coli* isolations are likely due to cross-contamination from contents of the intestinal tract.

The decision was made in 2008 to only culture liver and lung if the histology results warranted it. These results have yet to be reported.

Culture-Other

In 2007, one abscess was submitted and cultured. *Arcanobacterium pyogenes* was isolated. *Arcanobacterium pyogenes* is a bacterium commonly found in infected wounds and abscesses of ruminants and other animals. There were also samples from 2 spleens submitted for culture in 2007. No significant growth was documented in 1, and *Pantoea sp*. was isolated from the other.

Pulmonary *Mycoplasma* Culture

In 2007 a total of 119 lung samples were submitted for *Mycoplasma* culture. None was isolated. This was discontinued in 2008.

***Mycobacterium paratuberculosis* (Johne's)**

A combined total of 192 fecal samples were submitted for *M. paratuberculosis* culture in 2007 and 2008. All culture results were negative. In 2007, PCR was run on 118 fecal samples, with all results negative, and Biocor (serology) was run on 121 samples, with all of the results negative. PCR and Biocor testing were not continued in 2008

The negative fecal cultures and PCR results indicate that those moose were not actively shedding the bacterium. The negative Biocor results indicate that these animals had not been exposed to the bacterium.

All species of ruminants are believed to be susceptible to Johne's and it is frequently diagnosed in cattle and sheep (Manning and Collins, 2006). Clinical signs in wild ruminants are similar to those seen in sheep, and 1 moose with diarrhea, which resulted in death, was diagnosed with Johne's (Soltys et al., 1967). Serologic evidence of exposure to Johne's in moose has been documented, with 9/426 (2.1%) seropositive moose in Norway (Tryland et al., 2004).

Anaplasmosis

A combined total of 219 samples were screened for Anaplasmosis (*Anaplasma phagocytopila*, formerly *Ehrlichia phagocytophila*) with the card test in 2008 and 2009. One of

these samples was positive (1/219 = 0.5%). Positive test results indicates that exposure to this bacterium is occurring. See Table 1 for a breakdown of results by year and titer levels.

Moose are known 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* (Jenkins et al., 2001). In sheep, this disease produces significant effects on the immunological defense system, increasing their susceptibility to disease and secondary infections (Larson et al., 1994).

A. phagocytophilum is known to occur in MN. In fact, from 1998-2005, 790 human cases were reported in MN and in recent years the MN Department of Health has documented an expansion in the areas in which MN residents are exposed to vector-borne diseases (MN Department of Health). The NE MN population of moose overlaps with the primary area of tick-borne disease risk determined by the MN Department of Health and NE MN moose often have a significant infestation of winter ticks.

Borreliosis (Lymes Disease)

A combined total of 221 samples were screened for lymes disease with an immunofluorescence assay (IFA) in 2007 and 2008. Positive results were reported for 41 of the samples (41/221 = 18.6%). See Table 1 for a breakdown of results by year and titer levels.

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.

Brucellosis

A combined total of 205 samples were submitted in 2007 and 2008 for *Brucella* screening with the card test. All of the results were negative. These negative results indicate that these animals were not likely exposed to the bacterium. See Table 1 for a breakdown of results by year.

While naturally occurring fatal *Brucella* infections have been documented in free ranging moose (Honour and Hickling, 1993) and serologic evidence suggests that moose are being exposed to *Brucella sp*. (Zarnke, 1983), evidence suggests that the prevalence is low (Honour and Hickling, 1993).

Bovine Viral Diarrhea Virus (BVD) 1 & 2

A combined total of 230 samples were submitted for serum neutralization (SN) testing for BVD 1 & 2 in 2007 and 2008. Positive results were reported for 3 of the samples (3/230 = 1.3%). These results indicate that the moose population is being exposed to BVD. See Table 1 for a breakdown of results by year and titer levels.

BVD is considered a major disease of cattle and is thought to be the most common infectious cause of reproductive failure in beef herds in the western U.S. BVD is also considered a disease of wild ruminants such as moose, caribou (*Rangifer tarandus*), and deer. Some clinical signs of BVD include diarrhea, dehydration, fever, impaired vision and hearing, depression, abortions, and weakened neonates. Serologic evidence of BVD has been documented in 4 of 22 moose sampled in Alberta (Thorsen and Henderson, 1971).

Bovine Herpes Virus 1 (BHV)

A combined total of 230 samples were screened for BHV using a SN test in 2007 and 2008. One results was reported as positive (1/230 = 0.4%). See Table 1 for a breakdown of results by year and titer levels.

BHV is a disease of the respiratory tract. It is believed to infect all ruminant species and has been isolated from a large number of wild species. It is most commonly isolated in feedlot cattle.

Blue Tongue Virus (BTV)

A combined total of 231 samples were screened using a Competitive Enzyme-Linked Immunoabsorbent Assay (cELISA) for BTV in 2007 and 2008. All results were negative. See Table 1 for a breakdown of results by year.

BTV is a hemorrhagic disease transmitted by a biting midge that is known to cause illness and death in white-tailed deer. While it is known to be infective to a variety of domestic and wild ruminants, clinical disease is quite variable.

Epizootic Hemorrhagic Disease (EHD)

A combined total of 231 samples were screened for EHD using an Agar Gel Immuno Diffusion (AGID) test in 2007 and 2008. All results were negative. See Table 1 for a breakdown of results by year.

EHD is a hemoorhagic disease transmitted by a biting midge that is known to cause illness and death in white-tailed deer. While it is known to be infective to a variety of domestic and wild ruminants, clinical disease is quite variable.

Leptospira sp.

