

PRELIMINARY RESULTS FROM THE 2007 HUNTER HARVESTED MOOSE 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 what diseases the NE MN moose population is being exposed to as well as to provide some comparisons for similar testing completed with non-hunting moose mortalities from the same population. Positive results were reported for eastern equine encephalitis, West Nile Virus, malignant catarrhal fever, anaplasmosis, 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, with a variety of results. All results were negative for *Mycobacterium paratuberculosis*, brucellosis, bovine herpes virus 1, blue tongue virus, epizootic hemorrhagic disease, *Neospora*, chronic wasting disease, and bovine tuberculosis.

INTRODUCTION

The estimated 2006 non-hunting mortality of 34% for adult moose in this population is higher than reported elsewhere in North America. Recent population survey results suggest a declining moose population, with a 23% decline noted between 2006 and 2007. In addition, hunter success rates have declined from 84% in 1993 to an all time low of 58% in 2005. Significantly lower cow:calf ratio was reported in 2006, lower than the average estimated for the previous 21 years. There have also been increased reports of clinically ill animals. Parasites have been documented, including *Parelaphostrongylus tenuis*, *Echinococcus granulosus*, *Eelaphora schneideri*, and *Sarcocystis spp.*, liver flukes (*Fascioloides magna*) and winter ticks (*Dermacentor 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 been noted in some bull mortalities.

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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 and an incisor for aging. We provided a presentation and instructions relative to the moose health survey at the mandatory MNDNR Moose Hunt Orientation Sessions. Hunters were mailed a sample kit with instructions prior to 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.

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Blood was centrifuged and serum was extracted. Liver and lung samples were split, with half placed in a formalin jar, while the other half was fresh-fixed (and later frozen) in whirlpaks bags. The hunter collected blood from the chest cavity as soon after death as possible, using a large syringe from which samples were placed in serum tubes and kept cool. If the hunter found anything unusual, those samples were collected and split between the preservative methods. We provided hunters with all equipment needed for sample collection/preservation. Also, retropharyngeal lymph nodes and/or obexes were removed by trained MNDNR staff at the registration stations with permission of the hunter (Figure 1).

Portable freezers were located in advance at the stations to maintain the tissue samples. Stations were staffed with Wildlife Health Program employees, tribal employees, and 'volunteer' students (as per CWD/bovine TB station protocol).

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.

Samples were submitted to the University of Minnesota Veterinary Diagnostic Laboratory (U of M VDL), where much of the testing occurred. The National Veterinary Services Laboratories (NVSL) in Ames, IA performed additional tests that could not be conducted at the U of M VDL.

RESULTS AND DISCUSSION

A total of 135 sampling kits were turned in by moose hunters (110 state, 25 tribal) at MNDNR registration stations throughout moose range in northeastern Minnesota (Figure 1). Of the kits submitted, 118 were complete, with the remainder being partial submissions. The quality of the samples were 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 total of 116 samples were submitted to NVSL for Virus Neutralization (VN) testing. A total of 5 were positive ($5/116 = 4.3\%$). Two of these positive samples had titers at 100, while 3 had titers greater than or equal to 100.

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 total of 117 samples were submitted to NVSL for VN testing. A total of 45 samples were positive ($45/117 = 38.5\%$). Thirty-two of the positive samples had titer levels at 10, 6 had titer levels at 100, and 7 had titers greater than or equal to 100.

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. This indicates that the virus is more serious for some species than others.

Malignant Catharral Fever (MCF)

A total of 117 samples were submitted to NVSL for peroxidase-linked assay (PLA) testing. If the PLA test came back positive, the samples were screened with a VN test. A total of 8 samples tested positive on the PLA test ($8/117 = 6.8\%$). Four of these 8 were positive at 1:100 and 4 were positive at 1:20. Of the 8 that were positive on PLA, 5 were negative on the VN and the serum was unsuitable for VN in 3.

The PLA test is more sensitive than the virus isolation, meaning it is much better at identifying positives, while the VN is more specific which means it is better at identifying true negatives. There were a couple of problems 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 brain worm as the animals often exhibit neurological deficits, go blind, and often 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).

