Sveriges lantbruksuniversitet
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## SUMMARY

Moose Wasting Syndrome in moose (*Alces alces*) was first discovered in Sweden in the 1980's. It was characterised by atrophied lymphoid organs, ulcers and erosions of the mucus membranes of the digestive tract, e.g. glossitis, gingivitis, esophagitis, rumenitis and abomasitis. Clinical signs seen in affected moose were diarrhoea, dehydration, alopecia, weakness, anorexia, impaired vision, emaciation and central nervous system disturbances. The etiology of the wasting syndrome is still unknown. There are many hypothesis as to the cause of the disease; however, none have been definitively proven.

In this project, an experimental study of seven moose calves born by mothers suspected of being affected with MWS is compiled and reviewed. The calves were born between May 15 and June 15, 1992. They were captured in the south of Sweden where MWS was known to occur. The calves were stabled in a specific-pathogen-free surrounding as laboratory animals between 7 to 11 months and any diseases contracted by the calves were treated. They were given controlled deer milk formula and feed with known ingredients. Extra minerals and vitamins were given at a regular basis and they had constant access to saltstones and water. The calves were observed daily to detect any abnormalities. Investigations for blood biochemistry, hematology, gross pathology and histopahology were done. Results show that the calves developed some clinical, gross pathological and histopathological findings similar to those found in MWS affected moose. Some of the clinical signs shown by the calves were diarrhoea, alopecia, inappetence and lesions in the mouth. No pronounced neurological disturbances were shown by the calves. Gross pathology showed enlarged and congested liver and spleen, hemorrhages in the adrenal cortex, lung consolidation, hyperemic trachea, discolouration of the renal cortex and medulla and lesions in the mouth, myocardium, lungs and cerebral meninges. In the intestinal tract congestion, discolourations, bleedings, flaccidity of the intestinal walls and thin Peyer's patches were seen. Histopathology revealed hyperplasia of lymph nodes, alveolar emphysema, mononuclear cell infiltration in the myocardium and mucosa of some areas of the intestinal tract among other findings. The brain did not display any histopathological lesions indicating classical spongiform encephalopathy. Hematology and biochemistry showed increases and decreases in total leukocyte, lymhocyte, neutrophil and eosinophil count but no uniform changes were seen. In conclusion, there were indications that MWS was contracted by the calves via their mother's directly or transplacentally, suggesting an infectious cause of MWS. It is possible that other, more severe MWS characteristic clinical and gross pathological lesions would have appeared if the calves had been stabled for a longer time and been investigated when the disease had progressed further. No evidence of classic prion disease (BSE) was shown in this study, however, with the long incubation period for prion diseases like CWD, as Benestad & Telling (2018) declare, it is possible that more pronounced lesions would have developed in the moose calves, given more time. Future studies are needed, using up to date technology and methods to determine if prions is the cause of MWS.

## SAMMANFATTNING

Moose Wasting Syndrome upptäcktes första gången i Sverige under 1980-talet. Det karaktäriserades av atrofierade lymfoida organ, ulceration och erosioner i digestionkanalens slemhinnor, d.v.s. glossit, gingivit, esofagit, rumenit och abomasit. Kliniska tecken noterade hos MWS-drabbade älgar var diarré, dehydrering, alopeci, svaghet, anorexi, synnedsättning, utmärgling och defekter i centrala nervsystemet. Etiologin bakom wasting syndromet är fortfarande okänd. Det finns många hypoteser om vad som orsakar sjukdomen; dock har ingen slutligen bevisats.

I detta projekt sammanställs och granskas en experimentell studie av sju kalvar, födda till mödrar misstänkta att vara drabbade av MWS. Kalvarna föddes mellan 15:e maj och 15:e juni 1992. De fångades i södra Sverige där sjukdomen veterligen förekom. Kalvarna stod uppstallade i en SPF-miljö mellan 7 till 11 månader och om de uppvisade tecken på sjukdom behandlades de. De gavs kontrollerad hjortmjölk och foder med känt innehåll. Extra mineraler och vitaminer gavs på regelbunden basis och de hade konstant tillgång till saltsten och vatten. Kalvarna observerades dagligen för att upptäcka avvikelser. Undersökningar för blodbiokemi, hematologi, patologi och histopatologi gjordes. Resultaten visar att kalvarna utvecklade vissa kliniska, patologiska och histopatologiska fynd som liknar de som ses hos MWS-sjuka älgar. Några av de kliniska fynd som sågs hos kalvarna var diarré, alopeci, inappetens och lesioner i munnen. Inga uttalade neurologiska avvikelser sågs hos kalvarna. Vid obduktion sågs bl.a förstorad och stasad lever och mjälte, blödningar i binjurebark, konsolidering av lunga, hyperemisk trachea, missfärgning av njurbark och –märg samt lesioner i munnen, myokardiet, lungor och hjärnhinnor. I gastrointestinalkanalen sågs bl.a stas, missfärgningar, blödningar, slapphet av tarmväggen samt tunna Peyerska plack. Histopatologi visade hyperplasi av lymfknutor, alveolärt emfysém, mononukleär cellinfiltration i myokardiet och i slemhinnan i vissa delar av gastrointestinalkanalen m.m. I hjärnan sågs inga histologiska fynd som indikerade klassisk spongiform encefalopati. Hematologi och biokemi visade både ökade som sänkta nivåer av totalantal leukocyter, lymfocyter, neutrofiler och eosinofiler men inga ensartade förändringar sågs. Sammanfattningsvis kan sägas att det fanns indikationer på att kalvarna smittats med MWS av sina mödrar, direkt eller transplacentalt, vilket tyder på att MWS har en infektiös orsak. Det är möjligt att andra eller mer allvarliga MWS-karaktäristiska kliniska och patologiska fynd hade setts om kalvarna hade hållits uppstallade en längre tid och blivit undersökta när sjukdomen fått fortskrida längre. Inga tecken på prionsjukdom sågs i denna studie, men med den långa inkubationstid för prionsjukdomar så som ses vid CWD, som Benestad och Telling (2018) uppger, kunde potentiellt mer uttalade lesioner utvecklats efter ett längre tidsförlopp. Vidare studier, med modern teknik och moderna metoder, krävs för att avgöra om prioner är orsaken till MWS.

## CONTENT

## ABBREVIATIONS

| | |
|---|---|
| MWS | Moose Wasting Syndrome |
| CWD | Chronic Wasting Disease |
| PrP | Prion protein |
| BSE | Bovine Spongiform Encephalopathy |
| BVD | Bovine Viral Diarrhoea |
| BVDV | Bovine Viral Diarrhoea Virus |
| GLDH | Glutamate Dehydrogenase |
| GGT | Gamma-glutamyl Transferase |
| FFA | Free Fatty Acids |
| CK | Creatine Kinase |
| $T_4$ | Thyroxine |
| PBDE | Polybrominated Diethyl Ethers |
| ALOV | Alces Leucotropic Oncovirus |
| LCO | Luminescent Conjugated Oligothiophenes |
| CNS | Central Nervous System |
| PLARCA | Proximity Ligation Assay – Rolling Circle Amplification |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| DMF | Deer Milk Formula |

## INTRODUCTION

In Sweden in the mid 1980's there was an outbreak of an unknown disease affecting the moose population (*Alces alces*). People reported of moose found dead or in poor physical condition. Most cases were reported from the south west of Sweden in Älvsborg County (Broman *et al*., 2002b), hence the name 'Älvsborg's disease', although in the literature the disease is also known as 'Moose Wasting Syndrome' (MWS), 'erosive/ulcerative alimentary disease' and 'bovine viral diarrhoea/mucosal disease-like syndrome'. This paper will henceforth use the name Moose Wasting Syndrome (MWS). Affected moose showed signs of a bovine viral diarrhoea/mucosal disease-like syndrome. Many animals were dehydrated, emaciated, alopecic and had diarrhoea and neurological disturbances (Rehbinder *et al*., 1991). In 1996 a total of just over 1400 moose had been reported sick, euthanised or having died from MWS, still there were no signs of the disease tapering off (Steen & Rehbinder, 1996). The disease peaked 1992 (Broman *et al*., 2002a).

Since the outbreak a handful of hypotheses has been presented trying to elucidate the cause of the disease. The hypotheses have been broadly divided into two groups: food related and host-parasite related (Broman *et al*., 2002b). The food-related causes are in turn divided into changes in trace element concentration, pollution and decreased browse availability, while the host-parasite related causes infer viruses, bacteria and prions. There are some evidence that support these hypotheses, equally there are evidence that disprove them, thus none has been definitely proven.

In this project, I will compile and review an experimental study of seven moose calves born to mothers suspected of being affected with MWS. The study was conducted in 1992-1993 on the premiss that MWS can be transmitted from mother to offspring, and the main goal here is a study conducted to investigate if a disease can be transferred from mothers to offspring by checking the moose calves under a period of 1992 to 1993 to finish with a postmortem study. The routes of transmission are unknown, as is the cause of the disease, and recently more evidence are pointing towards prions as being the cause of MWS.

## LITERATURE REVIEW

### Moose Wasting Syndrome

Moose wasting syndrome goes by many names, such as ’Moose disease’, ’Älvsborg’s disease’, ’erosive/ulcerative alimentary disease’ and ’bovine viral diarrhoea/mucosal disease-like syndrome’ (Broman *et al*., 2002b; Cederlund *et al*., 1994). The disease was first recorded in Sweden in the mid 1980’s when an increasing number of moose were found dead or in poor condition. The number of routine investigations performed at the National Veterinary Institute (SVA) increased tenfold during this time period (Broman *et al*., 2002a). Most cases were reported from southwest of Sweden, in Älvsborg County, however the syndrome was observed in moose all over the country (Stéen *et al*., 1989). The syndrome has been seen in moose as young as 0-6 months old, but seems to be more common among older animals (Stéen *et al*., 1993). In a study by Broman *et al*. (2002a) the risk of dying from non-traumatic causes, including MWS, was shown to increase with age. Veterinary investigations of diseased moose showed signs of a new complex BVDV-like wasting syndrome.

Atrophied lymphoid organs, ulcers and erosions of the mucus membranes of the digestive tract, e.g. glossitis, gingivitis, esophagitis, rumenitis and abomasitis mainly characterize the syndrome. Clinical findings seen among affected moose were diarrhoea, dehydration, alopecia, weakness, anorexia, impaired vision, emaciation and central nervous system problems with circling, ataxia, hypersalivation, teeth grinding, lowering of the head, drooping of the ears and behavioral aberrations (Stéen *et al*., 1989; Stéen *et al*., 1993; Rehbinder *et al*., 1991), see Fig. 1. In diseases causing neurologic dysfunction it is not uncommon to see aspiration pneumonia (Williams, 2005). Although, in necropsied moose with MWS pneumonia has only been an incidental finding, and it has not been established whether aspiration was the cause or not (Rehbinder *et al*., 2004; Cederlund *et al*., 1994). The alopecia does not seem to have a characteristic pattern, although in the study by Stéen *et al*. (1993), the alopecia in three moose were bilateral and located on the body, head and ears.

Figure 1. *Alopecia with skin lesions were seen in MWS moose.*

Diseased moose showed no uniform hematological picture. When compared to healthy moose, the hemoglobin concentration of diseased moose varied immensely, some showed obvious signs of anemia while others had a high hemoglobin concentration. The varying results were suggested to be related to hydration status. Healthy and sick moose showed no difference in lymphocyte count, although diseased moose sometimes showed an extremely high or low lymphocyte count. The neutrophil count and total number of leukocytes were frequently higher in moose with MWS (Kockum-Adolfsson, 1995). Blood biochemical changes seen in moose affected with MWS was decreased concentrations of thyroxine ($T_4$), glutamate dehydrogenase (GLDH), bilirubin, and gamma-glutamyl transferase (GGT) and increased concentrations of insulin, glucose, urea, creatine kinase (CK) and free fatty acids (FFA) (Frank *et al*., 2000a; Frank *et al*., 2000b).

Common gross pathological findings included signs of emaciation with loss of muscle mass and serous atrophy of the coronary fat and bone marrow (Rehbinder *et al*., 2004). Necrotizing lesions of the upper alimentary tract with ulcers and erosions was also seen, see Fig. 2 and 3. Ulcers, varying from a few mm to couple of cm, were found in the nostrils, mouth, esophagus, rumen and abomasum (Stéen *et al*., 1993). In the abomasum, dilation of the lymphatic vessels, severe hyperemia and edema was seen. A characteristic finding in the duodenum was macroscopically apparent dilation of Brunner glands (Rehbinder *et al*., 2004). The intestinal mucosa showed petechiae and dilated blood vessels, and the intestinal wall was often thin and fragile (Bergsten, 1992). Hemorrhagic or catarrhal enteritis was also common, with hyperemia and edema of the intestines and watery to hemorrhagic content. Atrophy of the Peyer's patches, a small and thin spleen, as well as a small and shrunken liver was characteristic. Macroscopically, the body lymph nodes appeared normal in size and color, except for the mesenteric lymph nodes sometimes being edematous and discolored (Rehbinder *et al*., 2004; Stéen *et al*., 1993). The myocardium was sometimes substantially dilated and flabby and the bones brittle. Another relatively common finding was uni- or bilateral opacity in the eyes (Stéen *et al*., 1993; Frank, 1998), see Fig. 4. In addition to the typical findings, incidental findings such as pneumonia, arthritis, abscesses and metritis were seen (Cederlund *et al*., 1994).

