An erosive/ulcerative alimentary disease of undetermined etiology in Swedish moose (*Alces alces* L.)

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Abstract: During the years 1985 to 1987, 689 moose (*Alces alces* L.) collected throughout Sweden were necropsied at the National Veterinary Institute in Uppsala, Sweden. Sixty-eight of those investigated had catarrhal to hemorrhagic enteritis, atrophied lymphoid organs, and/or numerous erosive, uclerative, necrotizing lesions of the digestive mucosa. Histopathology of the mucous membranes revealed marked inter- and intracellular oedema, erosions, ulcers and intracytoplasmic inclusion bodies. Neither Bovine Virus Diarrhoea/Mucosal Disease (BVD/MD) or Infectious Bovine Rhinotracheitis (IBR) virus could be isolated from the diseased animals. It is suggested that the syndrome resembling BVD/MD complex, may have been caused by an yet unidentified virus.

Key words: *Alces alces* L., Bovine Virus Diarrhoea/Mucosal Disease (BVD/MD), Infectious Bovine Rhinotracheitis (IBR) virus, intracytoplasmic inclusion bodies, moose, Sweden.

Introduction

A disease outbreak in farmed fallow deer (*Dama dama* L.) resembling Bovine Virus Diarrhoea/Mucosal Disease (BVD/MD), was reported (Diaz *et al.*, 1988). The disease was characterized clinically by diarrhoea. At necropsy ulcerative lesions in the digestive tract and very thin intestinal wall were found. Histology revealed intracytoplasmic basophilic inclusion bodies (IB). BVD-virus was demonstrated from one of the animals. Similar morphological findings have been reported in reindeer (*Rangifer*

tarandus L.) (Rehbinder *et al.*, 1985), and in a roe deer (*Capreolus capreolus* L.) and a moose (*Alces alces* L.) (Feinstein *et al.*, 1987). The findings were characterized by multiple erosions and ulcers of the mucous membranes of the digestive tract, and catarrhal to haemorrhagic enteritis.

Since 1985 an unknown disease, similar to BVD/MD, has been observed in moose in Sweden. More than 1000 animals have been found with the syndrome during the period 1985–1992. This paper reports on a retrospective stu-

dy on 68 moose necropsied during the years 1985–1987, which showed typical lesions. Virological studies have been performed in order to investigate the etiology. Rehbinder *et al.* (1991) reported on an investigation of the central nervous system (CNS) from 12 adult female moose with this syndrome. This clarified that the disease was not associated with a spongiform encephalopathy, and was probably not associated with virological diseases which occur in Sweden.

Material and methods

Animals

During the years 1985–1987, 689 moose from different parts of Sweden were submitted to the National Veterinary Institute, Uppsala, for routine necropsy investigations. Diarrhoea and atrophied lymphoid tissues of the mucous membranes of the digestive tract, and/or ulcers and erosions characteristic of a distinct syndrom were seen in 68 of these animals. The origin of the animals is shown in Figure 1. Thirty-one of the animals were observed still alive, with signs that included all combinations of: anorexia, weakness, lack of wariness toward man, impaired vision, diarrhoea, circling and emaciation. Thirty-seven of the moose were submitted after having been found dead. In 10 of the 68 cases only some organs and/or the head were obtained. Eleven moose with this syndrome were not studied microscopically.

Pathological investigation

Tissue samples collected from the digestive tract, lymphoid organs, and parenchymatous organs were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin.