A combined total of 231 samples were screened for 6 species of *Leptospira* using a microscopic agglutination test (MAT) in 2007 and 2008. Positive results per species are reported below. See Table 1 for a breakdown of results by year and titer levels.

- *L. bratislava*:
  - 4/231 (1.7%)
- *L. canicola*:
  - 2/231 (0.9%)
- *L. grippothyphosa*:
  - 4/231 (1.7%)
- *L. hardjo*:
  - 0/231
- *L. interrogans* serovar *icterohaemorrhagicae*:
  - 16/231 (6.9%)
- *L. pomona*:
  - 13/231 (5.6%)

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, and moose are known to frequent these habitats.

Neospora sp.

A combined total of 232 samples were screened for *Neospora* with an ELISA test in 2007 and 2008. Positive results were reported for 9 samples (9/232 = 3.9%). See Table 1 for a breakdown of results by year and titer levels.

While clinical disease due to infection is best described in domestic animals, reports of ill effects due to *Neospora* infection in wildlife do exist. Systemic neosporosis was diagnosed in a California black-tailed deer (*Odocoileus hemionus*) that was found dead (Woods et al., 1994) and the parasite was identified in the brain of a full-term stillborn deer from a zoo in France (Dubey et al., 1996).

Antibodies to Neospora have been found in numerous species of wildlife, including 8/61 moose from NE MN (Gondim et al., 2004).

Parainfluenza Virus 3 (PI)

A combined total of 232 samples were screened for PI using a hemagglutination inhibition (HI) test in 2007 and 2008. There was 1 positive result (1/232 = 0.4%). See Table 1 for a breakdown of results by year and titer levels.

The positive result indicates that NE MN moose are being exposed to PI. Domestic ruminants are considered the main source of infection for free-ranging ruminants. However, studies of white-tailed deer, which were geographically isolated from livestock, indicate that large wild ruminant populations can maintain PI and latency of the viruses allows them to be maintained in a restricted host population for a long period (Sadi et al. 1991).

Chronic Wasting Disease (CWD)

In 2007, a total of 14 obex samples and 23 retropharyngeal lymph nodes were screened for CWD using immunohistochemistry (IHC). In 2008, 32 obex samples and 33 lymph node samples were screened for CWD. All results were negative.

CWD is a transmissible spongiform encephalopathy that causes neurological disease in cervids. CWD is known to occur in moose, but has never been documented in wild cervids in MN.

Bovine Tuberculosis

In 2007, 23 sets of head lymph nodes (parotid, retropharyngeal, and submandibular) were collected and cultured for *Mycobacterium bovis*. In 2008, 33 sets of head lymph nodes were submitted. All results were negative.

Bovine tuberculosis is a chronic, progressive bacterial disease that infects a wide array of mammals. Bovine tuberculosis has been found in wild white tailed deer in small, localized area in northwestern MN, but has not been found in any wild animals within the moose hunt permit areas.

Brain Histopathology

A total of 23 brains were collected and submitted for histopathology. These results are pending at this time. This examination is meant to help identify if there are chronic migration tracts (presumably due to *P. tenuis*) present in the brains of apparently healthy animals.

Liver Histopathology

In 2007, a total of 114 liver samples underwent histological examination. There were no significant findings with 57 of the samples. Thirty-nine of these samples had a diffuse, hepatocellular lipidosis, of which 27 were classified as mild and 12 were classified as moderate.

Fourteen of the samples exhibited varying types and degrees of hepatitis. Perihepatitis was described in 3 samples. Four of the samples exhibited evidence of fluke infection, either currently or previously. Three samples exhibited fibrosis. There were single cases of lymphoid hyperplasia, hydatid cysts, and possible capsulitis/peritonitis. The results from samples collected in 2008 have yet to be evaluated.

Lung Histopathology

In 2007, a total of 126 lung samples underwent histological examination. There were no significant findings in 93 of the samples examined. Pulmonary hemorrhage, likely related to the gunshot, was documented in 10 of the samples. Hydatid cysts, likely *Echinococcus*, were found in 5 samples. Lymphoid hyperplasia was observed in 6 samples. Four samples had chronic pleuritis. Varying types and degrees of pneumonia were found in 4 samples. Single cases of bronchitis, emphysema, an eosinophilic granulama, and intrabronchial foreign material (likely agonal aspiration) were reported. The results from samples collected in 2008 have yet to be evaluated.

Other Histology

In 2007, a total of 24 brainstem samples underwent histologic examination. Twenty-three had no significant findings and 1 had mild hemorrhaging, which was likely related to the gunshot. Twenty-one lymph nodes were examined. Twenty exhibited no significant findings and 1 of them had blood resorption, which was likely related to the gunshot. Fifteen spleens were examined. None of them exhibited any significant findings. One sample of cerebellum, kidney, heart, and brain were examined, with no significant findings. One sample of the colon and small intestine were examined and found to have enteritis. The results from samples collected in 2008 have yet to be evaluated.

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Table 1. Serology results from the moose herd health assessment broken down by year.