Figure 2. *Ulcers on the hard palate of a moose with MWS.*

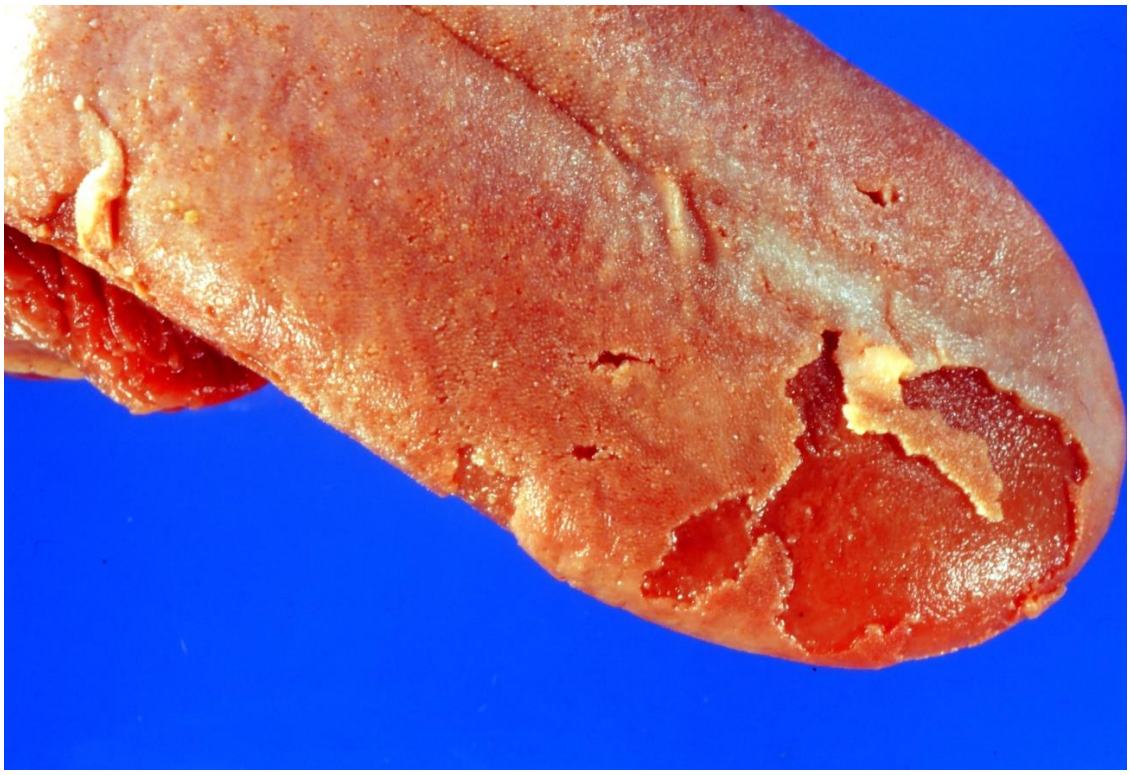


Figure 3. *Ulcers on the tongue of a moose with MWS.*

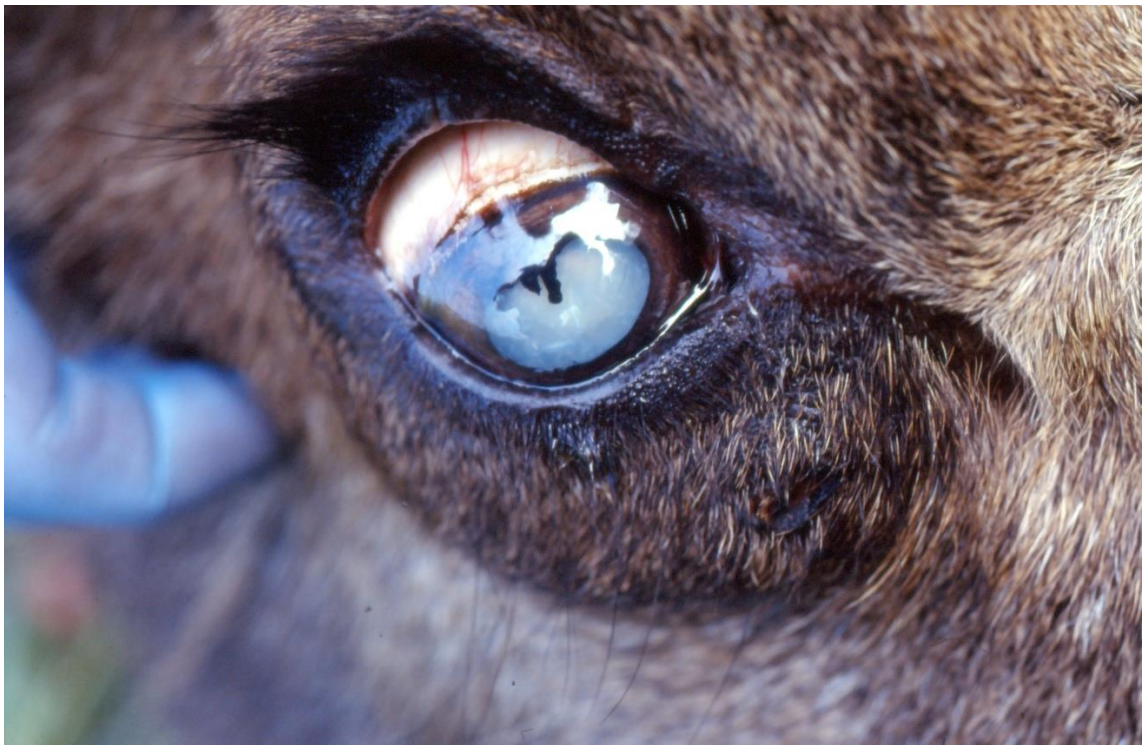


Figure 4. *Opacity in the eye of a moose with MWS.*

Histological findings were intra- and intercellular edema and mild mononuclear cell infiltration of the submucosa and lamina propria of the mucus membranes of the upper alimentary tract, including the esophagus and rumen. Vesicles were seen in the epithelium, as well as vacuolar degeneration of groups of cells in the stratum basale and stratum spinosum (Stéen *et al*., 1993). Intracytoplasmic inclusion bodies were seen in epithelial cells of the stratum germinativum and stratum spinosum of the upper alimentary tract. The inclusion bodies ranged from 2 to 10 µm and were slightly basophilic (Feinstein *et al*., 1987). Similar lesions were seen in the alopecic skin areas. Depletion of the white pulp, congestion and hemosiderosis was typically seen in the spleen, as well as depletion of lymphoid follicles in all the body lymph nodes (Stéen, *et al*., 1993). However, depletion of lymphoid follicles was an inconsistent finding as reactive hyperplasia was also seen (Rehbinder *et al*., 2004). Hemosiderosis has been noticed in the lymph nodes of MWS diseased moose (Stéen *et al*., unpublished). In the intestines, lesions similar to those found in Cu-deficient cattle has been seen, e.g. villous atrophy of the duodenum and jejunum (Rehbinder *et al*., 2004). Lymph- and blood stasis, necrotized, stunted and sloughed villi, hyperemia, pseudomembranes, bleedings and mononuclear and occasionally polymorphonuclear cell infiltration in the lamina propria has been seen in the small intestine of sick moose. In the colon an edematous and eroded mucosa and submucosa, with mononuclear cell infiltration in the lamina propria and submucosa has been seen (Stéen *et al*., unpublished). Heptocytes of sick moose were often small and pleomorphic, containing lipofuscin granules. Proliferation of intralobular lymph canaliculi and perilobular lymph vessels was sometimes seen, as was mild centrolobular connective tissue proliferation. In the kidneys nephrosis was present, with sclerosis of the glomerular membranes and degeneration of the distal tubuli. Signs of abiotrophy were seen in the cerebellum, characterized by Purkinje cell degeneration. The perikaryons, axons and dendrites were swollen but sometimes the perikaryons were pyknotic, malformed or

shrunken. In addition, loss of dead Purkinje cells was seen, creating empty spaces with Bergman cells filling and surrounding the empty spaces, see Fig. 5. Focal myocyte degeneration could be seen in the myocardium. Osteoporosis was also occasionally found, with fractured trabeculae separated by loose connective tissue seen in the primary and secondary spongiosa of the metaphysis. The growth plate cartilage showed an erratic mass of matrix and chondrocytes and no separation in zones (Rehbinder *et al*., 2004).

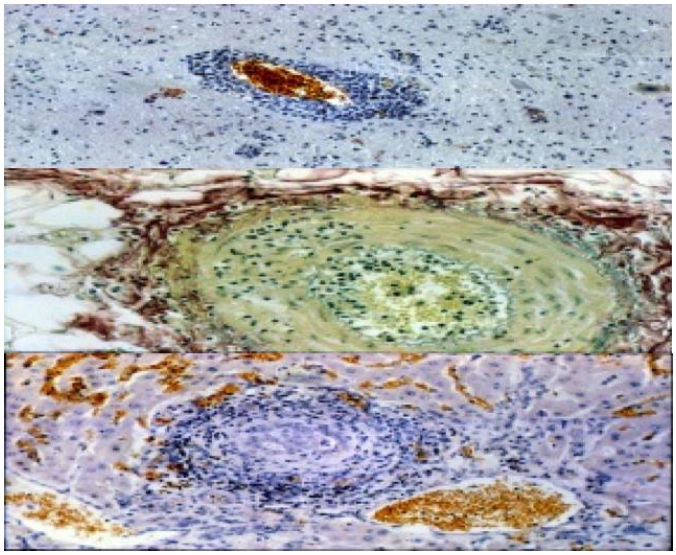


Figure 5. *CNS presented non-purulent meningoencephalitis with gliosis, neurophagia, increase of astrocytes, glial-fibrillary-acidic-protein and intra-cytoplasmic granules in neurons. Other histological findings were inter- and intracellular edemas in mucous membranes, erosions and ulcers. Liver displayed lymphocyte cholangitis with proliferation of the bile duct epithelium.*

There is no pathognomonic definition of the syndrome, neither is there any pathognomonic instrument or method to detect the disease or differ healthy from diseased animals (Naturvårdsverket, 1995). Diagnosis of the syndrome is made from typical clinical and pathological findings, however since there has not been any ultimate pathognomonic criterion a definitive diagnosis has been difficult to obtain.

## Current hypotheses

To date there is no answer to what causes MWS, but there are many plausible hypotheses that have been presented throughout the years. Either of these hypotheses still need to be proven, as there are both evidence that support and disprove them. Two broad groups of potential causes have been propounded; food related and host-parasite related. Food-related hypotheses includes changes in trace element concentrations, decreased browse availability and pollution, while the host-parasite related hypothesis include viruses, bacteria and prions (Broman *et al*., 2002b).

### *Food related hypotheses*

#### *Trace elements*

It has been suggested that MWS is caused by changes in trace element concentrations, mainly copper deficiency and/or molybdenosis, but also general mineral deficiency. Since most cases of MWS was found in Älvsborg County, the most acidified area in all of Sweden at the time, environmental factors was suspected as a probable reason for the disease (Naturvårdsverket, 1995). Acidification is causing soil cations to be lost by leaching, which affects copper concentration among other minerals, in the soil and hence the minerals available for the plants (Thelin *et al*., 1997). Equally liming, which intensively occurred in Älvsborg County during the time to neutralize the acidification, affects soil minerals (Frank *et al*., 1994). Liming on acid soils

causes plants to absorb more molybdenum (Stout *et al*., 1951). Both primary copper deficiency and secondary copper deficiency due to molybdenosis has been suggested as plausible causes of MWS (Frank *et al*., 2000a; Frank *et al*., 2000b). In addition, in the study by Frank *et al.* (2000b) it is suggested that Cu-deficiency and Mo-toxicosis may lead to diabetes mellitus causing some of the clinical signs seen in moose with MWS, such as motor disturbances, neuropathy and eye lesions. There are however both supporting and contradicting evidence to this Cu-deficiency hypothesis. The clinical and pathological picture in MWS to some extent coincide with the clinical and pathological picture in sheep and cows with copper deficiency. Clinical findings in sheep and cows with copper deficiency are unthriftiness, chronic diarrhea, ataxia, anemia and sudden death due to myocardial degeneration. Necropsy findings are emaciation, anemia, hair and wool abnormalities, darkening of the spleen and liver due to hemosiderosis, osteoporosis, flabby and pale heart (Constable *et al*., 2017). However, the clinical and pathological findings in moose with MWS are not identical to those found in cows and sheep with Cu-deficiency, with the biggest difference being necrotizing lesions, erosions and ulcers of the upper alimentary tract, which was seen in moose with MWS but not in Cu-deficient sheep and cows. It has been shown that copper and molybdenum concentrations in liver of diseased and healthy moose differ considerably, with moose affected by MWS having a marked decrease in Cu-concentration and elevated Mo-concentrations compared to healthy moose (Frank *et al*., 2000a). In the study, sick moose were sampled in 1991 and 1993 while the control animals were sampled in 1982. From 1982 it was also seen that the Cu-concentration in liver in healthy yearlings decreased to 50% by 1994 (Frank, 1997). Equally, the molybdenum concentration in liver of healthy moose has increased significantly from 1982 to 1992 (Frank *et al*., 1994). The fact that copper and molybdenum concentrations in liver was decreased and increased, respectively, in both healthy and sick moose, shows that there is no definite causal relationship between Cu- and Mo-concentrations and MWS. Also no significant differences has been seen in any trace elements in bark samples between areas where no cases of MWS has been reported and areas with most reported cases (Faber & Pehrson, 2000). Concerning other trace mineral deficits, Frank *et al*. (2000a) showed that diseased moose had lower concentrations of Cu, Cd, and Mg in the liver, and decreased concentrations of Cd, Co, Mg and Mn in the kidney compared to healthy moose. Additionally, hepatic and renal levels of Al, Ca, Fe, Pb and Zn was shown to be increased compared to healthy moose. However, when trace mineral concentration was measured in moose browsing species in southern Älvsborg (with many reported MWS cases) and Grimsö Wildlife Research Area (with no reported MWS cases) no difference was shown (Cederlund *et al*., 1994).

### *Decreased browse availability*

In Älvsborg County the MWS coincided with bark stripping of Norway spruce both in time and space, although no general overgrazing was shown. This was thought to indicate a decreased amount of available food and minerals, since spruce was usually rejected by moose (Faber & Pehrson 2000; Cederlund *et al*., 1994). The intake of quantitatively and qualitatively nutrient-poor plants was suspected to be able to cause MWS by either starvation or intoxication like effects (Broman *et al*., 2002b). However, moose calves born in areas with much bark stripping had higher slaughter weights than calves born in areas without bark stripping. Similar tendencies were shown among older moose as well. In addition, the nutritive content was equal when ruminal substance was measured. Fat deposited in musculus flexor carpi ulnaris was used to

give an indirect measurement of total body fat deposits. When measured in moose in areas with a high frequency of MWS and where much bark stripping occurred compared to areas with a low frequency of MWS and no bark stripping, the moose in the high MWS-frequency area had lower amount of fat, which is in agreement with the decreased browse availability hypothesis (Cederlund *et al*., 1994).

#### *Pollution*

To date, there is no specific pollutant believed to be the ultimate cause of MWS, however the textile industry has been suggested as a potential source of the pollutions since the majority of the Swedish textile industry took place in areas where most MWS cases were reported (Broman *et al*., 2002b). During textile processing, toxic chemicals such as dioxine-like compounds and polybrominated diethyl ethers (PBDE) are released and when samples of aquatic species were taken in different areas in Älvsborg County it showed high or very high concentrations of PBDE (Sellström *et al*., 1993). Excessive consumption of these toxic compounds inhibits collagen synthesis leading to osteoporosis, which has been noticed in moose with MWS. However, only 10% of diseased moose showed signs of osteoporosis and there was no statistical difference in the concentration of dioxine-like compounds in sick compared to healthy moose (Örberg, 1999; Stéen & Broman, 2013).