The best test for MCF is a polymerase chain reaction (PCR) on whole blood. This would allow identification of active infection as well as determining which strain is present. If possible, whole blood should be collected from all euthanized moose as well as hunter-harvested animals.

Fecal Examination for Parasites

A total of 123 fecal samples were screened for evidence of parasites. 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*. 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

A total of 12 fecal samples underwent fecal sedimentation. Sedimentation is used to determine the presence of a patent liver fluke infection. None of the samples were positive for liver fluke ova.

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. Samples will not be submitted for fecal sedimentation next year as moose are not expected to shed fluke ova in their feces.

Liver and Lung Culture

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.

Culture-Other

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. Samples from 2 spleens were submitted for culture. No significant growth was documented in 1, and *Pantoea sp.* was isolated from the other.

Pulmonary *Mycoplasma* Culture

A total of 119 lung samples were submitted for *Mycoplasma* culture. None was isolated.

Mycobacterium paratuberculosis (Johne's)

A total of 90 fecal samples were submitted for *M. paratuberculosis* culture. At this time, 53 of the results have been reported as negative, while 37 are pending. 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.

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, though 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 total of 117 samples were screened for Anaplasmosis (*Anaplasma phagocytophila*, formerly *Ehrlichia phagocytophila*) with the card test. One of these samples was positive (1/117 = 0.9%). Positive test results indicates that exposure to this bacterium is occurring.

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 *Klebsiella pneumoniae*, to which the calf's death was attributed, though the *Klebsiella* 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 often has a significant infestation of winter ticks.

Brucellosis

A total of 112 samples were submitted 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.

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 total of 120 samples were submitted for serum neutralization (SN) testing for BVD 1 & 2. Two of these results were positive (2/120 = 1.7%). One was positive at 1024/4096 and 1 was positive at 128/256. These results indicate that the moose population is being exposed to BVD. These 2 positives were surprisingly high.

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 total of 120 samples were screened for BHV using a SN test. All results were negative

Blue Tongue Virus (BTV)

A total of 121 samples were screened using a Competitive Enzyme-Linked Immunoabsorbent Assay (cELISA) for BTV. All results were negative.

Epizootic Hemorrhagic Disease (EHD)

A total of 121 samples were screened for EHD using an Agar Gel Immuno Diffusion (AGID) test. All results were negative.

Leptospira sp.

A total of 121 samples were screened for 6 species of *Leptospira* using a microscopic agglutination test (MAT).

- ❑ *L. bratislava*: Four total positives (4/121 = 3.3%); 2 had titer levels at 100, 2 had titer levels at 200.
- ❑ *L. canicola*: Two total positives (2/121 = 1.7%); 1 had a titer of 100, 1 had a titer at 200.
- ❑ *L. grippothyphosa*: Three total positives (3/121 = 2.5%); 2 had a titer at 100, 1 had a titer at 200.
- ❑ *L. hardjo*: None of the samples tested positive.
- ❑ *L. interrogans* serovar *icterohaemorrhagicae*: 2 total positives (2/121 = 1.7%); 1 had a titer at 100, 1 had a titer level at 200.
- ❑ *L. pomona*: Ten total positives (10/121 = 8.3%); 4 had a titer at 100, 1 had a titer at 200, and 5 had a titer at 400.

Positive results indicate exposure to the bacterium is occurring. Leptospirosis is known to be present in Alaskan moose. Randall Zarnke found serologic evidence of exposure in 39/618 of moose on the Kenai Peninsula, while all 34 caribou, 11 Dall sheep (*Ovis dalli dalli*) and 15 wolves (*Canis lupus*) screened were negative (2000).

Neospora

A total of 122 samples were screened for *Neospora* with an ELISA test. All samples were negative.

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 total of 122 samples were screened for PI 3 using a haemagglutination inhibition (HI) test. There was 1 positive result (1/122 = 0.8%). It had a titer of 10. Positive results indicate that exposure to the virus has occurred.

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)

A total of 14 obex samples were screened for CWD using immunohistochemistry (IHC). All were negative. Twelve additional samples were submitted, but were unable to be tested due to incorrect tissue. A total of 23 retropharyngeal lymph nodes were screened for CWD using IHC. All were negative. An additional 1 sample was submitted, but was not tested due to incorrect tissue.

