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An Outbreak of a Disease in Farmed Fallow Deer (*Dama dama L*) Resembling Bovine Virus Diarrhea/Mucosal Disease

By Regina Diaz, Margaretha Steen, Claes Rehbinder and Stefan Alenius

Diaz, R., M. Steen, C. Rehbinder and S. Alenius: An outbreak of disease in farmed fallow deer (*Dama dama L*) resembling Bovine Virus Diarrhea/Mucosal Disease. Acta vet. scand. 1988, 29, 369–376. – Farmed fallow deer suddenly developed disease showing lethargy, weakness, anorexia and several of them died. The animals showed macroscopic lesions in the digestive mucosa characterized by erosions, ulcers and necrotizing lesions. Histo-pathology of the mucous membranes revealed marked inter- and intracellular oedema, erosions, ulcers and intracytoplasmic inclusions bodies. BVD-virus was demonstrated in 1 deer using an indirect immunofluorescence method. It is suggested that the disease may have been caused by Bovine Virus Diarrhea virus alone or in conjunction with a simultaneous infection by another unidentified virus.

intracytoplasmic inclusion bodies.

Introduction

This paper deals with the pathological lesions found in 6 animals brought to the National veterinary Institute, Uppsala, Sweden, from a farmed fallow deer herd in N. E. Uppland, Sweden. The pathological investigation was supplemented by virological, parasitological and bacteriological investigations of tissue samples obtained at necropsy.

Material and methods

Animals

During the early winter 1985 several farmed fallow deer (*Dama dama L*) suddenly developed lethargy, weakness, anorexia, and died. The herd was kept in an enclosure erected in 1978, when 2 females and 1 male were in-

troduced from a zoo. The herd was supplementary fed. Deaths occurred during late autumn and early winter 1984–1985 when the herd consisted of 21 animals. All 7 calves in the herd died. Several were found dead under the snow. One of these was necropsied and acute infection with *Yersinia pseudotuberculosis* was diagnosed. More animals, however, continued to die, including adults.

The animals were without any contact with other ruminants except sheep (deer and sheep drank out of the same brook, at both sides of the fence). One animal was observed while showing clinical signs of disease. It was kyphotic, lethargic, dull and with a ruf-

fled hair coat. The animal died the day after being observed.

The necropsied animals were 4 females and 2 males, aged between 6 months and 4 years. Age was estimated on basis of dental wear and/or the owners statement.

Pathological investigation

All the animals were submitted for necropsy to the National Veterinary Institute with the history of being found dead.

Mucous membranes were only procured for histology from 4 of the animals showing macroscopic lesions and not being too putrified.

For histological examinations tissues were fixed in 4 % formalin, embedded in paraffin and stained with haematoxylineosin, van Gieson and PAS and according to the Ayoub Shklar method for keratin and pre-keratin. From each case, material was taken for routine bacteriological and parasitological examinations.

Virus isolation

Tissue samples (spleen, lymph nodes and mucous membranes) and blood clots were collected at necropsy. Samples for virus isolation were kept at -70°C until processing. The supernatant of a 10 % suspension of ground tissues 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 bovine turbinate (BT) cells. After one passage 0.15 ml of the supernatant was inoculated on cover slip cultures of BT-cells. The cultures were examined for cytopathic 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).

Bacteriological and parasitological investigation

Samples from spleen, lymphnodes, liver, lung and intestine were submitted for routine bacteriological examination while material from lung and intestine were submitted for routine parasitological examination.

Results

Pathological investigation

The animals were poorly nourished and dehydrated. Macroscopical lesions on the digestive mucosa were found in 4 of the animals. Erosions ulcers and necrotizing lesions were observed in the oral mucosa (tongue, gingiva and palatum molle), and rumen. These lesions were deep, irregularly rounded, 5 to 15 mm in diameter with elevated margins and red bases (Plate 1). Some of the

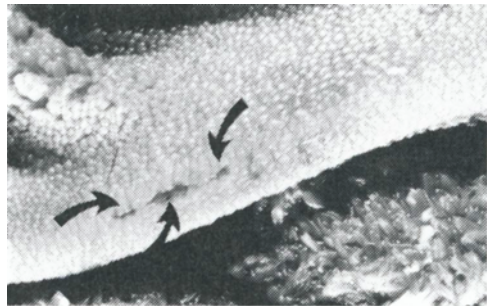


Figure 1. Anterior pillar of the rumen. Note the ulcers (arrows).

animals presented linear erosions in the oesophageal mucosa, covered by a layer of fissured necrotic material (Plate 2). The abomasal mucosa in all the animals was hyperaemic, showing large ulcers in 2 of them.