### ***Host-parasite related hypotheses***

#### *Viruses*

There was great reason to believe that MWS had a viral cause. The clinical and pathological picture of MWS to a great extent resembled Bovine Viral Diarrhoea/Mucosal Disease (BVD/MD) in cattle, a disease caused by a pestivirus (Rehbinder *et al*., 1991). Clinical signs in cattle affected with BVDV varies from being subclinical to severe and fatal with diarrhoea, fever, oral and nasal erosions, lameness, corneal edema, emaciation and rough, scurfy and dry hair coat. The consequences of an infection with BVDV depends on the immune status and the age of the animal when infection occurs, with older immunocompetent, seronegative cattle often developing a subclinical infection, while late embryonic to early fetal infection results in a persistent viremia with potential to develop mucosal disease. Other consequences of fetal infection with BVDV are congenital abnormalities, such as cerebellar hypoplasia, hydro-cephalus, cataracts and growth retardation (Constable *et al*., 2017). In a study by Feinstein *et al*. (1987) antibodies against BVDV was found in 8/16 moose all showing post-mortem findings in agreement with those found in MWS. Even though there are many similarities between BVD and MWS, congenital abnormalities were not characteristic of MWS and the neurological clinical signs commonly seen in moose with MWS are not seen in cattle with BVD. Also moose affected with MWS seemed to be older, while mucosal disease primarily affects younger cattle (Stéen *et al*., 1993; Constable *et al*., 2017). There has not been any successful attempts at isolating any known viruses originating from ruminants or other mammals (Bovine Viral Diarrhea Virus, Bovine Herpes Virus Type 1 and 4, Suid Herpes Virus Type 1 and Mammalian Reovirus type 1 and 2) in moose suffering from MWS (Rehbinder *et al*., 1991). Other plausible viruses, such as Bovine papular stomatitis virus, Orf virus and Herpes Virus Cervidae Type 1, able to cause similar lesions in the digestive tract were discussed, but was excluded as potential causes because the

lesions differed to much either histologically or macroscopically (Stéen *et al*., 1993). In diseased moose a prominent feature was involvement of the immune system, with atrophied lymphoid organs and sometimes very high or low lymphocyte counts (Stéen *et al*., 1993; Merza *et al*., 1994). Many known retro-viruses can cause these lymphoproliferative, immunodeficiency and wasting syndromes in other animals and it was suspected that a similar virus could be the cause of MWS. Since MWS showed an endemic rather than an epidemic appearance, an endogenic retrovirus was suggested as a likely cause (Simonsson *et al*., 1999). Merza *et al*., (1994) found that a possible retrovirus, Alces leukotropic oncovirus (ALOV), could be isolated from lymphocytes belonging to moose contracted with MWS. However, the results were questioned as there were only signs of retroviral proteins but no conclusive evidence of complete virus particles. In addition, endogenous retroviral protein sequences related to ALOV has been shown to exist in all moose, healthy and diseased (Simonsson *et al*., 1999).

### *Bacteria*

No specific infection has been shown to be the cause of MWS (Stéen *et al*., 1993; Rehbinder *et al*., 1991)

### *Prions*

Unaltered prion protein (PrP) is believed to have an essential, though yet unknown, function in an organism as it is highly conserved among different mammals. However, alterations in the PrP amino acid sequence can lead to it taking an insoluble form ($PrP^{sc}$) causing spongiform encephalopathy. The amino acid sequence in the PrP affects the pathology, incubation time, susceptibility and probably also transmissibility of the prion disease, consequently minor amino acid alterations at critical positions can have major effects (Wik *et al*., 2012; Wik *et al*., 2013). Moose with MWS have shown clinical signs similar to those seen in domestic ruminants with spongifom encephalopathies, giving reason to believe that MWS may be caused by a similar agent (Constable *et al*., 2017; Rehbinder *et al*., 1991; Benestad & Telling, 2018). Gross pathologic findings in cattle with Bovine Spongiform Encephalopathy (BSE) are non-specific and often no abnormalities are seen at necropsy (Constable *et al*., 2017). This differs from moose affected with MWS, where emaciation and necrotizing lesions of the upper alimentary tract among other gross pathological findings are characteristic for the syndrome (Rehbinder *et al*., 2004; Stéen *et al*., 1993). Bovine Spongiform Encephalopathy, affecting cattle, has never been recorded in Sweden except for an atypical variant diagnosed in 2006. Scrapie, affecting sheep, was recorded in Sweden in 1986 (Elvander *et al*., 1988; SVA, 2017). Recently, the emergence of Chronic Wasting Disease (CWD), a spongiform encephalopathy affecting cervids, strengthened the suspicions of a prions as a cause of MWS. Chronic Wasting Disease was first detected in moose and deer in Norway in 2016 (Veterinaerinstituttet, 2016), later in a moose in Finland in 2018 (SVA, 2018) and recently a novel type of CWD in moose has been discovered in Norway (Pirisinu *et al*., 2018). One of the early hypotheses was that MWS may be caused by an atypic prion strain (Broman *et al*., 2002b). Hammarström (2016) suggested MWS to be of CWD type or possibly an immature prion strain that potentially could develop into CWD. The incubation period for CWD is long, with the youngest animal ever diagnosed with clinical CWD being 17 months old and population level characteristics of MWS has shown that the risk of dying from non-traumatic causes, including MWS, increased with age, pointing to a long incubation period and suggesting prions as a potential cause (Broman *et al.*, 2002b; Williams *et al.*,

2002). Contradicting this hypothesis is the fact that MWS has been shown to affect moose younger than 6 months of age (Stéen *et al*., 1993). In a study by Nalls *et al*. (2013) it was shown that CWD can be transmitted from mother to offspring in Reeves' Muntjac deer, but this did not shorten the incubation period for the infected fawns. Mother to offspring transmission has also been shown to occur with CWD in free-ranging Rocky Mountain elk (Selariu *et al*., 2015). Equally to BSE, gross pathological findings in animals with CWD are non-specific and the characteristic necrotizing lesions of the upper alimentary tract seen in moose with MWS are not seen in CWD affected animals (Williams, 2005). When brains of diseased moose were histologically investigated an irregularly distributed astrocytic gliosis was seen, however no other microscopic findings typical of spongiform encephalopathies, of BSE-type, was found, such as vacuolation of the gray matter (Rehbinder *et al*., 1991; Rehbinder *et al*., 2004). However, typical histological lesions in the brain are not seen until relatively late in the disease, while detection of prion protein accumulations can be made much earlier, even before clinical signs develop (Benestad & Telling, 2018). Immunohistochemistry in the 1990's failed to demonstrate any PrP accumulations (Rehbinder *et al*., 1991). Recent histological investigations of tissues stained with luminescent conjugated oligothiophenes (LCO) probes have shown that LCO positive protein aggregates can be seen in MWS diseased moose, see Fig. 6 (Stéen *et al*., 2018). A group at Linköping University led by Per Hammarström has worked with novel approaches for detection of amyloid in tissue using amyloid dyes, LCOs, in combination with fluorescence microscopy in MWS moose. Seven MWS cases were examined by LCO fluorescence; three were positive for hallmarks of neurodegenerative disease with neurofibrillary tangles, dystrophic neurites and astrogliosis. Intracellular astrocytic aggregates were apparent in historical cases, see Fig. 6. Brain homogenates were used as seeds in the in vitro kinetic seeding assay using Bovine PrP (BoPrP) as substrate. Preliminary results from in vitro fibrillation assays demonstrate that one MWS case with LCO-positive histology displayed propensity to seed BoPrP conversion, see Fig. 7. Prion protein sequence (PrPs) in this assay is reluctant to cross-seeding, consequently will not be affected by amyloid seeds formed from other proteins, indicating that LCO positive MWS brains may contain aggregated PrP (Nyström & Hammarström, 2015).

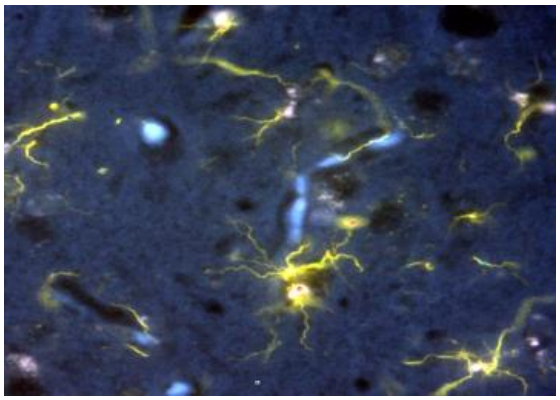


Figure 6. *Astrocytes are seen in a MWS case, filled with LCO positive aggregates. The moose, 15-years old female, displayed a severe degree of MWS clinical signs and was killed at site.*

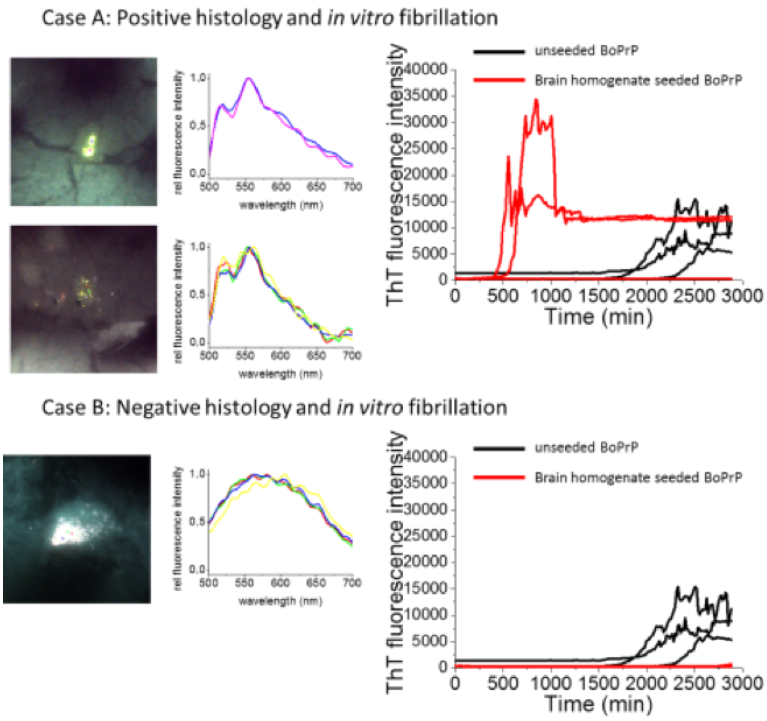



Figure 7. *A moose brain (a MWS case) investigated by LCO histology and seeding assay using BoPrP as substrate. Case A is positive for amyloid-like aggregates and positive in seeding assay. Case B is negative by both assays.*

Published and preliminary results from ongoing research have also shown that there is a possible correlation between a specific mutation in the moose PrP and MWS (Wik *et al*., 2012; Linné *et al*., unpubl: see Stéen *et al.*, 2018 p. 25). Wik and colleagues found a unique poly-morphism, a lysine (K) to glutamine (Q) amino acid change, in amino acid position 109 in the prion protein (PrP) sequence of Swedish moose. Genotyping of the mutation in Swedish moose populations from the 1980's-90's demonstrated a significantly greater proportion of KQ heterozygotes in moose with MWS. Amino acid position 109 is located at a highly conserved and positively charged cluster in the PrP sequence, only four amino acids N-terminal to the proteins α-cleavage site. Prion protein processing by α-cleavage has shown to prevent prion disease. Human PrP sequence variants in this cluster are associated with susceptibility to develop Gerstmann–Sträussler–Scheinker syndrome (GSS), and transgenic mice carrying mutations in the same cluster, develop neurodegenerative diseases. Johnson and colleagues (2006) found that polymorphism within the PrP sequence of another cervid species modulated susceptibility to CWD. In a study by McDonald *et al*. (2014) it was shown that $Cu^{2+}$ and $Zn^{2+}$ can affect α- and β-cleavage of the prion protein and moose with MWS have been shown to have an altered Cu- and Zn-balance (Frank *et al*., 2000a)

Preliminary results from a crude assay of protein processing analysis show that the homozygous 109 QQ genotype variant of the moose PrP possesses a different α-cleavage pattern compared to the homozygous 109 KK and heterozygous 109 KQ genotype variants, see Fig. 8. This gives an indication that the polymorphism may affect PrP genotype processing, and points to subtle PrP conformational differences between the different genotypes. These data suggest a possible connection between the PrP (K109Q) polymorphism in Swedish moose and MWS.

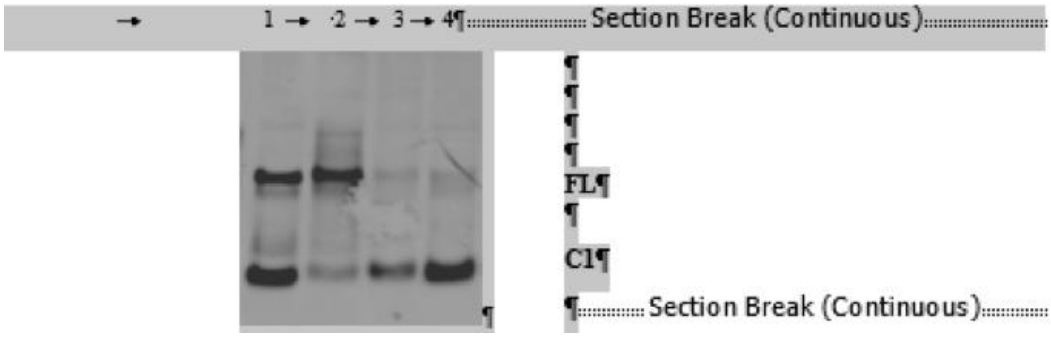



Figure 8. *Western blot detection of full length prion protein (FL) and the C-terminal fragment (C1). Lane 1: sheep brain extract (control); Lane 2: QQ-genotype brain extract; Lane 3: KQ-genotype brain extract; Lane 4: KK-genotype brain extract. Samples were treated with PNGase-F to remove N-linked oligosaccharides (Linné* et al.*, 2016, unpublished: See Arifrin, 2018).*

An ongoing project in a group at SLU have demonstrated that Proximity Ligation Assay – Rolling Circle Amplification (PLARCA) to detect PrP (Ebai *et al*., 2017). The group have used a highly sensitive PrP in vitro conversion assay to discriminate presence of small prion protein aggregates. The method relies on oligoconjugated antibodies binding two PrP epitopes, followed by ligation, rolling circle amplification, and detection of amplified DNA. This method allows for sensitive detection of small aggregated prion proteins, and selectively distinguishes the signal from native, unaggregated prion proteins. The group have used PLARCA on a small sample of MWS moose, thereby screening for potential TSE in the Swedish moose populations and mapping the existence of prions. The SLU group have recently demonstrated that the PLARCA, targeting aggregated prion protein highly increase detection sensitivity and can distinguish between normally occurring non-aggregated protein and infectious-causing aggregated prion protein in sheep (Scrapie). The assay format enhance specificity of CWD detection, reduce nonspecific background, and permit strongly amplified detection signals using standard assay formats and instrumentation. A validated CWD-adapted PLARCA, more sensitive than routine ELISA, can therefore potentially be a new tool to be used (Pineda and Andersson, 2018, unpublished: see Pelve and Stéen, 2019).

## MATERIAL AND METHODS

The experiment was conducted with permission from the Regional Ethical Review Board according to Swedish regulations (Swedish Board of Agriculture). And permission was obtained from the Swedish Environmental Protection Agency to capture moose calves.

### Animals

Seven moose calves, five males and two females, born approximately between May 15 and June 15 1992, were captured the same year between May and November. The calves were captured in the south of Sweden, where MWS was known to occur, at latitude 60-62 and longitude 12-17. Their mothers were suspected to have contracted with MWS. Two of the mothers were necropsied in the field and lesions similar to those found in MWS was observed. Another two mothers showed neurological signs such as strange behavior, swaying gait and confusion, one of who disappeared and the other one was killed in a car accident but not necropsied. Two of the calves were captured in vicinity to two dead female moose, probably their mothers, however the females were not necropsied. One calf was found alone by the local people and the destiny of the mother was unknown. All but one calf were captured by immobilization with Xylazine. They were shot intramuscularly with a CO2 immobilization Daninject rifle. The calf that was not immobilized was caught by hand by the locals. After immobilization and capture the calves were transported by horse-trailer for approximately one hour to a rebuilt cow stable, which had been empty for two years prior. During transport, more Xylazine was iterated if necessary to keep the calves calm. Each calf was named and numbered.