Year	Disease	n	Positive	Comments
2007	EEE	116	5 (4.3%)	Titers: 2 @ 100, 3 @ ≥100
2008	EEE	112	9 (8%)	Titers: 4 @ 10, 1 @ 100, 4 @ ≥100
2007	WNV	117	45 (38.5%)	Titers: 32 @ 10, 6 @ 100, 7 @ ≥ 100
2008	WNV	112	42 (37.5%)	Titers: 34 @ 10, 5 @ 100, 3 @ ≥ 100
2007	MCF	117	8 (6.8 %)	Titers: 4 @ 20, 4 @ 100
2008	MCF	112	82 (73.2%)	Titers: 71 @ 20, 11 @ 100
2007	Anaplasmosis	117	1 (0.9%)	
2008	Anaplasmosis	102	0	
2007	Borreliosis	111	38 (34.2%)	Titers: 7 @ 80, 7 @ 160, 12 @ 320, 3 @ 640, 9 @ 1280
2008	Borreliosis	110	3 (2.7%)	Titers: 1 @ 160, 1 @ 320, 1 @ 640
2007	Brucellosis	112	0	
2008	Brucellosis	93	0	
2007	BVD 1 and 2	120	2 (1.7%)	Titers: 1 @ 1024/4096, 1 @ 128/256
2008	BVD 1 and 2	110	1 (1%)	Titers: 1 @8/8
2007	BHV	120	0	
2008	BHV	110	1 (1%)	Titers: 1 @ 8
2007	BTV	121	0	
2008	BTV	110	0	
2007	EHD	121	0	
2008	EHD	110	0	
2007	L. bratislava	121	4 (3.3%)	Titers: 2 @ 100, 2 @ 200
2008	*L. bratislava*	110	0	
2007	*L. canicola*	121	2 (1.7%)	Titers: 1 @ 100, 1 @ 200
2008	*L. canicola*	110	0	
2007	*L. grippothyphosa*	121	3 (2.5%)	Titers: 2 @ 100, 1 @ 200
2008	*L. grippothyphosa*	110	1 (1%)	Titers: 1 @ 100
2007	*L. hardjo*	121	0	
2008	*L. hardjo*	110	0	
2007	*L. interrogans* serovar *icterohaemorrhagica*e	121	2 (1.7%)	Titers: 1 @ 100, 1 @ 200
2008	*L. interrogans* serovar *icterohaemorrhagicae*	110	14 (12.7%)	Titers: 11 @ 100, 3 @ 200
2007	L. pomona	121	10 (8.3%)	Titers: 4 @ 100, 1 @ 200, 5 @ 400
2008	*L. pomona*	110	3 (2.7%)	Titers: 1 @ 200, 2 @ 400
2007	Neospora	122	0	
2008	Neospora	110	9 (8.2%)	
2007	PI	122	1 (0.8%)	Titers: 1 @ 10
2008	PI	110	0	

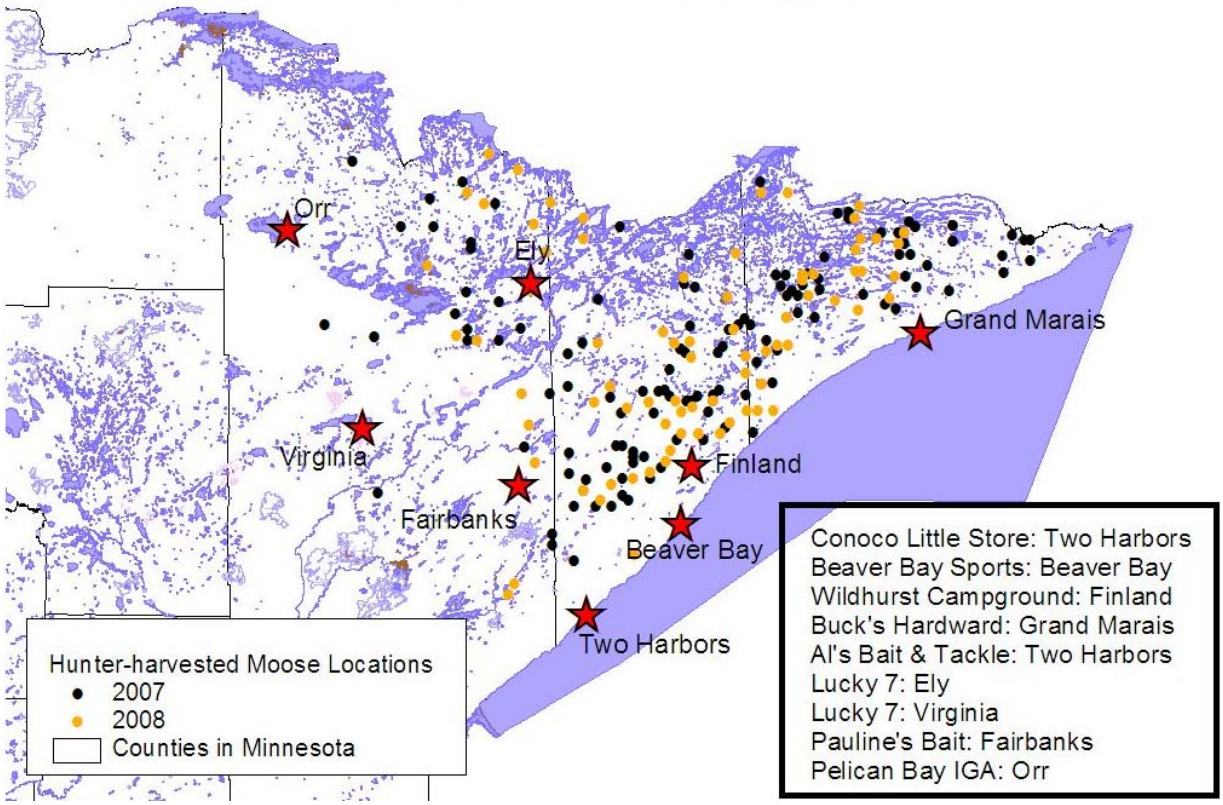

Figure 1. Locations of 2007 and 2008 hunter-harvested moose included in health assessment project in Minnesota.