Virological investigation

Virus reference strains and cell cultures used

For isolation and propagation of virus and serum neutralization test (SN), primary embryonic dermis (ED) cell and primary bovine embryonic turbinate (BT) cell were used. The cell cultures were supplemented with 1% fetal calf serum and maintained in Eagle's MEM. In the SN test a strain from Swedish cattle Bovine Herpesvirus Type 1 (BHV-1) (96) was used. Samples for virus isolation were kept at –70°C until processing.
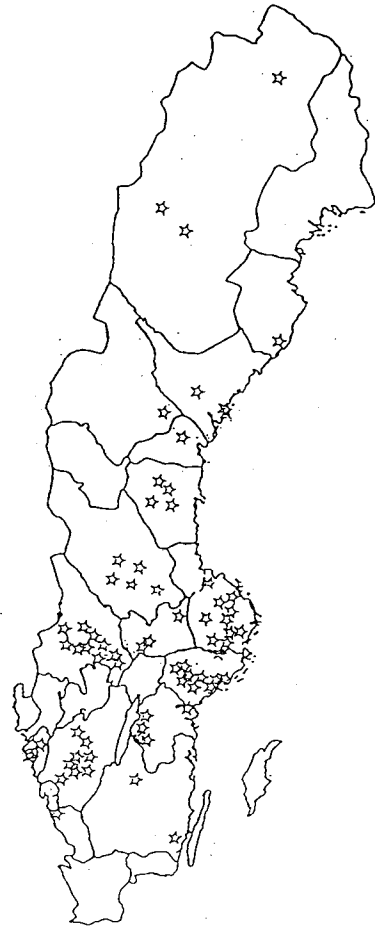

Fig. 1. A map of Sweden showing the distribution of 68 cases of a BVD/MD-like syndrome in moose collected between 1985–1987.

BVD/MD – Virus isolation

Tissue samples (spleen, lymph nodes, and mucous membranes) and/or heart blood from 58 animals and some of these tissues from another 10 animals were obtained at necropsy. The supernatant of a 10% suspension of ground tissue or blood clots were analysed for the presence of BVD virus. The virus isolation was performed by inoculation of 0.1 ml of the samples into 2 roller tube cultures of BT cells. After one passage 0.15 ml of the supernatant was inoculated on a cover slip culture of BT cells. The cultures were examined for cytopatic effects and 4 days later the cover slips were fixed in acetone and stained by an indirect immunofluorescent method using a hyper immune serum against BVD virus produced in swine (Bielefeldt-Ohmann *et*

al., 1981). The presence of non-cytopatic BVD virus was not investigated.

BHV-1 – Virus isolation
From a 10% suspension of organs and PBS (w/v) 0.2 ml was inoculated into tube cultures using two tubes per suspension. After 1 hour for absorption at 37°C, the tubes were rinsed with Eagle's MEM and new medium was added and further incubated at 37°C. Each day the tubes were controlled for presence of cytopatic effects (CPE). If negative after one week, 0.1 ml of the cell culture was collected and processed into 2 new tubes.

BHV-1 – Antibodies
A SN test was used to test for antibodies to BHV-1 virus in 20 animals in a microtiter system (Reid *et al.*, 1986). The mixture of serum and virus was incubated for 24 hours at 4°C (Bitsch, 1973).

Immunoperoxidase staining
Specimens from 12 animals were prepared for immunoperoxidase staining as described by Sainte-Marie (1962). Endogenous peroxidase activity was blocked by incubating sections for 30 min in 0.05 M TRIS-HCC buffer, pH 7.6, (which was used in all rinsing steps throughout the experimental procedure) with 0.3% H_2O_2. After a thorough rinse in buffer, sections were incubated with buffer containing 2% BSA. Sections were drained gently and incubated consecutively with swine antiserum to BVDV, diluted 1/80, and peroxidase conjugated rabbit antiswine IgG (DAKOPATTS), diluted 1/200. Dilutions were made in buffer containing 1% BSA and tissues were incubated for 30 min with each antiserum. After a short rinse in buffer, peroxidase activity was visualised by rinsing sections in 0.06% diaminobesidine (DAB, SIGMA) and 0.034% H_2O_2 in buffer for 8 min. Sections were rinsed in tap water, counterstained with haematoxylin, and finally mounted in Eukitt. Controls consisted of
1) normal swine serum instead of anti-BVDV serum; and
2) application of anti-BVDV serum on known positive and negative tissues.

Bacteriology
Samples were obtained from organ material collected at necropsy. Routine bacteriological investigations were performed.

Chemical analysis
The nutritional state of the moose was estimated by chemical analysis of the bone marrow fat according to the Schmid-Bondzynski-Ratzlaff method (Nordisk Metodikk-Kommitté för Livsmedel, 1955). The three categories used to describe the nutritional state of the animals were normal, undernourished, and emaciated (Borg, 1970).