### Experimental facilities and procedure

The stable was totally emptied of interior, minutely cleaned with water and washing detergent under high pressure and rebuilt for the purpose and thereafter painted. Each stall was equipped with a nameplate and automatic water cups were installed. Every animal had its own bottles, pails and buckets. Once a day the stalls were swept and cleaned and cutter shavings were laid on the stall floor. The cutter shavings were guaranteed microbe free and was stored at the loft of the stable and the feed was kept in a special food chamber in a rodent free environment. Every day the equipment was washed with hot water (70-80 $^{o}$C) and mild soap and once a week it was cleaned with chlorine. The personnel were wearing special laboratory cloths, worn only in the stable, rubber-boots or clogs (depending on season) and when entering the stalls, plastic socks were drawn over the foot-cover. In addition, the personnel was encouraged to shower once a day. They were forbidden to have contact with other ruminants, however, if that could not be avoided, their hygiene had to be minute before they were allowed to enter the stable. Control of rodents was done by placing boxes with rat poison at strategic places. Cats were not allowed to enter the stable.

All calves were kept indoors in separate stalls without possibility of any body contact, however for the animal welfare they could see each other via a net on top of each stall, see Fig. 9 and 10. The calves were maintained in the stall for 7 to 11 months and dewormed (with mebendazol) at the start of the experiment. If ectoparasites were observed initially, treatment for this was also given. The calves were fed up to a maximum of 4 L deer milk formula (DMF) for a total of 6-9 months with bottle feeding for 1 to 4 months and alternately in a bucket or bowl for one

week to five months until weaning. They were weaned between November and February and during weaning they were given dried sugar beet with molasses. In the autumn, limited amounts of apples were given. The calves were fed four to five times per day for the first few months, thereafter the number of feedings were successively reduced until about 3 months of age when they got food 2 times per day. The calves regularly received horse-pellets up to a maximum of 10 kg per day, hay (timothy/clover) ad lib, and limited quantities of browse (twigs branches) for occupation. After weaning the calves' received 30-50 g of minerals every day. They also had constant access to saltstones during the entire experiment. Iron, selenium and vitamins was given at arrival and thereafter regularly at a few months intervals during the experiment. B-vitamins were given one to five times. All calves had water at hand within easy reach. The feed and feeding routine was based on B-O Röken, Kolmården Zoo.

Figure 9. *The calves had no body contact but were able to see each other*.

Figure 10. *The staff were playing and stimulated the moose calves every day with fun games, like soccer, and the calves became very tame.*

The calves were observed daily to detect clinical signs typical of moose wasting syndrome, such as gait disturbances, behavioral abnormalities and postural aberrations, see Fig. 11.

Figure 11. *The tame calves were checked every day for lesions in the nostrils, mouth, tongue and pharynx.*

The calves filmed and photographed continuously. If the calves contracted any illness, treatment was given by the project veterinarian. Drugs used were stored in a refrigerator in a temperature between 4-8 ºC. Every second week the calves were immobilized with Xylazine using a Daninject rifle or a self-made or factory-made jab-stick, after which they were weighed, clinically examined, had their body temperature measured, and blood samples taken 5-10 minutes post immobilization for biochemistry, hematology and virology. The biochemistry panel included measurement of cholesterol, urea, alanine aminotransferase (ALAT), aspartate aminotransferate (ASAT), glutamate dehydrogenase (GLDH), creatine kinase (CK), protein, albumin and globulins. Investigations for pestivirus antibodies was performed (Frölich, 1993) and the blood was treated according to Kockum-Adolfsson *et al*. (1997). Iron, selenium and vitamins were given when sedated if deemed necessary. Daily journals were kept for each animal. At the end of the experiment the calves were sedated with Xylazine and euthanized with a single rifle shot in the neck and necropsied according to routine procedures (SVA, Uppsala, Sweden). The euthanasia and necropsy was carried out in and outside the facilities, see Fig. 12.

Figure 12. *The necropsy was carried out minutes after the euthanasia of the calves.*

Cultures for bacteriology were taken according to routine procedures (SVA, Uppsala, Sweden) in connection with the necropsy. The following organs were sectioned and fixed in 10% formalin: nostrils, mucous membranes of the nose and mouth, thyroid, lungs, heart, liver, pancreas, spleen, kidneys, adrenal glands, spinal cord, abomasum, rumen, parts of the small and large intestine, skin, m. longissimus dorsi, m. gracilis, m. splenus capitis, retropharyngeal, mediastinal, axillary, brachial, mesenterial, popliteal and lateral subiliac lymph nodes. The organs were

processed, cut to 4 µm thick slides and stained with haematoxylin and eosin. Additionally, cerebrum and cerebellum was collected from five calves, and in two of those calves brain stem was also collected. The brains were stored in formalin for 25 years before processing. Parts of the cerebrum, cerebellum and thalamus, and if possible obex was fixated for histology.

Table 1. *Nutriments given to moose (Alces alces) in experimental study*

| **Nutriment** | **Amount** | **Name** | **Adm.** | **Producer** | **Intervall** |
|---|---|---|---|---|---|
| Milk substitute | ≥ 4 L/day | Deer milk formula | Per os | Lactamin Sweden | From housing to Nov/Jan |
| Horse pellet | ≤ 10 kg/day | Horse-coarse | Per os | Lactamin Sweden | Regularly from weaning |
| NaCl | Ad. Lib | Saltstone | Per os | AKZO Sweden | Regularly |
| Hay | Ad. Lib | Timothy/clover | Per os | Lantmännen | Regularly |
| Browse | Ad. Lib | Twigs branches | Per os | Salix, birch, pine, mountain-ash | Regularly |
| Apples | Limited | Different sorts | Per os | Home produced | During autumn |
| Dried sugar beet with melasses | Limited | Betfor | Per os | | During weaning |
| Minerals | 30-50 g/day | | Per os | Lactamin Sweden | Regularly after weaning |
| Iron | 200 mg $Fe^{3+}$/ml<br>2-3 ml/inj | Pigeron | Intramuscular | Lövens Denmark | 7 days after stabling 1 to 2 times totally during the experiment |
| Selenium,<br>Vit. E | 1.0 ml/10 kg bw | Selevitan | Intramuscular | Pherrovet Sweden | 7 days after stabling, then regularly |
| Vit. B | 10.20 ml/inj | Beviplex | Intramuscular | Pherrovet Sweden | 7 days after stabling, then regularly |
| Vit. A-$D_3$-$E^x$ | 5-10 ml/inj | Ultrasan aq. | Intramuscular | Pherrovet Sweden | 7 days after stabling, then regularly |
| Vit A-$D_2$-$E^x$ | 2-5 ml/inj | Ultra-Plex | Intramuscular | Pherrovet Sweden | 7 days after stabling, then regularly |

Table 2. *Medical substances given and chemical substances used in experimental study*

| **Substance** | **Concentration** | **Dosage** | **Registered trademark** | **Adm.** | **Diagnosis/ action** |
|---|---|---|---|---|---|
| Xylazine | 500 mg/ampulla | 1.0 mg/kg bw | Rompun vet | i.m. | Immobilisat-ion |
| Atipamezol | 5 mg/ml | 1.0 ml per 40 mg Xy-lazine | Antisedan vet | i.m. | Antidote to Xylazine |
| Electrolyte infusion | | 40 ml/kg bw | Ringer-Acetat | i.v. s.c. | Rehydration |
| Glucose infusion | 500 mg/ml | Up to 50 ml | Glukos | i.v. | Energy support |
| Euthanasia infusion | Phenobarbitalum natricum 109.7 g, spiritus fortis 209 g aq. puris/100 ml | | Ex tempore | i.v. | Euthanasia |
| Analgeticum, antipy-reticum | 50 mg/ml | 2.0 ml/ kg bw | Finadyn | i.v. i.m. | Pain, fever |
| Enterofloxacin | 100 mg/ml | 2.5 mg/kg bw | Baytril | i.m. | Infections |
| Dihydristreptomycin-bensylpenicillin-procain | 0.25 g<br>200000 IE/ml | 1.0 ml/ kg bw | Streptocillin | i.m. | Infections |
| Dihydrostreptomy-cin-penicillinbensatin | 0.4 g/200 000 IE/8 g units | Based on needs | Siccalactin | cut. | Infections |
| Dihydrostreptomycin | 500 mg | 25 mg/kg bw | Dihydro-streptomycin | p.o. | Infections |
| Lactid acid bacteria | | Based on needs | Bacterie ba-lans | p.o | Diarrhoea |
| Anti-diarrhoeaticum | 3g Bismuth subnitr., 47 g bo-lus alba, 40 g carbo med./100 g | 10-20 g x 3 d | Carbo-pulbit | p.o. | Diarrhoea |
| Anti-diarrhoeaticum | | | Diakur | p.o. | Diarrhoea |
| Mebendazol | 4 g/20 g | 6.0 mg/kg bw/10 d, 10 to 30 days after stabling | Telmin | p.o. | Internal parasites |
| Ivermectin | 10 mg/ml | 0.2 mg/kg | Ivomec | s.c. | External parasites |
| Virion | | | | | Stable desinfection |
| Chlorine | | | Kloramin | | Stable desinfection |

# RESULTS

## Clinical findings

Four of the animals had diarrhea at least once during the stable period and often it was of long duration or intermittent, but they all recovered after treatment. The diarrhoea varied from mild with rather loose feces to more severe and watery, but no blood was seen. Three of the calves had a cough, lasting from a few days to 1 month. In one calf the cough was rattling, but in the other two the cough was dry. Two calves got small erosions at the edges of the nostrils that healed in a few days. One calf became stunted, had unnatural hair loss causing a thin haircoat and its condition was under average at 2 months of age, see Fig. 13. The conjunctiva of two calves became mildly hyperemic and edematous. One calf also developed aggressive and nervous behavior, and another calf started to lick and nibble the interior. Four of the calves had periods of inappetence during the stabling, which often coincided with diarrhoea. An erosion and discoloration just caudal to the dental pad was seen in one calf. Another calf developed a white spot 2 mm in diameter on the lower lip and a brown discoloration and erosion 2 x 3 cm in diameter located on the dental pad. In the same calf, a red area was located dorsally at the base of the tongue, and a 2 x 2 mm white spot was located ventrally on the tongue. One calf developed a 0.5 x 1 cm large erosion laterally on the coronary groove on the left front limb and a black point at a conical papilla on the tongue. The same calf also got a 4 x 4 x 2 cm nodule medially on right side of neck that healed in 10 days. In one calf two vesicles 2 mm in diameter was seen on the lower jaw medioventral to the first incisors. Five warts in a cluster was found ventrally on the tongue near the apex, and a 1 mm long erosion was seen on left side of tongue in the same calf. In another calf, an erosion 1 cm in diameter was seen dorsally on the second digit of the left hind limb. All calves developed alopecic spots on different areas of the body, beginning around the age of five months, see Fig. 13. The alopecic spots varied in size from a few cm up to 2 dm in diameter. Common areas with alopecia were the thighs, tibia, abdomen, pectoral region and back. Other areas where alopecia was found were the ears, metacarpus, neck, flanks and hips. The hair coat was woolly and the skin scurfy in two calves. One calf had especially extensive alopecia, with hair loss in areas on the tibia, pectoral region, abdomen, flank, hip, shoulders, upper parts of the limbs, ears and neck, see Fig. 13. Also, a 3 x 5 cm large erosion was seen on the shoulder and multiple greyish alopecic spots 2 x 3 cm in diameter was seen on the left side of the abdomen in the same calf.

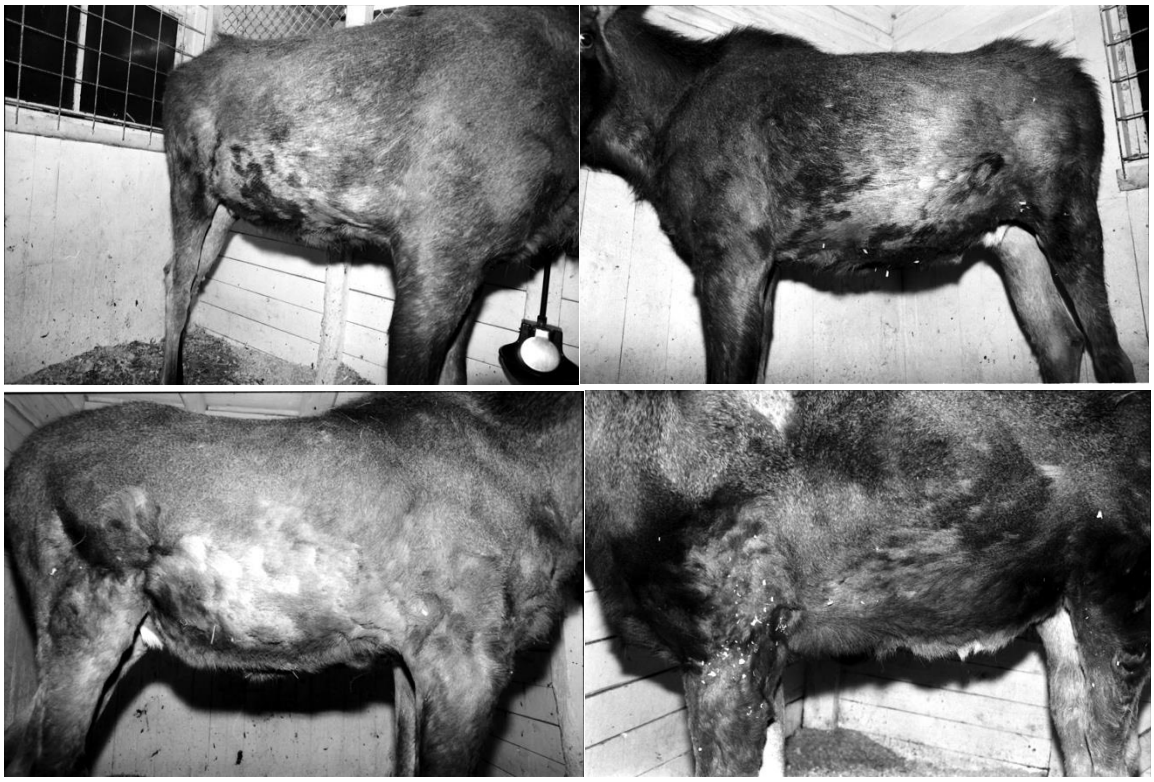


Figure 13. *The calves displays hairloss and alopecia.*

## Gross pathology

In two calves the cerebral meninges were heavily hyperemic. The muscle fascia on the right side of the sacrum was striated and had a yellowish color in one calf. Hyperemic and hemorrhagic mucosa of the dorsal and ventral concha was seen in three calves. In one calf a small erosion was seen on a conical papilla on the tongue, and in another calf a white spot was seen in the same location, see Fig. 14. The popliteal, axillary and subiliac lymph nodes varied from normal to soft, congested and having bleedings, prominent follicles and focal hard spots. In two calves the pericardium contained serous fluid, one of which also had a dilated left ventricle. Another calf had a slightly dilated right ventricle and the entire myocardium was slightly consolidated. The epicardium cut surface had large white focal spots 1 mm to 1.5 cm that penetrated into the myocardium in one calf. In five calves the trachea was hyperemic and it contained sanguineous froth in one calf. The lungs were affected in three calves, being mildly congested, darkish and consolidated or emphysematous and enlarged. In one calf the lungs had a slight fibrous pleuritis bilaterally, distally on the diaphragmatic lobes and there was fibrous exudation distally in the mediastinum and emphysema in all lobes. Viscous froth was found in the bronchi of four calves. The liver was enlarged, congested with rounded edges and sometimes hyperemic in all but one calf, which instead had moderate perihepatitis, see Fig. 15. Equally the spleen was enlarged, congested and had prominent white follicles in all but one calf. In all calves the kidneys were red to darkly discolored in the cortex and medulla, including the transition zone, and in two calves, the urine smelled peculiar and there were areas of focal necrosis over which the capsule were firmly adherent, respectively. There were small hemorrhages in the cortex of the adrenal glands in five calves, and the adrenal cortex was enlarged and had multiple 1 mm large calcified necroses in the cortex in one of those calves. In one calf, the ruminal content was