SURVEILLANCE FOR HIGHLY PATHOGENIC AVIAN INFLUENZA IN MINNESOTA'S WATERFOWL

Michelle Carstensen[1] and Michael DonCarlos

SUMMARY OF FINDINGS

As part of a national strategy for early detection of highly pathogenic avian influenza (HPAI) in North America, Minnesota Department of Natural Resources (MNDNR) and the United States Department of Agriculture (USDA) conducted surveillance for the virus in waterfowl in the state. A combined total of 1,547 birds were sampled for HPAI in Minnesota during 2008. Testing did not result in any positive cases of HPAI, especially the Asian strain of subtype H5N1, however numerous ducks (*n*=43) did test positive for a low pathogenic strain of avian influenza with the subtype H5. Approximately 65,000 wild birds were sampled throughout the United States in 2008, and no positive cases of HPAI were detected. It is likely that Minnesota will continue surveillance for the virus in the state's waterfowl next year, in cooperation with the Mississippi Flyway, Council of the U.S. Fish and Wildlife Service, and the USDA.

INTRODUCTION

Recent worldwide attention on the spread of a highly pathogenic strain of avian influenza, subtype H5N1, from Asia to Europe and Africa in 2006 has led to the development of a coordinated National Strategic Plan for early detection of HPAI-H5N1 introduction into North America by wild birds. Although movements of domestic poultry or contaminated poultry products, both legally and illegally, are believed to be the major driving force in the spread of HPAI-H5N1, migratory birds are thought to be a contributing factor.

This national plan outlined a surveillance strategy that targeted sampling of wild bird species in North America that have the highest risk of being exposed to or infected with HPAI-H5N1 because of their migratory movement patterns. Currently, these include birds that migrate directly between Asia and North America, birds that may be in contact with species from areas in Asia with reported outbreaks, or birds that are known to be reservoirs of AI. A step-down plan was developed by the Mississippi Flyway Council in 2006 identifying Minnesota as a key flyway state needed to participate in regional sampling for early detection of HPAI-H5N1 in migratory ducks, geese, and shorebirds.

In July 2008, the MNDNR entered into a $90,000 cooperative agreement with the United States Department of Agriculture's Wildlife Services (USDA-WS) to sample 800 wild birds (either live-caught or hunter-harvested) in Minnesota for HPAI-H5N1 during 2008. In addition to the 800 samples to be collected by MNDNR, USDA-WS was also planning to collect a similar number of samples in the state during the same period. Bird species that were targeted include those listed as priority species in the National Strategic Plan or approved for sampling in Minnesota by the Mississippi Flyway Council.

Avian influenza is a viral infection that occurs naturally in wild birds, especially waterfowl, gulls, and shorebirds. It is caused by type A influenza viruses that have 2 important surface antigens, hemagglutinin (H) and nuraminidase (N), that give rise to 144 possible virus subtypes. Influenza viruses vary widely in pathogenicity and ability to spread among birds. The emergence of an Asian strain HP-H5N1 virus in 1996 and subsequent spread of the virus in Asia, Africa, and Europe has killed thousands of wild birds and millions of domestic poultry. In 1997, HP-H5N1 became zoonotic in Hong Kong and to-date has infected at least 423 humans in Eurasia and Africa, resulting in over 258 deaths.

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METHODS

The MNDNR planned to sample 100 common goldeneye (*Bucephala clangula*), 100 ring-necked ducks (*Aythya collaris*), 50 mallards (*Anas platyrhynchos*), and 50 Canada geese (*Branta Canadensis*) during the summer months, primarily in conjunction with planned banding activities. In the fall, through hunter-harvested surveillance, sampling targets were as follows: 100 Northern pintails (*Anas acuta*), 100 mallards, 100 American green-winged teal (*Anas crecca*), 100 American blue-winged teal (*Anas discors*), 50 Northern shovelers (*Anas clypeata*), and 50 American wigeon (*Anas americana*). USDA-WS planned to sample a similar number of either the duck species mentioned above or others from their functional group (e.g., dabblers, divers, shorebirds) as well as 50 Canada geese. If sampling goals per species could not be met, other targeted waterfowl species within the same functional group could be sampled and counted toward the state's total. Sampling strategies were coordinated between the MNDNR and USDA-WS to maximize access to targeted birds species through existing banding operations and fall hunter-harvested surveillance.

Cloacal and oral-pharyngeal swabs were used to collect samples and they were submitted to the Veterinary Diagnostic Laboratory in St. Paul, MN for initial screening for the virus. If positive for avian influenza virus, samples were forwarded to the National Veterinary Services Laboratories in Ames, IA for strain-typing.

RESULTS AND DISCUSSION

From July 1, 2008 through March 31, 2009 MNDNR and USDA collected a total of 1,547 samples from wild-caught live birds (*n*=519), hunter-harvested birds (*n*=961), agency (USDA-WS) harvested (*n*=29), and mortality/morbidity events (*n*=38). USDA also collected 716 fecal samples. Thus, a combined total of 2,263 bird samples were screened for HPAI-H5N1 in Minnesota in 2008 (Figure 1, Table 1).

Testing did not result in any positive cases of HPAI-H5N1; however 10 different duck species tested positive for a low pathogenic strain of avian influenza with the subtype H5, and only 1 tested positive for a N1 subtype (Figure 2, Table 2). The testing protocol was limited to the screening for H5, H7, and N1 subtypes only; however in some cases other subtypes were identified and reported.