All the fallow deer had very thin intestinal walls and a watery content in the jejunum.

The spleens of 2 animals were thinner and smaller than normal but of normal size in the others. One animal presented very swollen and hyperaemic mesenteric lymph no-

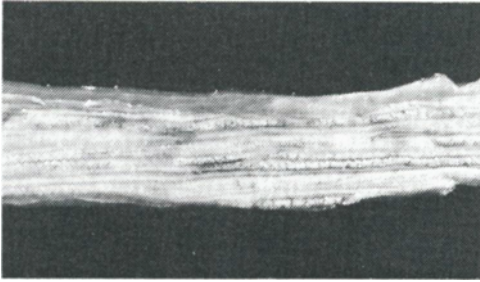


Figure 2. Oesophagus. Note the fissured necrotic material covering linear erosions.

Histologically the mucous membranes of the mouth, oesophageus, and rumen revealed marked inter- and intracellular oedema with some of the cells showing severe vacuolar degeneration. In addition the submucosa was markedly congested and oedematous. Vesicle-like formations appeared in different mucous membranes as circumscribed areas which were not raised above the adjacent epithelium. The presence of ulcers and erosions was confirmed microscopically. These areas were characterized by loss of epithelium and with the underlying submucosa covered by bacterial colonies and showing a purulent inflammatory reaction. A mild mononuclear inflammatory infiltrate was present in the lamina propria and submucosa except for the ulcerated areas. Numerous cells of the strata spinosum and granulosum showed changes. They were much bigger than normal, with clear cytoplasm, having nuclei that were either pyknotic or swollen, giving such cells an appearance of a "fried egg". These degenerated cells were often found to form a zone between normal epi-

des. The oral-mucous membranes of 2 animals appeared reddened, oedematous but without macroscopic ulcerations or erosions. These animals were, however, not suitable for histological studies due to pronounced postmortem changes.

A foecal pneumonia of the diaphragmatic lobe was observed in one animal. In addition the same animal presented shallow dermal erosions in the interdigital space of the forelimbs. A summary of the macroscopic lesions of the 6 fallow deer is shown in Table 1.

Table 1. Macroscopic findings.

No.	Mouth	Esophagus	Forestomach	Abomasum	Intestine	Spleen	Interdigital space	Remarks
V 276	erosion	erosion	erosion	poor digest content	thin *	normal	erosion	focal pneumonia
V 331	normal	erosion ulcers	ulcers	hemorrhag. ulcers	thin *	normal	normal	
V 332	ulcers	normal	ulcers	erosion hemorrhag.	thin *	thin small	normal	
V 436	normal	normal	hyperemia ulcers	hyperemia	thin *	thin	normal	positive virus isolation BVD-virus
V 445	hyperem.	normal	normal	hyperemia *	thin *	normal	normal	
V 446	hyperem.	normal	hyperem. poor digest content	normal	thin *	normal	normal	

* Watery content.

thelial cells below, and degenerated necrotized epithelial cells above.

Numerous cells in the stratum germinativum and spinosum contained intracytoplasmic basophilic inclusion bodies. In general, cells presented 1 inclusion body but in occasional cells 2 could be seen. Inclusion bodies were round, 2 to 10 μ in diameter, and often compressed the nuclei into a crescent shape. The inclusion bodies were surrounded by a clear halo. All the inclusion bodies were negative when stained with Ayob Shklar method for keratin and prekeratin (Fig. 3). The rumen of all animals, presented marked dysplasia and degenerative changes in all the epithelial layers. A common finding was congestion, oedema and depletion of the white pulp of the spleen.

The skin of the interdigital space in 1 animal revealed inter- and intracellular oedema, mild mononuclear infiltration of the dermis and minor erosions of the epithelium. The epithelial changes were similar to the lesions found in the mouth, oesophagus and rumen.