watery and had a very unpleasant smell. The abomasal mucosa was slightly to markedly thickened in two calves, and easily pealed off in another two calves. Reddish to dark brown discolourations, multiple craters and petechial to miliar bleedings were lesions that was seen in the abomasal mucosa of the calves, see Fig. 16. All calves had bleedings in the small intestinal mucosa, ranging from small petechial or miliar bleedings to large bleedings up to 2 cm in diameter. In five calves there were different areas of the small intestines that were thin and flaccid. The small intestinal mucosa in all calves varied from slightly reddish to heavily hyperemic and hemorrhagic. In one calf the jejunum was contracted in some areas and two calves had pseudomembranes in the duodenum and jejunum, respectively. The cranial part of the jejunal mucosa had moderate amount of elevations and craters in one calf. Congested and dilated blood vessels were also seen in parts of the small intestine in two calves. In all but one calf the Peyer's patches were mostly thin, sometimes reddish and in occasionally difficult to see. One calf showed Peyer's patches varying from slightly thin to large and prominent with congested vessels, see Fig. 17. The contents of the intestines varied, being mostly mucoid in three calves, varying from mucoid to watery in another two calves, and in yet another calf it varied from mucoid to hemorrhagic with blood clots. In two calves the cecal mucosa was slightly hyperemic, see Fig. 18.

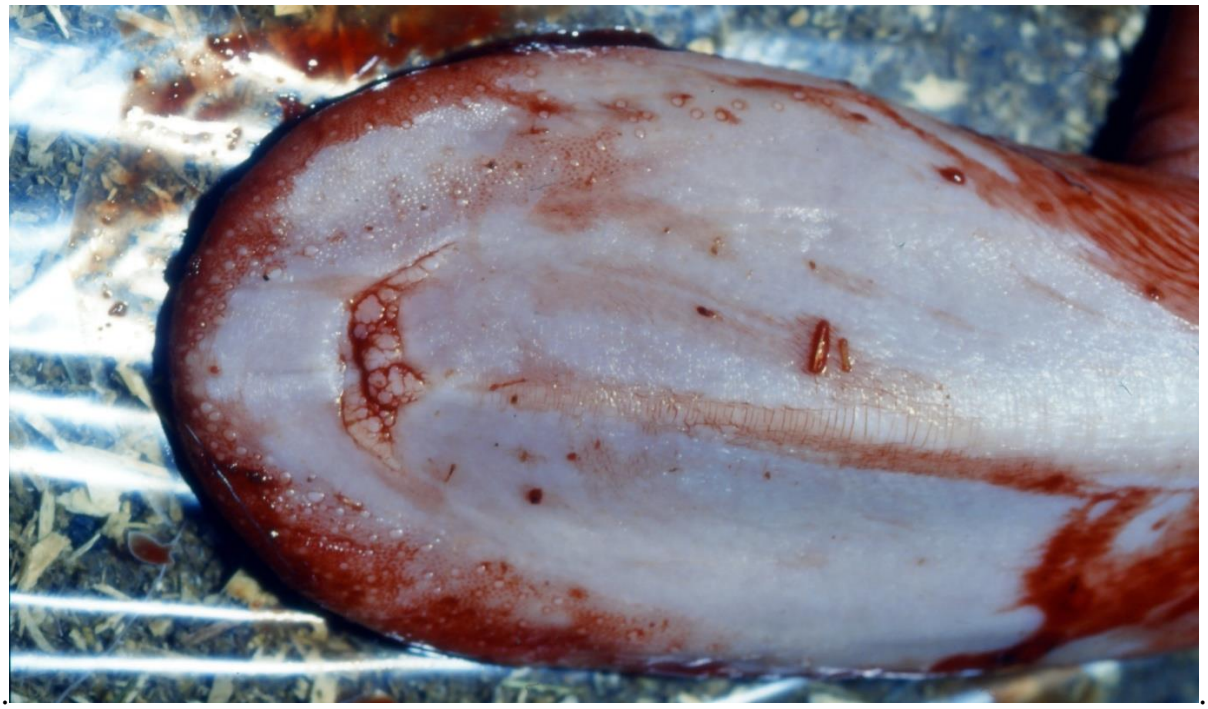


Figure 14. *Lesions seen in a calf on the back on the tongue.*

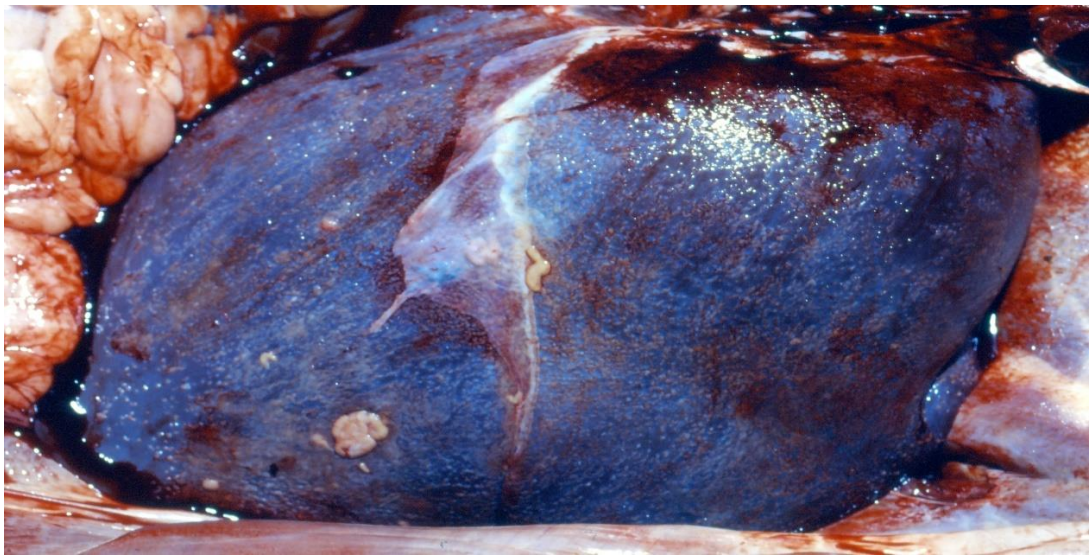


Figure 15. *The liver was enlarged, congested with rounded edges and sometimes hyperemic. One calf had moderate perihepatitis*.

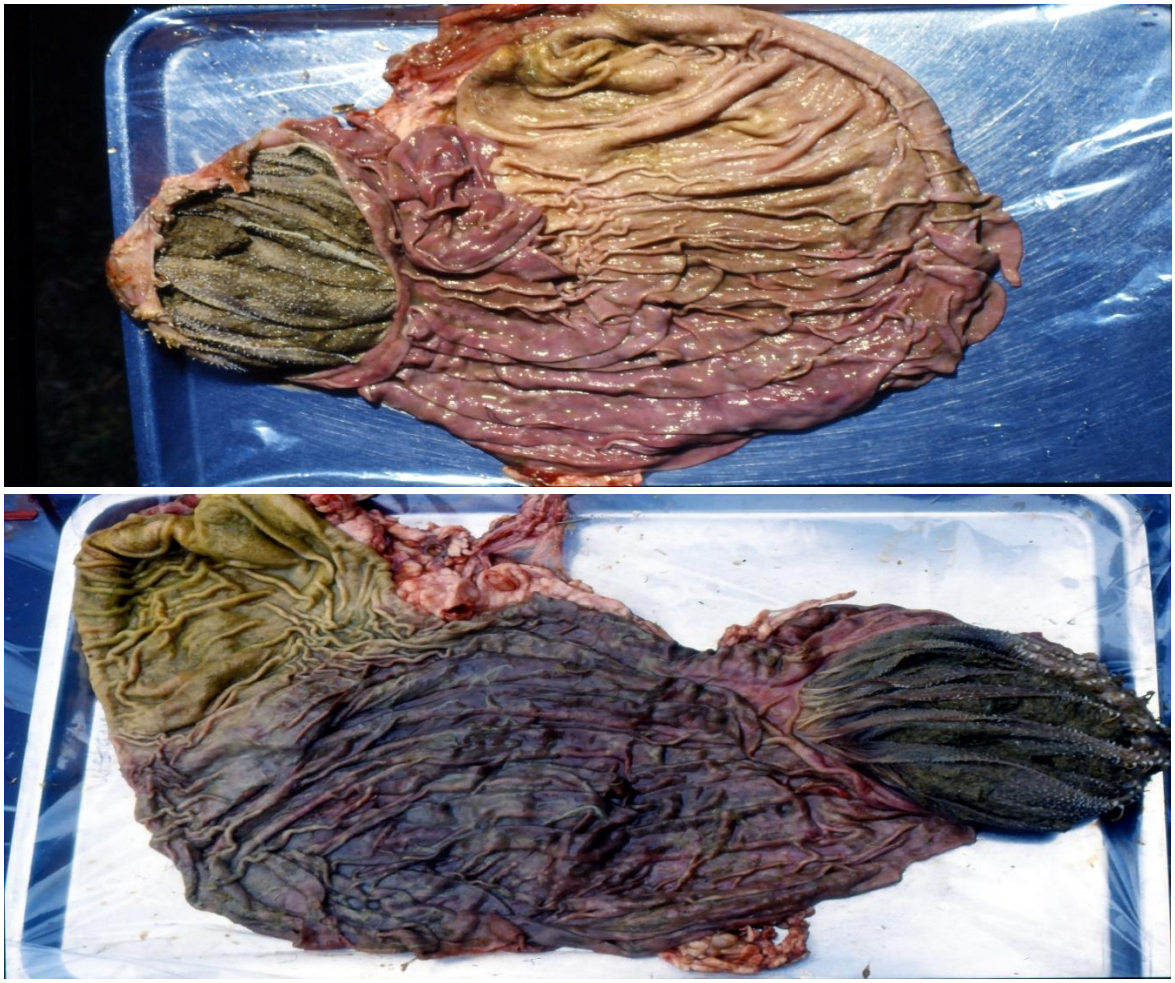


Figure 16. *Discolorations and other lesions were seen in the abomasum of the calves*.

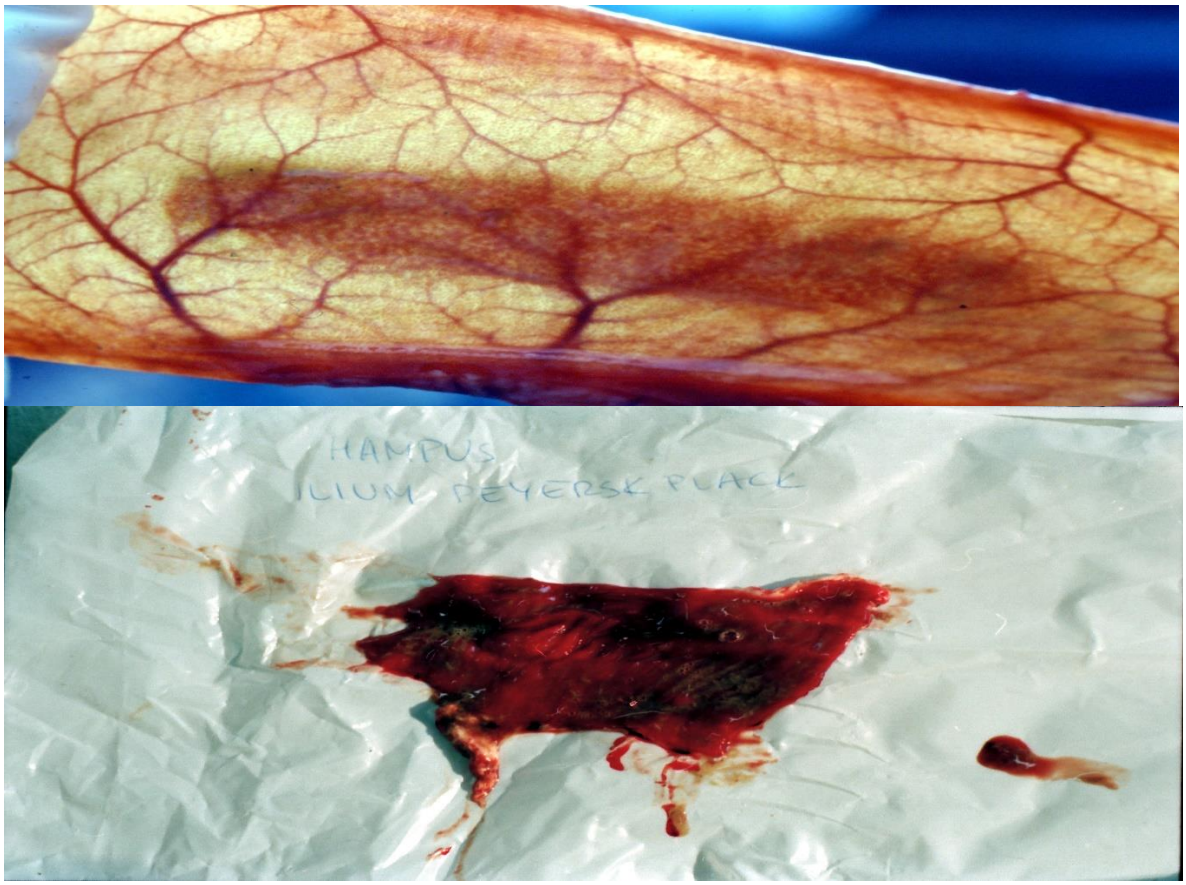



Figure 17. *The Peyer's patches varied from very thin to congested.*

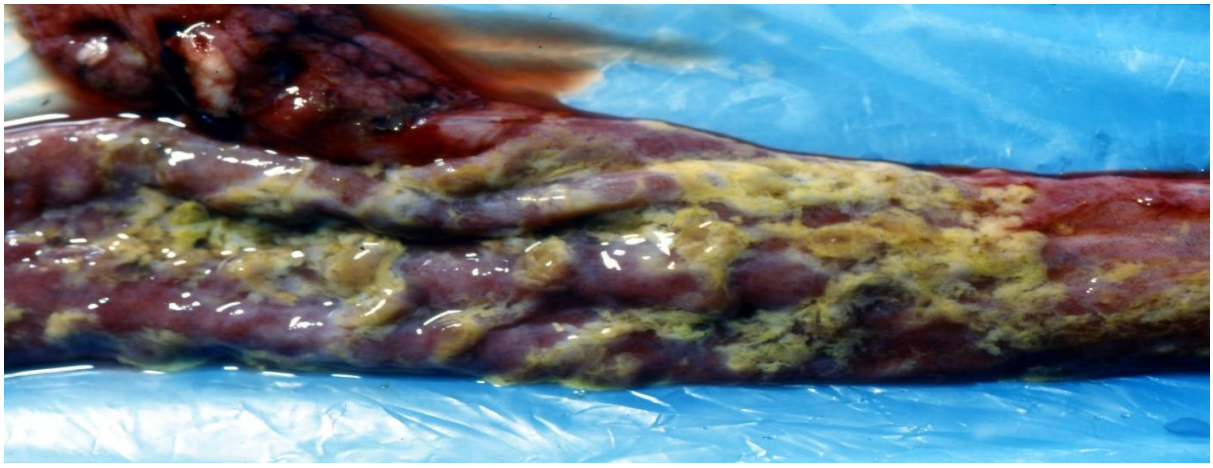


Figure 18. *The intestinal content varied from thin watery to mucoid, the intestine wall was in the most cases congested*.