According to the latest numbers on the United States Geologic Survey's website (http://wildlifedisease.nbii.gov/ai/), approximately 65,000 birds have been sampled for HPAI-H5N1 in the U.S. in 2008. No positive cases of HPAI-H5N1 have been found anywhere in North American to date. Since the majority of H5 positives (low pathogenic forms only) detected by USDA-WS in the United States since 2006 have been found in dabbling ducks, the primary focus of future sampling will be on these species (Genus *Anas*, *Aix*, *Cairina*, and *Dendrocygna).*

Surveillance for HPAI-H5N1 will likely continue in Minnesota, and other parts of the U.S. next year. The USDA has banked all samples taken from 2006 to 2008, and is currently accepting proposals from state agencies and universities for further avian influenza research. Minnesota remains prepared to assist with future surveillance objectives if needed. In addition, the MNDNR has developed a surveillance and response plan for HPAI in wild birds, which includes increased vigilance of mortality and morbidity events within the state.

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goals. Lastly, much of the hunter-harvested sampling was accomplished through assistance from Pat Reddig, University of Minnesota, and numerous students from both the Natural Resources program and the veterinary college.

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Table 1. Bird species sampled for highly pathogenic avian influenza H5N1 by Minnesota Department of Natural Resources and United States Department of Agriculture-Wildlife Services in 2008. Table includes live-bird, hunter-harvested, agency harvested, and mortality/morbidity. Fecal samples are excluded as they cannot be attributed to an individual species.

Species sampled	*n*
Ducks	
American Coot	11
American Green-Winged Teal	138
American Wigeon	78
American Blue-Winged Teal	153
Bufflehead	13
Canvasback	27
Common Goldeneye	112
Common Merganser	16
Gadwall	41
Greater Scaup	4
Hooded Merganser	4
Lesser Scaup	33
Mallard	305
Northern Pintail	58
Northern Shoveler	66
Red-Breasted Merganser	1
Redhead	21
Ring-Necked Duck	179
Wood Duck	74
Canada Geese	171
Other	
American White Pelican	16
American Woodcock	1
Double-Crested Cormorant	14
Lesser Snow Goose	1
Pied-Billed Grebe	1
Ruddy Duck	1
Ring-Billed Gull	6
Surf Scooter	1
Unidentified Duck	1
Total	1,547

Table 2. Results of avian influenza testing by the National Veterinary Services Laboratories (NVSL)[1] from samples submitted by Minnesota in 2008.

Species	Collection strategy	Test type[1]	Test result	Total
American Green-Winged Teal	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	4
American Green-Winged Teal	Hunter-harvested	AI NVSL-Subtyping	H3N2	1
American Wigeon	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	2
American Wigeon	Live Wild Bird	AI NVSL-AIV H5 RRT-PCR	H5	2
American Wigeon	Hunter-harvested	AI NVSL-Subtyping	H5N2	1
American Wigeon	Live Wild Bird	AI NVSL-Subtyping	H10N7	1
American Wigeon	Live Wild Bird	AI NVSL-Subtyping	H10N8	1
American Wigeon	Live Wild Bird	AI NVSL-Subtyping	H4N8	1
Bufflehead	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	1
Blue-winged Teal	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	4
Blue-winged Teal	Hunter-harvested	AI NVSL-Subtyping	H4N4, N8	1
Gadwall	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	2
Lesser Scaup	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	1
Mallard	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	24
Mallard	Hunter-harvested	AI NVSL-Subtyping	H3N4, N8	1
Mallard	Hunter-harvested	AI NVSL-Subtyping	H4N8	1
Mallard	Hunter-harvested	AI NVSL-Subtyping	H4N9	1
Mallard	Hunter-harvested	AI NVSL-Subtyping	H5N2	4
Mallard	Hunter-harvested	AI NVSL-Subtyping	H6N1	1
Mallard	Live Wild Bird	AI NVSL-Subtyping	H10N7	1
Northern Pintail	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	2
Northern Shoveler	Hunter-harvested	AI NVSL-AIV H5 RRT-PCR	H5	1
Northern Shoveler	Live Wild Bird	AI NVSL-Subtyping	H3N8	1

[1]Test results include AI NVSL Subtyping = identifies other strains of avian influenza that are not H5N1;
AI NVSL-AIV H5 RRT-PCR = test for the H5 avian influenza subtype only.