The lungs in all animals showed congestion, slight oedema and slightly increased number of alveolar macrophages. In addition in 2 animals foci of mild subacute alveolar pneu-

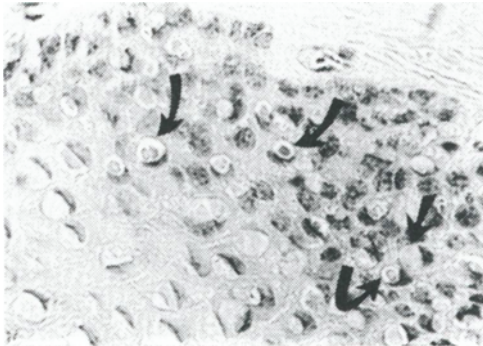


Figure 3. Mucous membrane mouth. Note the swollen cells and the numerous inclusion bodies. HE \times 450.

monia were recorded. In 5 animals the liver was congested, oedematous and showed hydropic degeneration of the hepatocytes. In 1 animal hemorrhagic areas and signs of chronic stasis in the centrolobular veins were observed. The brains showed congestion, oedema, very slight satellitosis and in 1 animal slight mononuclear perivascular cuffing was recorded.

The kidneys appeared without microscopic lesions. Slight degenerative changes were observed in the myocardial fibres in 2 animals.

Virus isolation

BVD-virus was demonstrated in 1 of the fallow deer. The virus was isolated from the blood clot sample. All other samples were negative. The virus did not produce cytopathic effect (CPE) on the BT cells used and the presence of the BVD-virus was demonstrated using the indirect immunofluorescent method.

Bacteriological and parasitological investigation

No specific infection by any significant bacteria was recorded. The parasitic burdens of gastrointestinal parasites were found to be none or low.

Discussion

According to the owner the disease appeared to be of an acute nature while the necropsy findings suggested it to be more of a chronic or subchronic nature. It is hard, however, to define the nutritional status of live deer during winter due to the thick fur.

Several diseases of wild and domestic ruminants are characterized by lesions in the digestive canal. This often makes the diagnosis difficult. In these cases consideration was given to several diseases, e.g. Bovine Virus Diarrhoea/Mucosal Disease (BVD/MD), bovine papular stomatitis, contagious ecthy-

ma (ORF), malignant catarrhal fever and bluetongue.

Infections with BVD is prevalent among wild ruminants, as indicated by different serological surveys (Romváry 1965, Thorsen & Henderson 1971, McMartin *et al.* 1977, Lawman *et al.* 1978, Couvillon *et al.* 1980, Elazhary *et al.* 1981, Weber *et al.* 1982, Doyle & Heuschele 1983, Zarnke 1983). Moreover, BVD virus has been isolated from fallow deer in an outbreak of a disease with lesions similar to mucosal disease (Weber *et al.* 1982).

In the present investigation the macroscopic changes were similar to those of BVD/MD but for the lack of changes in the Peyer's patches. The histological lesions were also in many respects similar to those of BVD/MD but for less oedema and less karyorrhesis, and the presence of numerous inclusion bodies.

The inclusion bodies were not similar to those reported by Hansen *et al.* (1962) from a case of BVD in Sweden, the latter appearing as irregularly shaped intracytoplasmic bodies in some swollen cells of the stratum granulosum of the skin. They stated these inclusion bodies to be of keratin nature.

In our investigation the inclusion bodies were negative to keratin staining and located differently.

Rehbinder *et al.* (1985) have reported on reindeer (*Rangifer tarandus L.*) showing lesions and inclusion bodies similar to those here described. Those animals were from herds known to be negative for BVD. A possible viral etiology was then suggested but not proved.

Macroscopic and microscopic lesions almost identical to those seen in fallow-deer and reindeer have also been seen in roe-deer (*Capreolus capreolus L.*) and moose (*Alces alces L.*). In roe-deer and moose BVD has not been

considered to be the causative agent (Feinstein *et al.* 1987).

BVD has an immunosuppressive effect (Diderholm & Dinter 1966, Peter *et al.* 1968, Johnson 1973, Muscoplat *et al.* 1973, Roth *et al.* 1981) and could promote the expression of an otherwise asymptomatic or mild disease. Bohac & Yates (1980) described a case in which it was possible that a calf was chronically infected with BVD which contributed to a severe and prolonged infection by bovine papular stomatitis virus.