## Histopathology

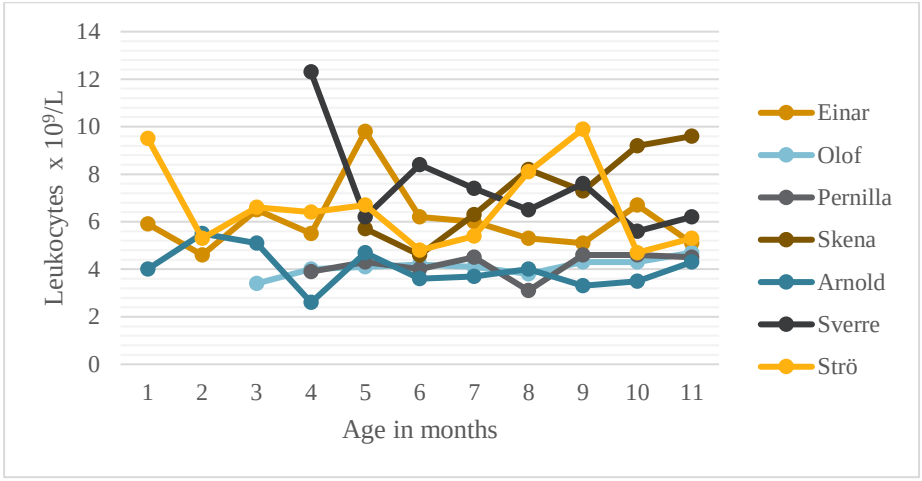
In one calf moderate mononuclear cell infiltration was seen in the salivary glands and its associated muscles. Mild hyperplasia of the axillary lymph node was seen in one calf. Another calf had moderate hyperplasia of the popliteal lymph. In one calf moderate eosinophilic cell infiltration was seen in the sinuses and capsule of the popliteal lymph node. The same calf also had mild hyperplasia and moderate hemosiderosis in the lateral subiliac lymph node. In addition, the calf had mild diffuse hyperplasia in the mesenterial, axillary and ileocecal lymph nodes.

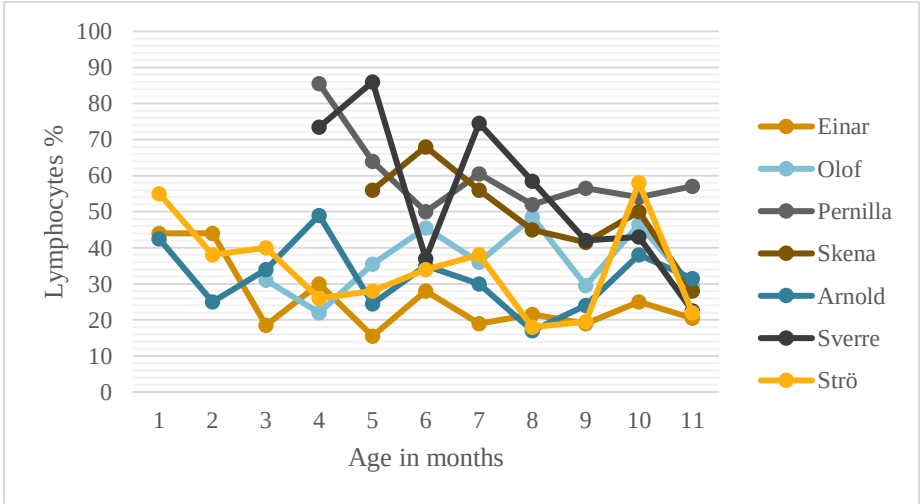
Slight hyperkeratosis was seen in the skin and the skin of the nasal septum in two calves, respectively. One calf had moderate perivascular neutrophilic infiltrations in the skin and in another calf severe mono- and polymorphonuclear cell infiltration was seen in the submucosa of the mucous membranes of the mouth, as well as microabcesses. In three calves there was mild to moderate alveolar emphysema in the lungs, with one calf also having mucoid fluid containing neutrophils in the bronchioli. Mild lymphocytic cell infiltration around the bronchioli and in the interstitium of the lungs was seen in another calf. The right papillar muscle and the interstitium of the heart and showed mild focal mononuclear cell infiltration in two calves, respectively. In addition, one calf had mild hyperemia in the left papillar muscle of the heart, as well as mild hyperemia in the pancreas and kidneys. Multiple focal necroses in the liver and mononuclear cellular infiltration in the portal triangles was seen in one calf. Chronic purulent nephritis with moderate fibrosis and mononuclear and neutrophilic cell infiltration was seen in the same calf, as well as slight destruction of the glomeruli and casts in the renal tubuli. In one calf mild hyperemia was seen in the liver, and in three calves the spleen was congested. Focal purulent inflammations was seen in the ruminal mucosa of one calf. Mild mononuclear cell infiltration was seen in the abomasal muscosa and submucosa in one calf and in another two calves moderate mononuclear cell infiltration was seen in the mucosa of the pyloric region and proximal duodenum. In the jejunum of four calves there was moderate mononuclear cell infiltration in the mucosa with additional lesions such as bleedings, edema, desquamation of epithelial cells and fusion, atrophy and destruction of villi. Moderate mononuclear cell infiltration was seen in the ileal mucosa in three calves. In addition, there was moderate destruction and slight atrophy of the villi with desquamation of epithelial cells. Bleedings and moderate hyperemia and edema was seen in the ileum in two calves. A mucoid-like proteinaceous material was seen in the mucosa of the ileocecal valve in one calf. In two calves, all parts of the small intestine showed moderate hyperemia and moderate to severe mononuclear cell infiltrations in the mucosa, with one of the calves also showing moderate atrophy and destruction of the villi and desquamation of epithelial cells. Moderate mononuclear cell infiltration in the colonic mucosa with mild destruction of the epithelium and a moderate hyperplasia of the lymphoid tissues was seen in one calf. No lesions were seen in the brains.

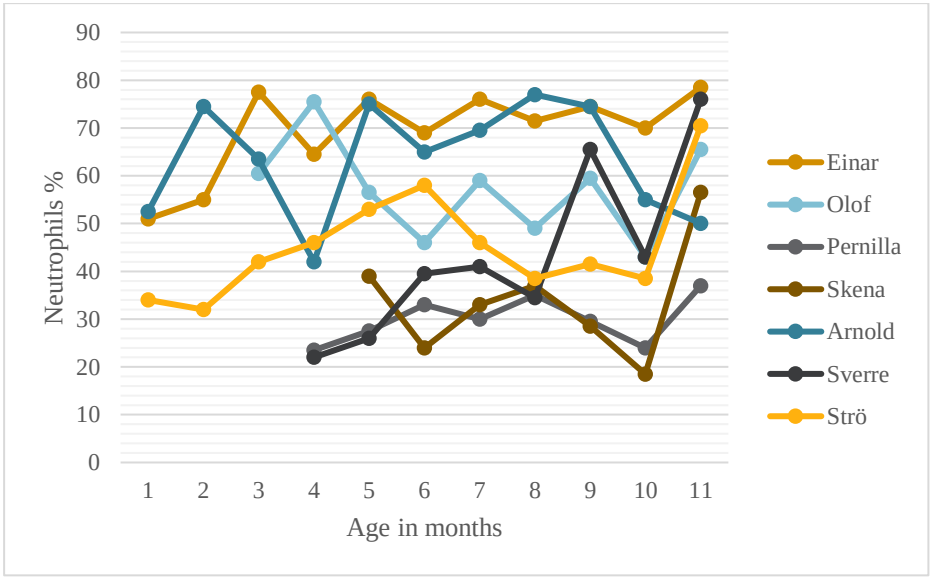
### Hematology and blood biochemistry

In one calf, the values were normal during the entire period of investigation, except for an increase in neutrophils at four months of age. Slightly reduced amount of lymphocytes and slightly increased amount of neutrophils was seen in one calf at two and between of five to nine months of age. In another calf, the leucocyte and eosinophil levels fluctuated between normal to moderately elevated and normal to markedly elevated, respectively, during the entire period of investigation. At four months of age the same calf had moderately high levels of $\beta_2$-globulins and at eight months of age moderately high levels of $\beta_2$-globulins was seen, simultaneously with a peak in eosinophil levels. Between five and six months of age the cholesterol levels was slightly to moderately increased and when the calf was eleven months old the blood showed moderately increased levels of neutrophils. Another calf showed slightly increased levels of neutrophils from three to eleven months of age. In addition, the same calf had slightly lower lymphocyte levels compared to the other calves between three and eleven months of age and at the age of eight months the same calf had moderately high levels of $\alpha_2$-globulins. In one calf,

the neutrophil levels were slightly decreased and the eosinophil levels slightly increased when compared to the other calves during the entire period of investigation. Additionally, during the entire experiment, the lymphocyte levels were increased, peaking at four months of age, in reference to the other calves. In the same calf, the levels of $\beta_2$- and $\gamma$-globulins was moderately increased at four months of age, and at eight months the $\gamma$-globulins was again moderately increased. At ten months of age the levels of $\beta_1$-, $\beta_2$- and $\gamma$-globulins was moderately increased, simultaneously with a slightly decreased albumin/globulin ratio. Another calf showed moderately decreased hematocrit and hemoglobin levels between four and a half and nine months of age, but the levels increased to near normal when compared to the other calves at ten to eleven months of age. In addition, during the same period the leucocyte levels were slightly to moderately increased but decreased simultaneously with increasing hemoglobin and hematocrit levels and were normal when compared to the other calves at eleven months of age. The neutrophil and lymphocyte levels fluctuated from normal to moderately increased during the entire period of investigation, with peaks at eleven and five months of age, respectively. Slightly to moderately increased levels of $\alpha_2$-, $\beta_2$- and $\gamma$-globulins and a low albumin/globulin ratio was seen during the entire period of investigation. One calf showed moderately increased $\gamma$-globulin levels from six months of age and moderately increased leucocyte and eosinophil levels from eight months of age until the end of the experiment. Additionally, a slight to moderate increase in lymphocyte levels were seen at six months of age, see Fig. 19.





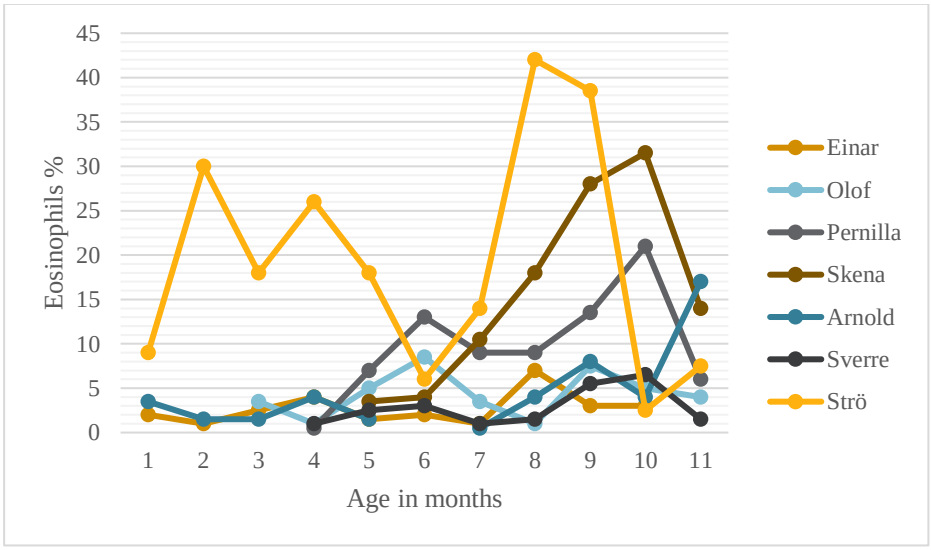




Figure 19. *Leukocyte, lymphocyte, neutrophil and eosinophil levels shown by the calves during the experiment.*

## Virology

All the calves were negative for Pestivirus antibodies.

## DISCUSSION

Moose Wasting Syndrome was first detected in Sweden in the 1980's, but to this date the cause of the disease is unknown. Many hypotheses have been presented though out the years with varying evidence to support them. In this study seven calves, born to mothers suspected of being affected with MWS, were studied to further investigate the disease.

Clinical signs frequently seen in moose affected with MWS are alopecia, diarrhoea, anorexia, emaciation, impaired vision and central nervous system disturbances (Stéen *et al*., 1993; Rehbinder *et al*., 1991; Stéen *et al*., 1989). Among the calves in this study, the two most common clinical findings were diarrhoea and alopecia, with all calves developing alopecia and four calves having diarrhoea or loose feces at least once during the experiment, see Fig. 13. The alopecia seen in the calves did not show a characteristic pattern and no calf developed simultaneous bilateral alopecia on the body, head and ears seen in some moose with MWS, see Fig. 1 and 13 (Stéen *et al*., 1993). However, alopecia was seen on the body and ears in some of the calves, see Fig. 13. Four of the calves also had periods of anorexia during the experiment, but not severe enough to affect the growth. Only one calf became stunted and showed a body condition below average at the age of 2 months. The lowered appetite and anorexia shown coincided with diarrhoea in two calves and one calf had simultaneously developed a vesicle in the mucosa of the lower jaw. No calf showed signs of emaciation. Signs of what could be interpreted as central nervous system disorders was only noticed in one calf, which developed an aggressive and nervous behaviour. No other central nervous system disturbances were seen in any of the calves, however, one calf nibbled and licked the interior. Characteristic clinical and pathological findings in moose affected with MWS is necrotizing lesions, ulcer and erosions of the mucous membranes of the upper alimentary tract, e.g. glossitis, gingivitis, esophagitis, rumenitis and abomasitis, see Fig 20 (Stéen *et al*., 1993). In this study, three of the calves developed these seemingly characteristic lesions in the mouth. Erosions located on the dental pad and at the edges of the nostrils was seen in two calves and an erosion was seen on the tongue in one calf, see Fig. 14. Other lesions, although not described as characteristic for MWS, seen in the mouths of the calves were warts, vesicles and small pigmented spots varying from white to black.