Results
Animals
During 1985–1987, the animals with this syndrome were distributed all over Sweden (Figure 1). The syndrome affects animals in different ages, sexes, and nutritional states, as seen in Table 1.

Necropsy findings
The majority of the animals (85%) had ulcerative, necrotizing lesions of the upper digestive

Table 1. Age, sex and nutritional state of 68 moose necropsied showing a BVD/MD-like syndrome in Sweden.

| | 0–6 mo. | | 7 mo.–2 yrs. | | 3 yrs.–>15 yrs. | | Unknown | |
	M	F	M	F	M	F		Total
Normal	3	2	1	1	2	9	1[b]	19
Undernourished	2	1	4	1	6	14	1[c]	29
Emaciated	1	2	2	4	1	8		18
Unknown				1[a]			1[d]	2
Total	6,	5	⌐7	7	9	31	3	68

[a] 7 mo. – 2 yr. old female of unknown nutritional state

[b] Normal, 0 – 6 mo. old animal of unknown sex

[c] Undernourished female of unknown age

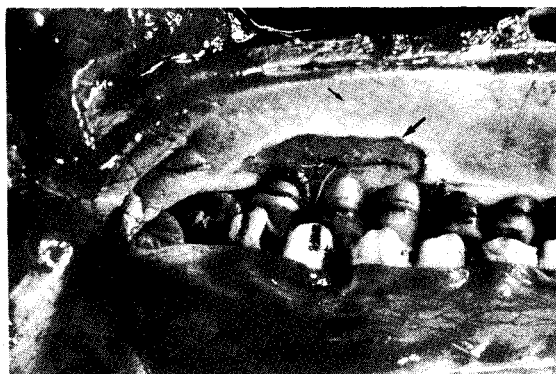[d] Male of unkown age and nutritional state

Fig. 2. Mouth. Note the elongated erosion (large arrow) and the smaller irregularly rounded lesion (small arrow) showing a dark necrotic bottom with white borders.
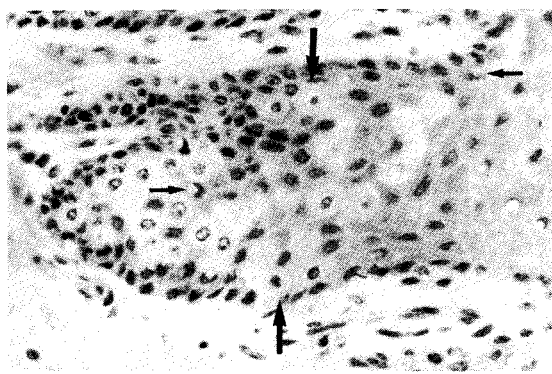

Fig. 3. *Papillae bucalis.* Note foci of swollen cells in *stratum basale* and *spinosum* (large arrows), and the inclusion bodies (small arrows). HEx400.

tract (glossitis, gingivitis, oesophagitis, and/or rumenitis). Catarrhal to haemorrhagic enteritis was present in 72% of the 58 moose upon which we were able to do a complete necropsy. Numerous erosions, ulcers and necrotizing lesions were observed in the nostrils, oral and/or oesophageal mucosa in 58 of the moose. In the nostrils the squamous mucosa was involved (Figure 2).

Additionally, 7 of the 58 animals had ulcers on the ruminal pillars and 2 also had small, pinpointed ulcers in the abomasal mucosa. The lesions were shallow, irregularly rounded to elongated, varying in size from about 3 mm to 4 cm, and had a red dark necrotic bottom with white borders (Figure 2). Oedema and hyperemia of the abomasal mucosa was common. The intestines of 42 animals appeared oedematous and hyperemic, with watery to hemorrhagic content. Depletion of the Peyer's patches was also a common finding. The mesenteric lymph nodes were black and oedematous. The spleen was smaller and thinner than normal in 29 moose, having pronounced trabeculae. A striking finding was bilateral alopecia of the body, head and ears in 3 moose. These 3 animals also had severe skin lesions with scabs and crusts on the body and around the eyes. In 18 of the animals uni- or bilateral corneal opacity was recorded.