Inclusion bodies are a common feature of poxvirus infections (Fenner 1979). Bovine papular stomatitis is a mild disease produced by a parapoxvirus. It is associated with erosions and ulcerations of the digestive mucosa and intracytoplasmic inclusion bodies in the epithelial cells (Griesemer & Cole 1960, 1961). The disease could be aggravated by stress and/or other concurrent infectious disease, e.g. BVD (Bohac & Yates 1980). Histologically, the inclusion bodies as induced by poxvirus are described as both eosinophilic and basophilic (Okada & Fujimoto 1975). In our investigation only basophilic inclusion bodies have been observed. Contagious ecthyma (ORF) is primarily a disease of domestic sheep and goats caused by a parapoxvirus. It has been diagnosed in several species of wild life from various parts of the world (Smith *et al.* 1982, Kummeneje 1979, Dietrich *et al.* 1981, Zarnke 1983, Munz *et al.* 1986). The lesions of ORF differ macro- and microscopically from the lesions found in our investigation (cauliflower-like papillomatous lesions in skin, eyelids and udder). Microscopically only parts of our findings are similar (ballooning of the cells and basophilic inclusion bodies) but there was not acanthosis and/or parakeratosis.

Malignant catarrhal fever is known to occur in deer in North America, Australia, Bri-

tain, and New Zealand (Senior et al. 1962, Clark et al. 1970, Denholm & Westbury 1982, Beatson 1985). Among the characteristic features of the disease are erosions and ulcers in large areas of the oral mucosa, hemorrhagic erosions of the abomasum and severe haemorrhagic inflammation of the trachea (Karstad 1970). In addition, widely disseminated vasculitis of small and medium arteries and veins and fibrinoid necrosis of vessel walls are considered pathognomonic lesions. It is known also that in malignant catarrhal fever eosinophilic inclusion bodies do occur. In this investigation some of the macroscopic and microscopic lesions were somewhat similar to those of MCF but the typical vascular lesions were missing.

Bluetongue is an infectious arthropod-borne viral disease producing excoriations of the epithelium of the lips, tongue buccal surfaces, the mucosa of the rumen, reticulum and abomasum. The histopathological lesions consist primarily of oedema, hyperemia, congestion haemorrhages, and intravascular thrombosis (Trainer 1970, Beatson 1985). These vascular lesions were not found in our material.

Rinderpest lesions show similarities to those described here, but since Rinderpest is an exotic disease that does not occur in Sweden, it will not be discussed further.

The macroscopical lesions present in the animals in this investigation were similar to lesions found in several diseases. Microscopically, however, the changes were mainly resembling the lesions found in animals affected by Bovine Virus Diarrhea/Mucosal Disease. The main differences are that in BVD/MD in cattle there are more focal areas of oedema, more pyknotic nuclei and no inclusion bodies. A species difference has to be considered, since the tissue response in cervidae may be different to that in cattle. On the other hand reindeer from herds hav-

ing no BVD antibodies have showed lesions similar to those found in these fallow-deer (Rehbinder et al. 1985).

It seems possible that several viral diseases may produce similar lesions in the same but also in different species. Bovine virus diarrhea virus was isolated from one of the fallow-deer, and the macro- and microscopical picture corresponded well with that of BVD/MD complex, one of the main differences being the presence of inclusion bodies. It cannot however be excluded that these inclusion bodies are a non-specific cellular response to degenerative cellular changes and thus may be present in different diseases and when other factors are affecting the mucous membranes. If this is relevant, considering the presence of intracytoplasmic inclusion bodies recorded in different species of cervidae (Rehbinder et al. 1985, Feinstein et al. 1987) it seems that the investigated outbreak may have been caused by BVD virus alone or in conjunction with a simultaneous infection by another unidentified virus.

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Sammanfattning

Ett sjukdomsutbrott bland hägnat dovilt (Dama dama L) tydande på bovin virus diarrre/MD.

Hägnande dovhjortar blev plötsligt sjuka uppvisande, slöhet, svaghet och upphörd foderlust. Ett flertal av dem dog. Djuren uppvisade slemhinneförändringar i digestionskanalen karakteriserade av erosioner, ulcerationer och nekrotiska förändringar. Histopatologiska undersökningar av slemhinneförändringarna påvisade inter- och intracellulärt ödem, erosioner ulcerationer och intracytoplasmatiska inklusionskroppar. Bovint virusdiarré-virus påvisades hos en hjort med hjälp av indirekt immunofluorescens. Det antas att sjukdomen kan ha orsakats av bovin virus diarré virus ensamt eller tillsammans med ett annat oidentifierat virus.

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