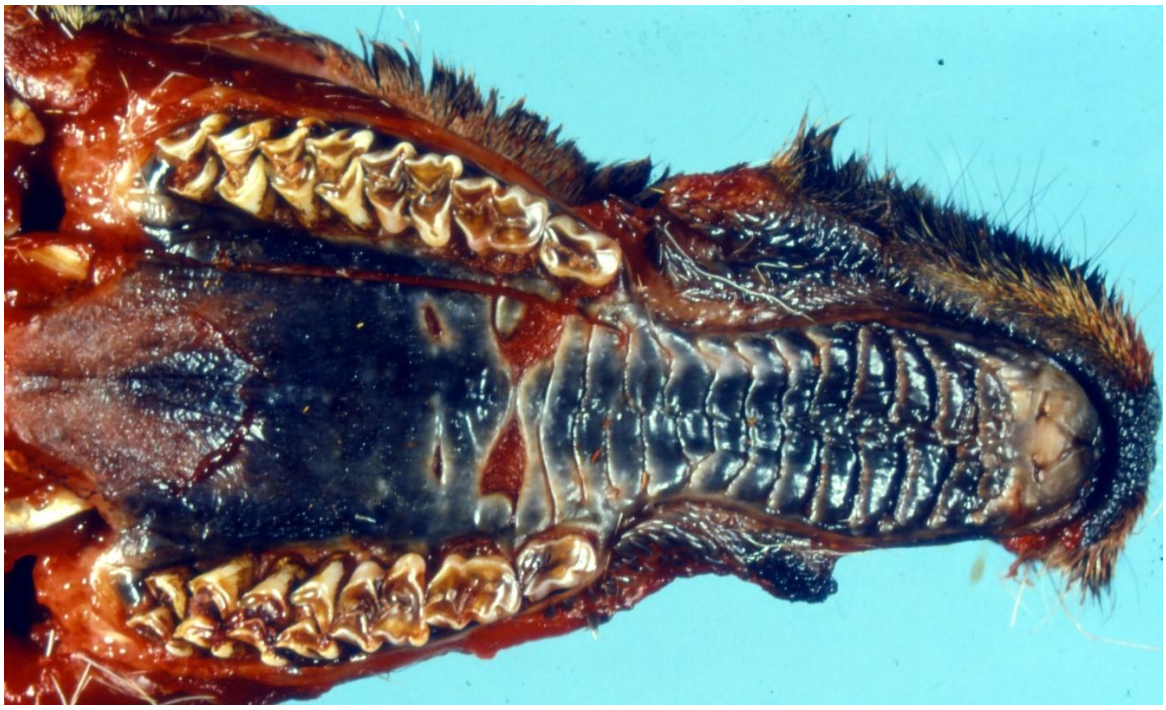


Figure 20. *Ulcer and erosions in the soft and hard palate in a MWS moose*.

Common gross pathological findings in moose with MWS are emaciation, lesions in the intestinal tract, small liver and spleen, dilated and flabby myocardium, corneal opacity and as already mentioned, necrotizing lesions of the upper alimentary tract (Rehbinder *et al*., 2004; Stéen *et al*., 1993; Bergsten, 1992; Frank, 1998). In the calves ulcers and erosions of the upper alimentary tract were not the most salient necropsy findings, which would be expected when they were affected with MWS, see Fig. 14 (Stéen *et al*., 1993). However, three of the calves showed a hyperemic and hemorrhagic mucosa in the dorsal and ventral concha, and in one calf there was a small erosion on a conical papilla of the tongue, see Fig. 14. In two calves the heart was affected, with slight dilation of the right and left ventricle, respectively, although in none of the calves was the myocardium flabby. In contrast to what is commonly seen at necropsy among MWS affected moose (Rehbinder *et al*., 2004) the liver in all but one calf was enlarged, congested and had rounded edges, see Fig. 15. The spleen showed similar changes, being enlarged and congested and also having prominent white follicles, in all but one calf. This is also opposite to what is usually seen in MWS affected moose in which the white pulp of the spleen is depleted, which can be depended on a chronic disease in its final stage. Lesions frequently seen in the intestinal tract of moose affected with MWS are hyperemia, petechiae, dilated blood vessels, edema, thin and fragile intestinal wall, hemorrhagic or catarrhal enteritis with watery to hemorrhagic content (Bergsten 1992; Rehbinder *et al*., 2004; Stéen *et al*., 1993). The lesions seen in the intestinal tract of the calves coincided with the characteristic lesions typically seen in MWS affected moose. Bleedings, slightly reddish to heavily hyperemic and hemorrhagic mucosa, thin and flaccid intestinal wall, dilated and congested blood vessels, mucoid to hemorrhagic intestinal content and thin Peyer's patches among other findings was seen at necropsy, see Fig. 17.

The histological findings seen in the calves in this study partially coincided with the typical histological lesions seen in moose with MWS as described in the literature by Stéen *et al*., (1993) and Rehbinder *et al*. (2004). The calves had histopathological lesions in their lymphoid organs, such as hyperplasia of body lymph nodes and thin Peyer's patches, which is commonly seen among MWS diseased moose. In addition, mononuclear cell infiltrations were seen in the mucosa and submucosa of the upper alimentary tract, another characteristic histopathological finding in MWS affected moose. In the jejunum of the calves' mononuclear cell infiltration in the mucosa with additional lesions such as bleedings, edema, desquamation of epithelial cells and fusion, atrophy and destruction of villi was seen. The ileal mucosa showed moderate mononuclear cell infiltrations, moderate destruction and slight atrophy of the villi with desquamation of epithelial cells. These lesions are similar to those found in MWS affected moose, see table 3.

Table 3. *Histological and pathological lesions typically seen in MWS affected moose compared to histological lesions found in the calves*

| **Characteristic lesions in MWS affected moose** | **Lesions seen in the calves** |
|---|---|
| Intra- and intercellular edema, congestion and mononuclear cell infiltration of mucous membranes of upper alimentary tract | Focal purulent inflammations in the ruminal mucosa of one calf |
| Intra- and intercellular edema, congestion and mononuclear cell infiltration of the skin | Moderate perivascular neutrophilic infiltrations was seen in the skin in one calf. Slight hyperkeratosis was seen in the skin and the skin of the nasal septum in two calves |
| Vacuolar degeneration of cells in stratum basale and stratum spinosum of the upper alimentary tract | Severe mono- and polymorphonuclear cell infiltration in the submucosa of the mucous membranes of the mouth, as well as microabcesses was seen in one calf. Vesicles were seen in one calf on gross pathology |
| Depletion of white pulp and hemosiderosis in the spleen | - |
| Depletion or hyperplasia and hemosiderosis of lymphoid follicles in body lymph nodes | Hyperplasia of the axillary, popliteal, mesenterial and ileocecal lymph nodes were seen in the calves. Moderate eosinophilic cell infiltration in the sinuses and capsule of the popliteal lymph node and mild hyperplasia and moderate hemosiderosis in the lateral subiliac lymph node was seen in one calf. |
| Small and pleomorphic hepatocytes containing lipofuscin granules | - |
| Proliferation of intralobular lymphcanaliculi and perilobar lymph vessels in the liver | Multiple focal necroses in the liver and mononuclear cellular infiltration in the portal triangles was seen in one calf |

| | |
|---|---|
| Nephrosis with sclerosis of the glomerular membranes and degeneration of distal tubuli | Chronic purulent nephritis with moderate fibrosis, slight destruction of glomeruli, mononuclear and neutrophilic cell infiltration, and casts in the tubuli was seen in one calf |
| Purkinje cell degeneration | - (?)<br><br>No conclusions could be drawn from the brain histology |
| Focal myocyte degeneration | Mild focal mononuclear cell infiltration in the right papillar muscle of the heart and a focal area of mononuclear cell infiltration was seen in the interstitium of the heart was seen in two calves, respectively |
| Osteoporosis | Not investigated |
| Lymph and blood stasis, necrotized, stunted and sloughed villi, hyperemia, pseudomembranes, bleedings and mononuclear and occasionally polymorphonuclear cell infiltration in the lamina propria in the small intestine | Moderate mononuclear cell infiltration, bleedings, edema, desquamation of epithelial cells, and fusion, atrophy and destruction of villi in the intestines was seen in the calves. |
| Edema and erosion of the mucosa and mononuclear cell infiltration in the lamina propria and submucosa in the colon | Moderate mononuclear cell infiltration with mild destruction of the epithelium was seen in the colonic mucosa in one calf. |

Histology of the brain did not show any lesions typical of MWS, such as Purkinje cell degeneration, loss of dead Purkinje cells, swollen axons and dendrites or swollen or shrunken perikaryons (Rehbinder *et al*., 2004). Since prions have been suggested prions as a potential cause of MWS, one purpose of this project was to investigate whether or not the calves showed signs indicating prion disease (Stéen *et al*., 2018). No certain histopathological conclusions could be drawn partly because the slides were of varying quality and only brain material from 6 out of the 7 calves were available. A major issue with the brain material was that it had ben fixated in formalin for 25 years making processing difficult and creating many artifacts. No vacuolation of the grey matter or any other lesion typical of BSE or classic CWD could be seen. It has been shown by Hammarström *et al*. (2018) diverse morphological features in moose with CWD compared to the classic CWD in reindeer, meaning that prion disease in moose brain and reindeer brain with CWD differ, see Fig. 21. This means that searching for classic CWD histological changes in the brain of MWS diseased moose probably will not yield the same histopathological picture.

Preliminary data of LCO stained CWD moose and reindeer show the presence of diverse morphology of aggregates positive for LCO, very similar to those positive with immune-histochemistry (Hammarström *et al*., 2018).

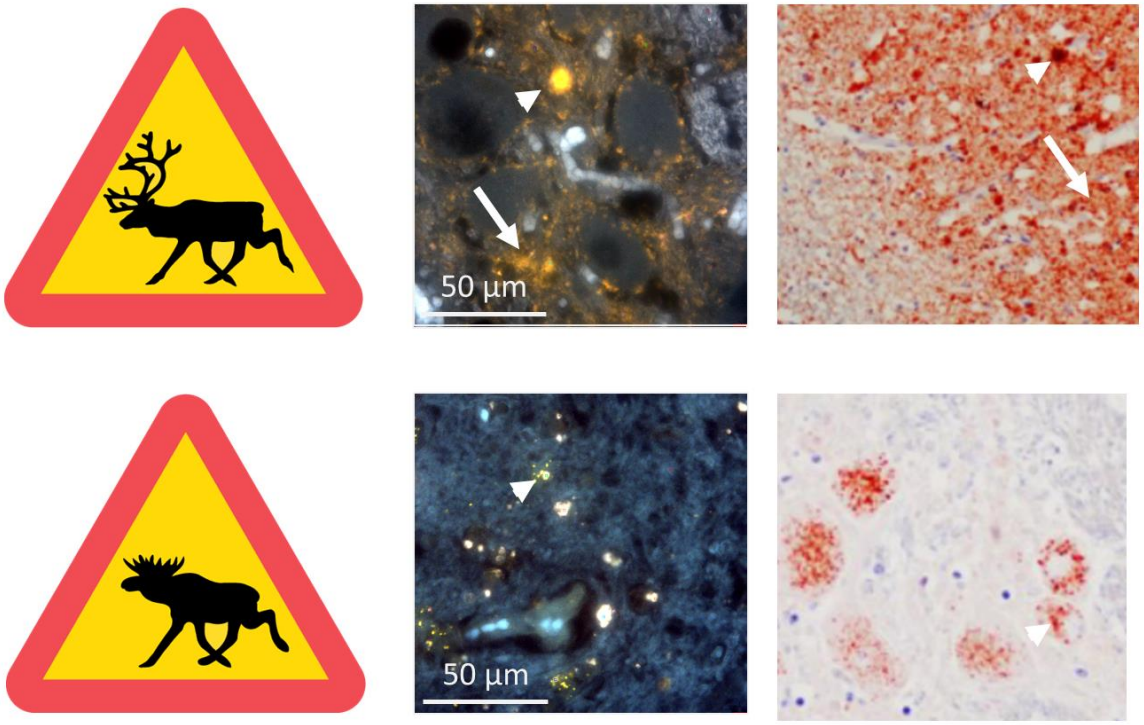



Figure 21. *PrP aggregate pathology in CWD from Norwegian reindeer (top) and moose (bottom). Arrows marks widespread fluorescent synaptic aggregates of PrP in reindeer similar to CWD prions found in North American deer. CWD prions in moose marked with arrowheads appear intracellular and are bright, small, round and punctuate. Very similar PrP pathology is observed by immunohistochemistry (Hammarström* et al., *2018).*

In a study by Kockum-Adolfsson (1995) it was stated that the hematological picture in MWS diseased moose varies greatly, but sometimes an extremely high or low lymphocyte count, high neutrophil count and high total number of leucocytes were seen. This is in agreement in what was seen in a study by Merza *et al*. (1994) in which leukopenia was seen in 6 and leukocytosis in 3 of 13 moose diseased with MWS. However, in both of those studies only hematology and no biochemical parameters was investigated. No extremes in the lymphocyte levels was noticed in the calves of this study, but three of the calves showed slight to moderate increases in the lymphocyte levels. In all but one calf, periods of moderate increases of neutrophils were seen and two calves showed moderate to marked increases in eosinophils. Additionally, some calves showed moderate increased in globulin levels, which in most cases seemed to coincide with periods of diarrhoea or coughing. When comparing the results to those shown in moose by Rostal *et al.* (2012), there were higher peak levels of total leucocytes, lymphocytes, neutrophils and eosinophils and lower nadir levels of lymphocytes in this study. The elevated eosinophil count seen in some of the calves could possibly be caused by migrating intestinal parasites, although no parasites were found in the intestines of the calves at necropsy.

In studies by Frank *et al*. (2000a; 2000b) blood biochemical changes seen in diseased moose was increases of urea, glucose, insulin and CK, and decreases of GLDH, bilirubin and $T_4$. However, only urea, CK and GLDH among the above-mentioned parameters was measured in the calves in this study. Urea, GLDH and CK levels did not differ significantly among the calves,

however, when comparing the overall urea levels to urea levels measured in free-ranging calves in a study by Rostal *et al*. (2012), they were slightly higher in the calves in this study. GLDH levels was never measured in the study by Rostal *et al*. (2012), and CK levels, when compared did not differ.

By conclusions drawn from the literature, hematology and biochemistry does not seem to be a very useful in diagnosing MWS, but this could however depend on the cause of MWS and the degree of illness and elongated disease time. In prion diseases like CWD, hematology and biochemistry has not been shown to be a useful diagnostic tool as the only expected change is low urine specific gravity in terminally ill cervids (Williams, 2005). Although in viral diseases such as BVDV, hematology could potentially be a useful tool as characteristic hematological findings would be severe leukopenia and lymphopenia (Constable *et al*., 2017). No leuko- or lymphopenia was seen in any of the calves in this study. If, as suggested by Frank *et al.* (2004, 2000a & 2000b) MWS is caused by molybdenosis and copper deficiency potentially leading to diabetes mellitus, anemia and hyperglycemia would be expected findings in the blood. However, increases in glucose is not only seen in diabetes. It is possible that the increases in glucose seen in the MWS disease moose in the study by Frank *et al.* (2000b) were caused by stress due to severe illness (Stewart, 2018). Glucose levels were not measured in the calves of this study and in only one calf signs of anemia was seen, having moderately decreased hemoglobin levels between four and a half and nine months of age.