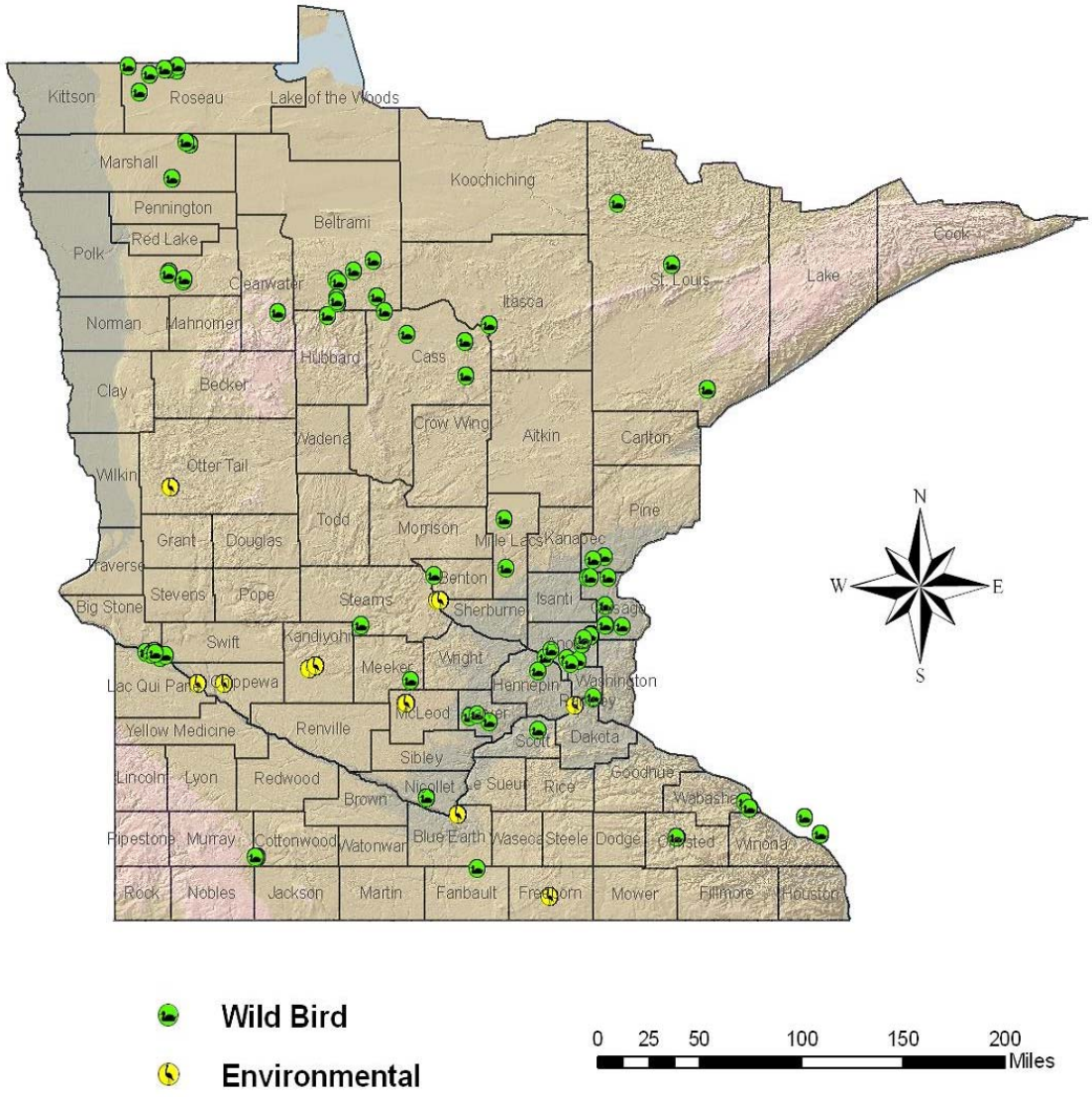

Figure 1. Collection sites from which live bird and environmental (fecal) samples (*n*=2,263) were tested for highly pathogenic avian influenza in Minnesota during 2008.

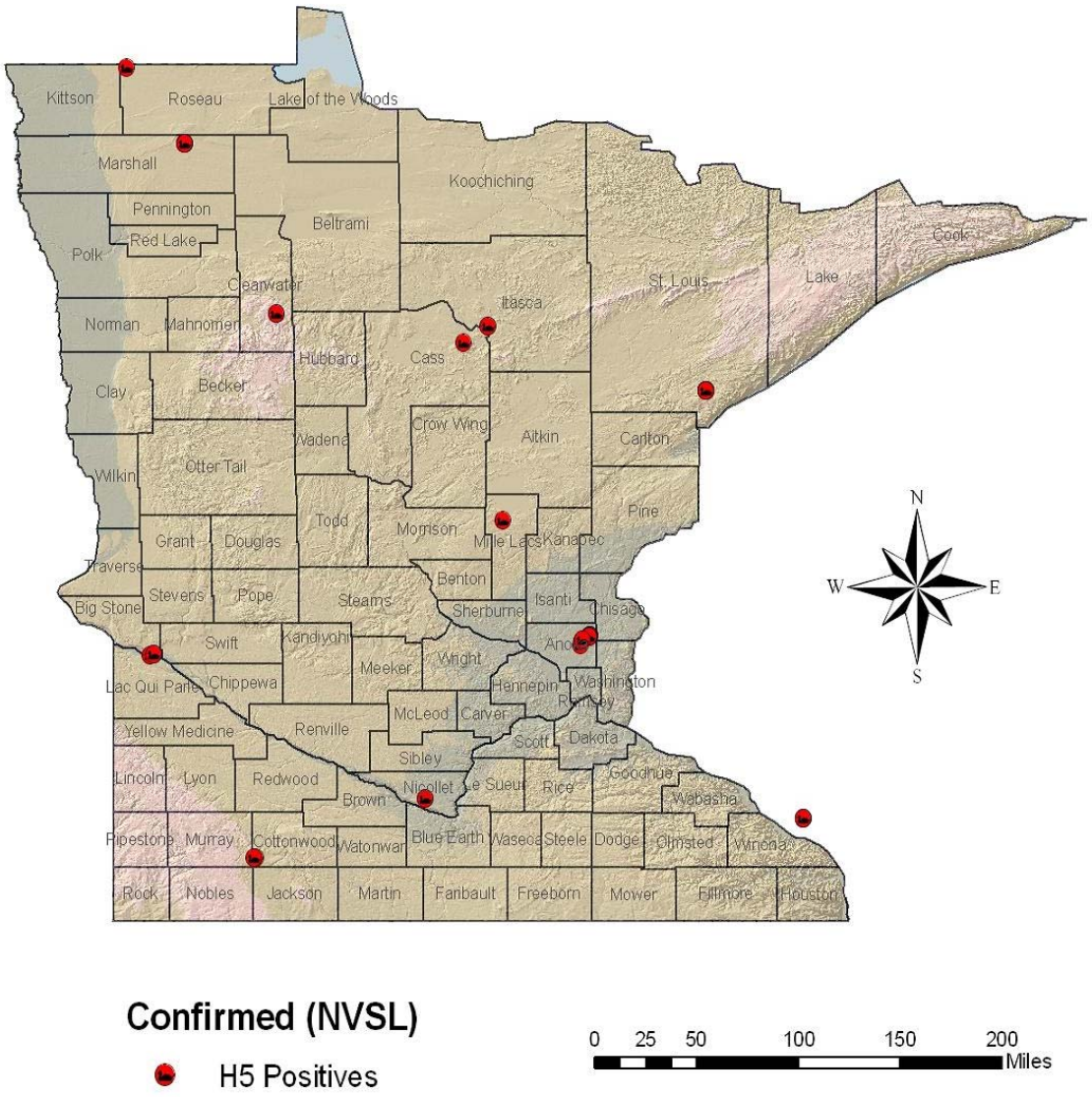

Figure 2. Collection sites where a low pathogenic H5 strain was detected (red dots) among the waterfowl (*n*=43) sampled in Minnesota during 2008.