Histological findings

The mucous membranes of the oral cavity, oesophagus and rumen had inter- and intracellular oedema, congestion and mild mononuclear cell infiltration of the *lamina propria* and submuco-
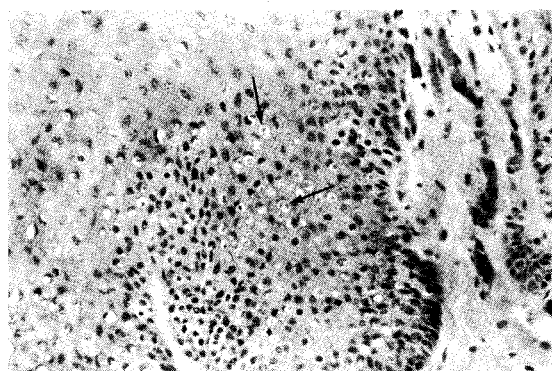

Fig. 4. Mucous membrane of the mouth. Note the swollen cells and the numerous inclusion bodies (arrows). HEx250.

sa. Ulcers and necrotizing lesions were confirmed. The ulcers frequently had a necrotic bottom, with bacterial colonies and purulent inflammatory reaction. In the 10 moose without gross ulcerative lesions of the upper alimentary mucosa, there were microscopic changes in those tissues, comprised of oedema, congestion and mild mononuclear infiltration of the *lamina propria* and submucosa. In the epithelium vesicles were evident as circumscribed areas filled with slightly eosinophilic fluid that separated the different epithelial layers. Groups of cells in the *stratum basale* and *stratum spinosum* showed a pronounced vacuolar degeneration and were very swollen, with strongly acidophilic cytoplasm and pyknotic nuclei (Figure 3 and 4). In 44 moose (65%), cells of the *stratum basale* and *stratum spinosum* taken from the mucous membranes of the mouth, oesophagus and fo-
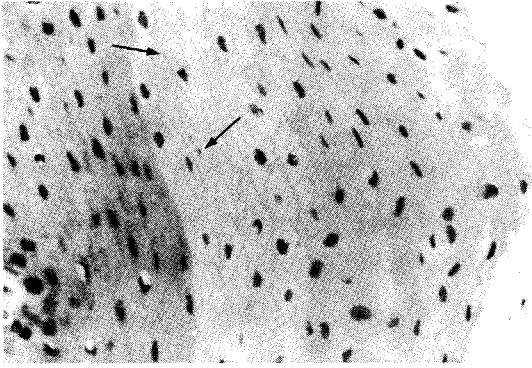
Fig. 5. *Papillae bucalis*. Note cells in *stratum spinosum* and *granulosum* showing clear cytoplasmas, irregular cell membrane and pyknotic nuclei (arrows). HEx400.

restomach, had pyknotic nuclei compressed into a crescent shape by intracytoplasmic IB (Figure 4). The IB were round, 2 to 10 μm in diameter and frequently surrounded by a clear halo. In 42 cases the IB were basophilic, while in 2 animals they were both basophilic and eosinophilic. In general, affected cells had one IB but in occasional cells two or three could be seen. In some cells a clear vacuole was present instead of the IB. Numerous cells in the *stratum spinosum* and *stratum granulosum* showed changes characterized by swelling, with clear cytoplasm and an irregular cell membrane. These cells had nuclei that were either pyknotic or swollen (Figure 5).

The ruminal mucosa had marked dysplasia and degenerative changes in all epithelial layers. Severe oedema of the *lamina propria* and submucosa gave rise to small cleavage vesicles along the basement membrane, which were filled with necrotic material and bacterial colonies. The *stratum corneum* appeared reduced in all the squamous mucous membranes. Intraepithelial microabscesses, with necrotic material and bacterial colonies were observed in the oral and ruminal mucosa in 8 of the animals. Microscopical changes of the intestines were not described because of the pronounced autolytic changes.