Gross pathology, histopathology, hematology, blood biochemistry and clinical signs seen in the calves were to some extent similar to those expected to be seen in moose affected with MWS. There is however no definitive pathognomonic criterion for MWS and diagnosis is mostly based on clinical and gross pathological findings, which in the calves did not completely correspond with what is expected to find in MWS affected moose. In this study, histology of the brains of the calves revealed no signs typical of spongiform encephalopathies, of BSE- or CWD-type. However, the tissue was only stained with hematoxylin/eosin, and as already mentioned, only 6 out of 7 calves could be investigated histologically and the brains had been stored for 25 years in formalin making processing problematic, creating many artifacts. It would have been desirable, but sometimes not plausible in the wild, if all the mothers to the calves had been investigated to the same extent as the calves, e.g. with necropsy, histology and hematology and blood biochemistry. Only two of the mothers were necropsied and diagnosed with MWS with certainty, but no other of the aforementioned investigations were done in those females or in any of the other females.

These calves were, in principle, kept in a specific-pathogen-free (SPF) environment as laboratory animals and the stable building was kept free of particular pathogens affecting ruminants. Even though reared under these circumstances, the calves showed obvious signs of illness, unlikely to have be caused by management factors or infections contracted in the stable. This indicates that clinical signs and pathological and histopathological lesions is due to infection before stabling. Since the calves were born between May 15 and June 15 and captured between May and November the same year, the age of the calves when captured varied <1 to 5 months, implicating that the calves contracted the disease at a very young age. The premises for this study was that MWS is caused by agents with the ability to transmit from mother to offspring

and that the calves could contract the disease this way. Assuming the calves were affected by MWS the most likely way of them contracting the disease would have been via their mothers' directly or transplacentally. The possibility of them having contracted MWS by a food-related route is rather unlikely since the feed, mineral and vitamin supply was carefully balanced, using the same formula as Kolmården zoo. They had food ad lib and the size of the antlers showed that they had grown better than they would have done in the wild, see Fig 22.

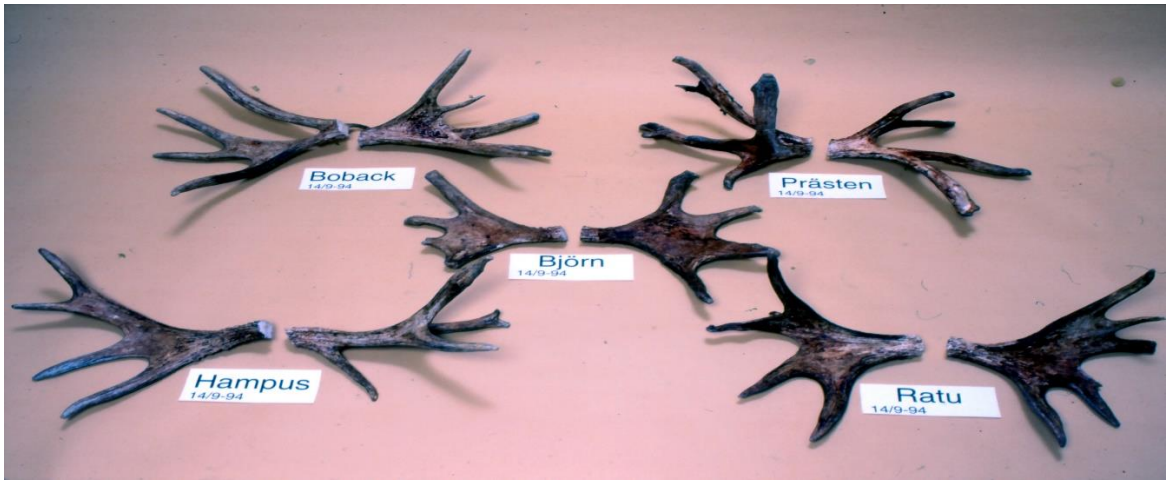



Figure 22. *The male calves developed bigger antlers with palmate shape than expected at their age in the wild, showing that the feed, minerals and vitamin supply were optimal*.

The deer milk formula and feed given to the calves were controlled and the ingredients known. They were given minerals every day, had constant access to saltstones and were given iron, selenium and vitamins at regular intervals. This contradicts the food-related hypotheses, such as the calves being subjected to pollution, decreased browse availability or trace mineral deficits, and rather indicates that MWS is caused by an infectious agent. Although it was presumed in this study that MWS can be vertically transmitted, the route/routes of transmission for MWS, or even if is MWS a transmissible disease is unknown. Both viruses, such as BVDV, and prions have been shown to transmit vertically (Constable *et al*., 2017; Nalls *et al*., 2013). Ever since the outbreak, and when this experimental study was carried out, virus has been on the top list of suspected causes of MWS, much because of the similarities to BVDV (Feinstein *et al*., 1987; Rehbinder *et al*., 1991). In this study, all the calves were negative for Pestivirus antibodies, making that an improbable cause of the disease shown by the calves. Although it is still possible that another virus could be the cause of disease albeit not detected in this study. Moose Wasting Syndrome is, in addition to atrophied lymphoid organs and ulcer and erosions of the upper alimentary tract, characterized by atrophied lymphoid organs. This indicates that the disease has a negative impact on the immune system, potentially leading increased susceptibility to infections. In turn, this could result in a plethora of possible clinical signs and pathological and histopathological lesions depending on underlying agent causing them making it difficult to characterize and finding pathognomonic diagnostic criterion for the syndrome. This also makes it difficult to compare the clinical, pathological and histopathological picture between different MWS diseased moose. It is possible that some of the changes seen in the calves were not directly caused by MWS, but rather normal flora or opportunistic pathogens infecting the calves, before entering the stable, because of an attenuated immune system. The discovery of CWD in

Norway in 2016 gave reason to believe that MWS may have been caused by a similar, or even the same agent (Hammarström, 2016). Considering the long incubation period for prion diseases, for the calves to develop clinical disease from a similar agent they must have been infected at a very young age, or perhaps prenatally. Mother to offspring transmission has been shown to occur with CWD in free-ranging Rocky Mountain elk (Selariu *et al*., 2015), and evidence of transplacental transmission of Scrapie has been shown by Spiropoulus *et al*. (2014). Spiropoulus *et al*. (2014) suggested that transplacental transmission requires peripheral distribution of prion proteins, which in turn depends on the PrP amino acid sequence. Stéen *et al*. (2018) showed that LCO positive protein aggregates are seen in diseased moose when tissues are stained with LCO probes. In two earlier studies by Rehbinder *et al*., 1991 and 2004, no support was provided of prions as being the cause of MWS, with the diagnostic tools available at that time. However, in the first study immunohistochemistry, by a perhaps outdated method, was only carried out in two animals, while the second study only looked at brain histology slides stained with hematoxylin/eosin. Studies carried out at SLU with the assay PLARCA give some indications of a Scrapie-like disease occur. In addition, as stated by Benestad & Tellin (2018) histological lesions in the brain does not appear until relatively late in CWD and in moose histopathology differ from the classical CWD-histology. With the long incubation period for CWD, as Benestad & Telling (2018) declare, makes it possible that more pronounced lesions would have developed in the moose calves. It is possible that other, more severe MWS characteristic clinical and gross pathological lesions would have appeared if the calves had been stabled for a longer time and investigated when the disease had progressed further.

## ACKNOWLEDGEMENT

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## POPULÄRVETENSKAPLIG SAMMANFATTNING

I Sverige i mitten på 1980-talet sågs ett ökat antal döda och sjuka älgar med liknande kliniska och patologiska fynd som de som ses hos nötkreatur drabbade av Bovin Viral Diarré. 1996 hade drygt 1400 älgar hade insjuknat, dött eller avlivats p.g.a. sjukdomen. Syndromet fick många namn, så som Älvborgssjukan, erosive/ulcerative alimentary disease, bovine viral diarrhoea/ mucosal disease-like syndrome och Moose Wasting Syndrome. De sjuka älgarna hade diarré, håravfall, nedsatt aptit, synnedsättning, var uttorkade, avmagrade och svaga. Hos de sjuka älgarna sågs också neurologiska problem så som cirkelgång, ataxi, beteendeförändringar, tandgnissling och hängande öron. Vid obduktion sågs f.f.a. förtvinade lymfoida organ och sår och erosioner i slemhinnorna i övre delen av magtarmkanalen, vilket var karaktäristiskt för sjukdomen. Andra obduktionsfynd som hittades hos sjuka älgar var hemorrhagisk enterit, dilaterat och slappt myokardium, opaciteter i ögonen och osteoporos. Hematologi visade inga uniforma förändringar hos de sjuka älgarna, men ibland sågs extremt höga eller låga lymfocytnivåer. Biokemiska förändringar som har setts hos sjuka älgar är förhöjda nivåer av bl.a. insulin, glukos och urea och minskade nivåer av bl.a. thyroxin och bilirubin. Histologiska undersökningar har visat inflammation i hud och slemhinnor i övre magtarmkanalen, med infiltration av mononukleära celler i mukosa och submukosa och intra- och intercellulärt ödem. Atrofi och nekros av villi, lymf- och blodstas, avskavda epitelceller, pseudomembran, blödningar och hyperemi är lesioner som setts i tarmarna hos sjuka älgar. Lesioner har även setts i lever, njure, skelett och hjärna. Eftersom det saknas diagnostiska instrument och metoder för att ställa en definitiv diagnos har diagnosställandet baserats på typiska kliniska, patologiska, histopatologiska, hematologiska och biokemiska fynd, trots att sjukdomen saknar patognomona fynd. I dagsläget finns fortfarande inget svar på vad som orsakar MWS men många olika teorier har presenterats genom åren. Två grupper av potentiella orsaker har föreslagits; födorelaterade och infektiösa. Till de födorelaterade orsakerna hör förändringar i spårämneskoncentrationer, minskad tillgång på föda samt föroreningar och till de infektiösa orsakerna hör virus, bakterier och prioner. För alla av teorierna finns det evidens som talar både för och emot men ingen har hittills definitivt bevisats. Gällande förändringar i spårämneskoncentration har primärt koppar-brist och/eller molybdenförgiftning misstänkts då en studie har visat att sjuka älgar haft lägre koncentration av koppar och högre koncentration av molybden i levern jämfört med friska älgar. Problemet med studien var att de sjuka älgarna provtogs mellan 1991 och 1993 medan kontrollgruppen provtogs 1982, och under samma tidsperiod sågs att koppar- och leverkoncentrationerna förändrades på samma sätt hos friska älgar. Virus misstänktes starkt väldigt tidigt p.g.a. likheten med BVDV. Dock har aldrig något virus isolerats hos sjuka älgar, men antikroppar mot BVDV har hittats hos vissa sjuka älgar. Älgar med MWS har visat liknande kliniska tecken som setts hos andra djur med prionsjukdomar, f.f.a. avmagring och neurologiska problem. Tidigare undersökningar har inte kunnat visa några tecken på klassisk prionsjukdom hos sjuka älgar men sedan Chronic Wasting Disease upptäcktes i Norge väcktes ett nytt intresse för att studera om MWS eventuellt kunde bero på prioner. Nya studier där LCO-färgning använts har visat att LCO-positiva proteinaggregat kan ses hos sjuka älgar. Det har också visats att den histologiska bilden hos älgar med CWD skiljer sig från renar med CWD, vilket även skulle kunna vara fallet vid MWS och förklara varför inte klassiska prionförändringar har setts histologiskt hos MWS-sjuka älgar. I detta projekt har en experimentell studie, där sju kalvar födda till mödrar misstänkt vara drabbade av MWS, granskats och sammanställts för att försöka besvara om sjukdomen kan

överföras från moder till avkomma och om kalvarna uppvisade några tecken på prionsjukdom. Kalvarna stod uppstallade i SPF-liknande förhållanden mellan 7 till 11 månader och utfodrades på samma sätt som älgarna på Kolmårdens djurpark. De observerades dagligen och undersöktes för blodbiokemi, hematologi, patologi och histo-patologi. Resultaten visar att kalvarna utvecklade några av de histopatologiska, patologiska och kliniska fynd som ses hos älgar sjuka i MWS. De två vanligaste kliniska fynden hos kalvarna var håravfall och diarré, vilket alla utvecklade åtminstone en gång under experimentet. Kalvarna uppvisade också nedsatt aptit men ingen av kalvarna magrade av. Inga neurologiska avvikelser noterades. Tre kalvar utvecklade lesioner i munnen karaktäristiska för MWS, men hos kalvarna sågs även andra förändringar i munnen så som vårtor, vesikler och små pigmenterade fläckar, vilket inte ses hos MWS-sjuka älgar. Obduktionen visade kraftiga förändringar i tarmarna med blödningar, hyperemi, tunn och slapp tarmvägg, dilaterade och stasade kärl, slemmigt till blodigt innehåll samt små och tunna Peyerska plack. Till skillnad från vad som ses hos MWS-sjuka älgar, där mjälte och lever brukar vara liten och skrumpen, hade kalvarna en förstorad lever och mjälte. Opaciteter i ögonen och tecken på utmärgling sågs inte. Histopatologiskt sågs inflammation i slemhinnorna i övre magtarmkanalen, med mono-nukleär cellinfiltration i mukosa och submukosa, vilket ofta ses hos MWS-sjuka älgar. Inflammation sågs även i hjärta, njure, lever, hud, lymfknutor och tarmar. I hjärnan hos en kalv sågs en del fynd som liknar de som kan ses vid MWS, med skrumpna neuroner och purkinjeceller. Dock kunde inga definitiva slutsatser dras från histologi av hjärnan p.g.a. att hjärnmaterialet förvarats i formalin väldigt länge och därmed uppstod många artefakter som gjorde materialet svårtolkat. Inga lesioner typiska för klassisk prionsjukdom, såsom CWD eller BSE, sågs hos kalvarna. Sammanfattningsvis kan sägas att kalvarna visade uppenbara tecken på sjukdom, och detta trots att de hölls i en SPF-liknande miljö. De kliniska patologiska och histopatologiska fynden hos kalvarna stämde till stor del överens med de som ses hos MWS-sjuka älgar, d.v.s. kalvarna var med stor sannolikhet sjuka i MWS. Med tanke på att älgkalvarna utfodrades på samma sätt som de gör på Kolmårdens djurpark är det osannolikt att sjukdomen som sågs hos kalvarna berodde på foder-relaterade orsaker så som foderbrist, föroreningar eller kopparbrist. Snarare tyder det på en infektiös orsak och mest sannolikt blev kalvarna infekterade före uppstallning med tanke på att de som tidigare nämnts levde i en SPF-liknande miljö. Förmodligen infekterades kalvarna via deras mödrar direkt eller transplacentalt. I denna studie sågs inga tecken på prionsjukdom hos kalvarna, men p.g.a. att hjärnvävnaden hade förvarats länge i formalin och hade en hel del artefakter var det svårt att dra några definitiva slutsatser. Det är också möjligt att den histologiska bilden vid MWS skiljer sig från den klassiska histologiska bilden vid prionsjukdomar. Med tanke på den långa inkubationstid som ses vid prionsjukdomar är det fortfarande tänkbart att kalvarna hade en prionsjukdom och att förändringar hade uppstått om sjukdomen hade fått fortskrida under en längre tid.

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