MINNESOTA DEPARTMENT OF NATURAL RESOURCES CWD SURVEILLANCE PROGRAM 2008

Michelle Carstensen[1], Erika Butler, Michael DonCarlos, and Lou Cornicelli

SUMMARY OF FINDINGS

In 2008 and early 2009, the Minnesota Department of Natural Resources (MNDNR) sampled 1,440 hunter-harvested white-tailed deer (*Odocoileus virginianus*) for chronic wasting disease (CWD). The majority of these samples (66%) were collected in northwestern Minnesota, in conjunction with surveillance efforts for bovine tuberculosis; the remainder (34%) of samples were collected along the MN-WI border. All of the samples were negative for CWD. In addition, MNDNR submitted samples from 56 deer through targeted surveillance, which included sick animals, escaped captive cervids, and roadkills; these samples were also negative for the disease. MNDNR plans to conduct hunter-harvested surveillance in southeastern MN in fall 2009, in response to a recently detected CWD-positive captive elk facility in Olmsted county and the continued risk of disease spread from CWD-infected wild deer from Wisconsin.

INTRODUCTION

Chronic wasting disease is a transmissible spongiform encephalopathy (TSE) that affects elk (*Cervus elaphus*), mule deer (*Odocoileus hemionus*), white-tailed deer, and moose (*Alces alces*). TSEs are infectious diseases that alter the morphology of the central nervous system, resulting in a "sponge-like" appearance of this tissue. The etiological agent of CWD is an infectious protein, called a prion. Precise mechanisms and rates of CWD transmission remain unclear, although animal-to-animal contact and environmental contamination are thought to promote the spread of the disease. Incubation time of the disease, from infection to clinical signs, averages 16 months but can range from a few months to nearly 3 years. There is a limited distribution of infection in the body (primarily brain, spinal column, spleen, and lymph nodes) although a recent study demonstrated that prions can also be found in muscle. Clinical signs may include a loss of body condition and weight, excessive salivation, ataxia, and behavioral changes. Currently, there is no known treatment for the disease and it is always fatal. There is also no documented evidence of transmission of CWD to other species, including humans.

To date, CWD has been diagnosed in 3 captive elk herds and 1 captive white-tailed deer herd within the state of Minnesota. Two of the elk herds (Stearns and Aitkin counties) were discovered in 2002 and depopulated; no additional CWD positive animals were found. In spring 2006, a captive white-tailed deer was found infected with CWD from a mixed deer/elk herd in Lac Qui Parle county. That herd was also depopulated without additional infection being detected. However, over 40 additional premises within the state have been impacted as a result of trace-forwards and trace-backs conducted by the Minnesota Board of Animal Health (BAH). Currently, nearly all of these herds have undergone surveillance protocols. In all of these cases, the original source of the CWD has not been identified. In early 2009, a third captive elk herd (Olmsted county) was found infected with CWD. This most recent herd is expected to be depopulated once an indemnity agreement can be reached between the herd owner and the United States Department of Agriculture (USDA). MNDNR and BAH are working cooperatively to address the impact of CWD in these captive facilities, as well as management options to control its spread.

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Over the past 7 years, MNDNR has tested in excess of 30,000 deer across the state for CWD, all of which have been negative. Consequently, in recent years, sampling has been scaled back to address 3 main components:

1. Sampling of animals exhibiting symptoms of CWD (targeted surveillance);
2. Sampling of animals in response to elevated risk factors (e.g., detection of positive animals in captive cervid farms, or proximity of Minnesota to positive CWD cases in other states); and
3. Sampling of hunter-killed deer for CWD in conjunction with surveillance for bovine tuberculosis.

METHODS

MNDNR continues to sample deer exhibiting clinical symptoms consistent with CWD (targeted surveillance). Information has been disseminated to wildlife staff regarding what to look for regarding symptomatic deer. Staff were provided the necessary equipment and training for lymph node removal and data recording. The number of samples expected through targeted surveillance is estimated to be less than 100 animals annually, as few reports of sick deer are taken.

Hunter-harvested surveillance occurs at deer registration stations during the firearm hunting seasons. At the stations, hunters were asked to voluntarily submit retropharyngeal lymph node samples for CWD testing. Samples were submitted to the Veterinary Diagnostic Laboratory at the University of Minnesota for disease screening. Any presumptive positive samples were submitted to the National Veterinary Services Laboratories (Ames, IA) for official confirmation of the disease. Hunter information was recorded, including the hunter's name, address, telephone number, MNDNR number, and location of kill. Maps were provided to assist the hunters in identifying the location (Township, Range, and Section) of the kill. Cooperating hunters were given a cooperator's patch.

During fall 2008, MNDNR also collected approximately 1,250 lymph node samples from hunter-harvested deer in the northwest as part of a surveillance program for bovine tuberculosis. MNDNR had planned to screen approximately 1,000 of these samples for CWD as well. The registration stations that were selected to screen for both diseases include those in the northwest part of the state. Registration stations were also selected along the MN-WI border, as the disease exists in wild populations in WI, and screened for CWD only. The sampling goal along the WI-MN border was 500 samples.