Bovine Tuberculosis

Lymph node samples were submitted for bovine tuberculosis culture. The number of samples submitted is currently unavailable, however, all results have been reported as negative.

Liver Histopathology

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.

Lung Histopathology

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 granuloma, and intrabronchial foreign material (likely agonal aspiration) were reported.

Other Histology

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.

ACKNOWLEDGEMENTS

This project would not have been possible without assistance from a number of MNDNR employees, tribal biologists, and volunteers. We would like to especially thank Region 2 staff that worked moose registrations stations: Tom Rusch, Jeff Hines, Penny Backman, Dave Ingebrigsten, Bob Kirsch, Nancy Gellerman, Walt Gessler, Dan Litchfield, Martha Minchak, Tom Engel, Kevin Carlisle, Carolin Humpal; as well as Julie Adams for making our area maps. We'd also like to thank tribal biologists Mike Schrage, Andy Edwards, and Seth Moore for their help with the project. Finally, we appreciate the help of volunteers Annie Widdel (U of M veterinary student) and Bill Delanis.

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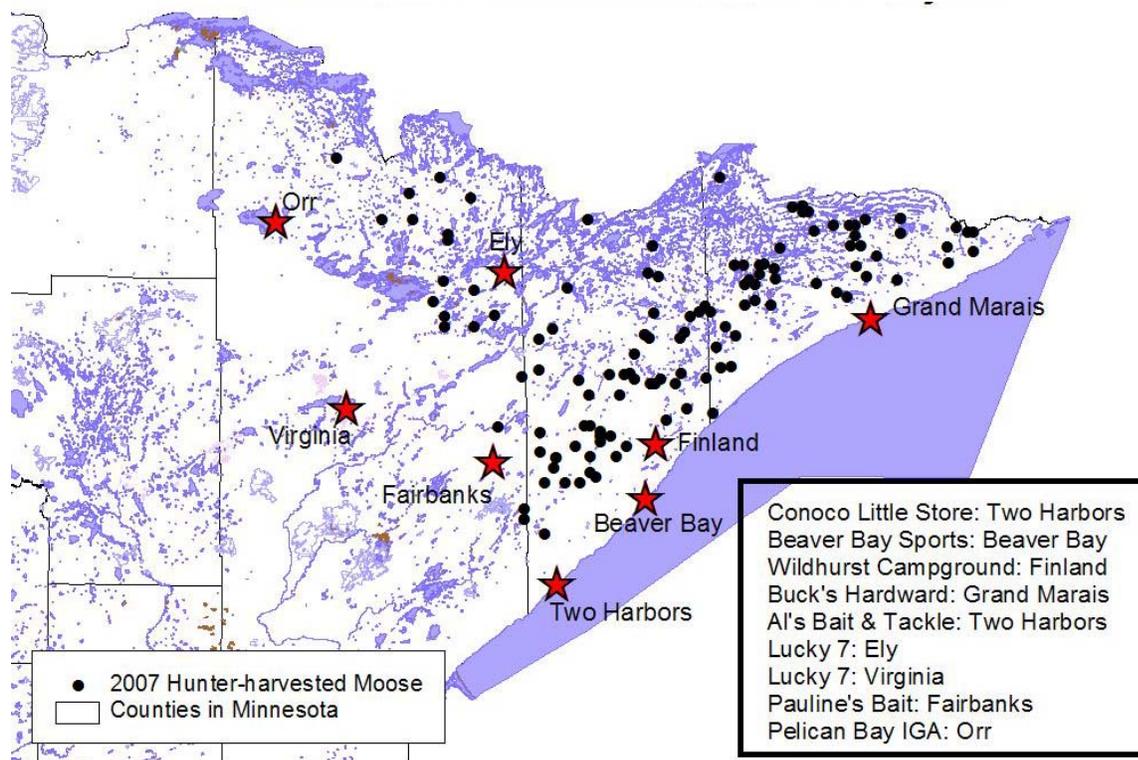


Figure 1. Locations of 2007 hunter-harvested moose included in health assessment project