The spleen in 32 animals had slight to severe depletion of the white pulp, heavy hemosiderosis, and congestion. The lymphoid follicles of all the body lymph nodes also appeared depleted of lymphocytes. In the 14 corneas examined, only pronounced oedema was observed, except in one case where a purulent keratitis

was observed in connection with *Aeromonas hydrophila* infection. In all 3 moose with alopecia, changes in the stratified squamous epithelium of the skin were found that were similar to those observed in the mucous membranes of the digestive tract.

Virological findings
BVD-virus was not isolated for any of the 68 moose from which virus isolation was attempted. All the 20 moose tested for IBR-virus and antibodies were negative.

Immunoperoxidase staining
BVD viral antigen was not demonstrated by immunoperoxidase staining in any of the 12 moose examined. BVD infected control tissues stained positively.

Bacteriology
No specific infection was encountered.

Discussion
Our investigation shows a clinical disease in moose affecting the central nervous system, and the mucous membranes of the nostrils, mouth, oesophagus, the stomach and the intestine. Macro- and microscopical lesions and IB were found mainly in the mucous membranes of the digestive mucosa. Electronmicroscopical studies by Feinstein et al. (1987), of a moose included in the present study, revealed that the IB were enclosed in a cytoplasmic vacuole and consisted of electron dense granular material surrounded by a single layered membrane. The membrane of the IB and of the vacuole ran parallel in close apposition creating the impression of a double membrane. No virus particles could be found. The IB were not similar to those reported by Hansen et al. (1962) from a case of BVD. The latter appeared as irregular shaped intracytoplasmic IB in the *stratum granulosum* of the skin, and stated to be of keratin in nature. The IB here were negative to keratin staining and located differently. Similar IB have been reported in an outbreak of a disease resembling BVD in farmed fallow deer (Diaz et al., 1988). To reveal the origin of the IB a study was performed by Diaz et al. (1990) to see if other injuries, besides virus diseases, such as autolysis could play a role in the genesis of the IB. They concluded that IB could be a result of unspecific degenerative or post-mortal changes.

The moose originated from 18 different counties throughout Sweden indicating that the distribution of the syndrome is present in the entire country (Figure 1). The first cases, received in 1985, originated in southern Sweden and during 1986–87 affected animals from the northern counties were also received. In the 10 years preceeding this study there had been a dramatic increase in the Swedish moose population. In large populations, social and physiological stresses occur, and stress can be a predisposing factor to disease (Karstad and Hanson, 1957; Brown 1986).

In wild ruminants some diseases are known that affect the digestive tract, including BVD/MD, Bovine papular stomatitis (BPS), Contagious ecthyma (Orf), IBR and Herpes Virus Cervidae Type 1 (HVC-1). BVD infection is prevalent among wild ruminants as indicated by serological surveys (Romvary, 1965; Thorsen and Henderson, 1971; McMartin et al., 1977; Elazhary et al., 1979; Couvillion et al., 1980; Elazhary et al., 1981; Weber et al., 1982; Zarnke, 1983; Feinstein et al., 1987).

In this investigation BVD-virus could not be demonstrated by either virus isolation or immunoperoxidase staining. The correlation between clinical symptoms and lesions of most of the moose involved in this investigation showed clearly that they belong to two main categories:

i) mucosal disease-like, affecting adult animals, with characteristic erosive and ulcerative lesions of the digestive mucosa and atrophied lymphoid organs; and

ii) viral diarrhoea-like syndrome, affecting young animals, showing pronounced catarrhal to hemorrhagic enteritis, atrophied lymphoid organs and occasional ulcerative lesions of the digestive tract.

The symptoms and macro- and microscopical lesions were compatible with those of BVD/MD complex, except for the presence of numerous IB. BPS is a mild disease produced by a parapox virus. It is associated with erosions and papules of the digestive mucosa and intranuclear and intracytoplasmic IB in the epithelial cells (Griesemer and Cole, 1960; 1961). The disease could be aggravated by stress and/or other concurrent infectious disease e.g. BVD (Bohac and Yates, 1980). In this investigation, the macroscopic lesions bear similarities to those of BPS, but histologically they differ from the diffuse areas of hydropic degeneration and marked hyperplasia of the epithelium, except for the IB. Orf is another poxviral disease associated with intracytoplasmic IB in the alimentary epithelium, but the macro- and microscopical changes are very different from those described here.