RESULTS AND DISCUSSION

From January 2008 to May 2009, MNDNR collected a total of 56 samples from targeted surveillance efforts. This includes samples from 13 escaped captive cervids, 29 free-ranging sick deer, and 14 car-killed deer (collected within 10 miles of recent CWD-positive elk facility in Olmsted county). All samples were negative for CWD.

MNDNR collected a total of 1,440 samples from hunter-harvested deer for CWD screening during fall 2008. The vast majority of these samples (n=951) were collected in conjunction with bovine tuberculosis surveillance in the northwest, and the remaining samples (n=489) were collected along the MN-WI border (Figure 1). All samples were negative for CWD.

Since the agency has now collected in excess of 30,000 negative samples in statewide surveillance efforts, we feel that future resources for CWD surveillance, in addition to targeted surveillance, are better spent addressing changing risk factors. Specifically, it is important to monitor the CWD surveillance activities occurring in our bordering states, and conduct periodic surveillance in Minnesota in response to CWD status changes in these states. Additionally, periodic surveillance in the vicinity of previous cases of CWD in captive cervids in Minnesota may be prudent. Given the most recent case of a CWD-infected cervid farm in Olmsted county,

MNDNR plans to conduct extensive surveillance during the fall 2009 firearm hunting season in the southeast portion of the state. Targeted surveillance of suspect deer is expected to continue throughout the state.

ACKNOWLEDGEMENTS

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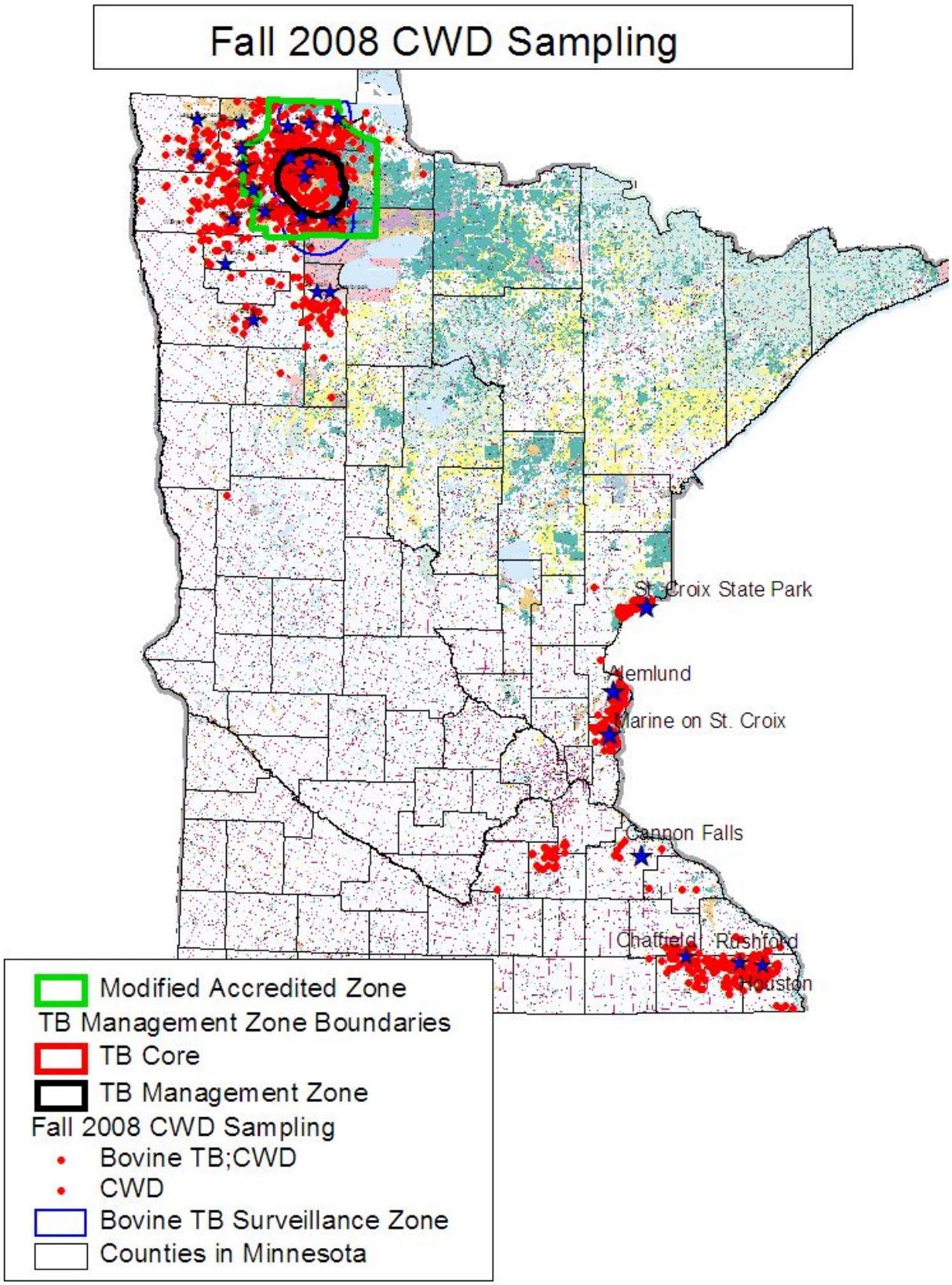

Figure 1. Sampling distribution for hunter-harvested deer tested for chronic wasting disease in Minnesota, fall 2008.