IBR is a herpesvirus infection of cattle which has diverse clinical manifestations. It is primarily a respiratory disease causing rhinitis, tracheitis and fever, but also plays a prominent role among causes of undifferentiated bovine respiratory disease and abortion. It also causes conjunctivitis, infectious pustular vulvovaginitis, balanoposthitis, and rarely, encephalitis. A systemic form of the disease is described in neonatal calves and feedlot cattle, characterized by hyperemia, erosions and ulcers of the digestive tract and foci of necrosis in liver, adrenal glands, lymph nodes and spleen (Jubb et al., 1985). The virus is highly contagious and has a worldwide distribution (Kars, 1977). Different serological surveys have shown that antibodies against BHV-1, or a serologically related virus, are found in deer and other wild ruminants in North America, England and Finland (Chow and Davis, 1961; Friend and Halterman, 1967; Barrett and Chalmers, 1975; Lawman et al., 1978; Elazhary et al., 1979; 1981; Zarnke and Yuill, 1981; Ek-Kommonen et al., 1982; Zarnke, 1983; Kocan et al., 1986).

In our investigation, macroscopic lesions were somewhat similar to those of the systemic form of the disease. Microscopically only parts of our findings were similar (swelling of the cells and vacuolated cytoplasms) but necrosis of the glandular epithelial cells of the abomasum and foci of coagulative necrosis in liver, spleen or adrenal cortices were not seen. A herpesvirus has been isolated from an outbreak of ocular disease in red deer (Cervus elaphus) that antigenically resembled BHV-1, although neutralization tests suggested that the deer herpesvirus differed from the Oxford strain of IBR-virus (Inglis et al., 1983). Lesions appeared to be limited to the upper respiratory tract, cornea, conjunctiva and chronic ulcers on the posterior dorsal surface of the tongue and the soft palate. Ek-Kommonen et al. (1986) reported on the isolation and characterization of a herpesvirus from reindeer in Finland, following the administration of dexamethasone. The authors suggested that the herpesvirus isolated from reindeer is different from

BHV-1 and could be related with the HVC-1. In Denmark, Rönsholt et al. (1987) reported on the reactivation of a latent herpesvirus infection in a red deer that was seropositive for IBR-virus after glucocorticoid treatment. The virus was transmitted to a seronegative deer with a fatal outcome.

In Sweden, reindeer developed a disease in an outbreak of a BVD/MD-like syndrome elicited by stress (from transporting the animals) (Rockborn et al., 1990). In this case HVC-1 related to BHV-1 was isolated. It is unlikely that the syndrome described in this paper has been produced by the HVC-1 virus. The lesions (exept for the ulcers in the mouth) were different to those described in the outbreaks of HVC-1 (Inglis et al., 1983; Reid et al., 1986). In addition, none of the 20 moose tested for IBR-virus isolation resulted positive.

In the investigation by Rehbinder et al. (1991) on the CNS of female moose with the BVD/MD-like disease, virology and peroxidase and anti-peroxidase technique for BVDV, BHV-1, Bovine Herpesvirus Type 4 (BHV-4), Suid Herpes Virus-1 (SHV-1) and mammalian reo virus types 1 and 2, were performed. An indirect immunofluorescence test was also applied to find BVDV. All attempts to isolate and detect viruses gave negative results. Immunohistochemical studies were also carried out for accumulations of prion related protein (PrP) in brain tissue. Additionally, brain tissues were tested for the presence of scrapie-associated fibrils using the methods of Diringer et al. (1983). The results were negative for spongiform encephalopathy.

It seems possible that several viral diseases may produce lesions similar to those described here. In addition, the disease described here may be caused by an yet unidentified virus considering a morhpological picture similar to a large group of viral diseases in ruminants.

Acknowledgements

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