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Congenital cerebellar hypoplasia associated with BVD-MD virus infection in a naturally infected calf - a case report

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ABSTRACT

The objectives of the present study were to describe pathomorphological and immunohistochemical features of congenital cerebellar hypoplasia associated with natural BVD-MD virus infection in a Holstein-Friesian calf. The characteristic macroscopical lesion was cerebellar hypoplasia, almost completely absent, with hydrocephaly. Cerebellar changes were cortical destruction, destructive changes of granule and Purkinje cells and irregular cavity formation of the folia. The positive antibody reaction against the BVD-MD virus was observed in the CNS, but not in the eyes and peripheral nerves.

Key words: cerebellar hypoplasia, bovine viral diarrhoea-mucosal disease, calf

Introduction

The effect of viruses on the developing foetus has received considerable attention as a cause of congenital birth defects. Numerous viruses have been shown to produce birth defect in animals (HAZIROGLU, 2000). Akabane virus (HAZIROGLU, 2000), bluetongue virus (HAZIROGLU, 1990; HAZIROGLU, 2000), bovine viral diarrhoea-mucosal disease (BVD-MD) virus (WARD, 1969; KAHRS et al., 1970a; KAHRS et al., 1970b; BROWN et al., 1973; SCOTT et al., 1973; ALLEN, 1977; WILLSON et al., 1983; HEWICKER-TRAUTWEIN et al., 1995; HAZIROGLU, 2000) and Chuzan (Kasba) virus (MIURA et al., 1990; HAZIROGLU,

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2000) have been suggested as causative agents of congenital central nervous system abnormalities in calves. Among them, Akabane, bluetongue and BVD-MD virus infections are well known in Turkey (HAZIROGLU, 1990; HAZIROGLU, 2000). These virus infections have occasionally caused epidemic outbreaks, especially in the southern part of Turkey (HAZIROGLU, 1990). BVD-MD virus has been strongly implicated as a cause of congenital abnormalities, including cerebellar and ocular lesions, in experimentally infected calves (WARD, 1969; KAHRS et al., 1970a; KAHRS et al., 1970b; BROWN et al., 1973; SCOTT et al., 1973; ALLEN, 1977; WILLSON et al., 1983; HEWICKER-TRAUTWEIN et al., 1995). However, such lesions associated with BVD-MD virus have not been demonstrated in tissue sections of naturally infected cases.

The present paper describes pathological observations of the central nervous system and demonstrates the virus in the cerebellar and cerebral tissue sections of a Holstein-Friesian calf naturally infected with BVD-MD virus.

Materials and methods

A 45-day-old Holstein-Friesian female calf was presented for post-mortem examination to the Department of Pathology, Faculty of Veterinary Medicine, University of Ankara. The calf was recumbent and blind and had BVD-MD serum antibody detected by virus neutralization test at the Etlik Animal Disease Research Institute (ADRI) in Turkey. The calf was necropsied systemically. Following fixation with 10% buffered formalin the brain was sliced transversally and gross-subgross lesions were recorded. The organ samples taken from all viscera were processed for histological examination by conventional methods. Sections were stained with haemotoxylin and eosin (HE). Selected central nervous system (CNS) sections were also stained with Klüver & Barrera's luxol fast blue (LFB) for myelin. A commercial kit (LSAB 2, DAKO A/S) was used to demonstrate the BVD-MD virus in the CNS tissue sections. The sections were de-waxed in xylol and rehydrated in decreasing alcohol series. The slides were rinsed in distilled water and immersed in antigen retrieval solution (DAKO A/S), pH 6.0, for 20 min at 90 °C. The slides were allowed to cool to room temperature. The samples were then incubated in 3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. Immunohistochemistry was performed with rabbit anti-cow BVD-MD (working dilution 1/50) antibody using streptavidin-biotin peroxidase technique with the commercial kit in the manufacturer's manual.

Results

Clinical signs were not apparent in the dam of calf. However, serum neutralization titers of the cow and calf indicated high BVD-MD titers (1/3200). Head tremors of the calf were observed occasionally.

At necropsy, the domed cranium was not seen macroscopically. After removal of the calvarium, cerebellum was virtually absent, consistent with cerebellar hypoplasia (Fig. 1). There was no recognizable cerebellar cortex. Only remnants of cerebellar peduncles were present. Lingula, rudimenter lobus ansiformis, lobus flocculus, lobus paraflocculus and uvula were observed sub-grossly using operation microscope (Olympus MTX). Later, in the 10% buffered formalin fixed brain, central nervous system malformations, including cystic septum pellucidum, slight bilateral internal hydrocephalus, dilation of the third-fourth ventricles and mesencephalic canal were detected. However, the central canal in the last part of the rhombencephalon and spinal cord could not be seen macroscopically or sub-grossly.

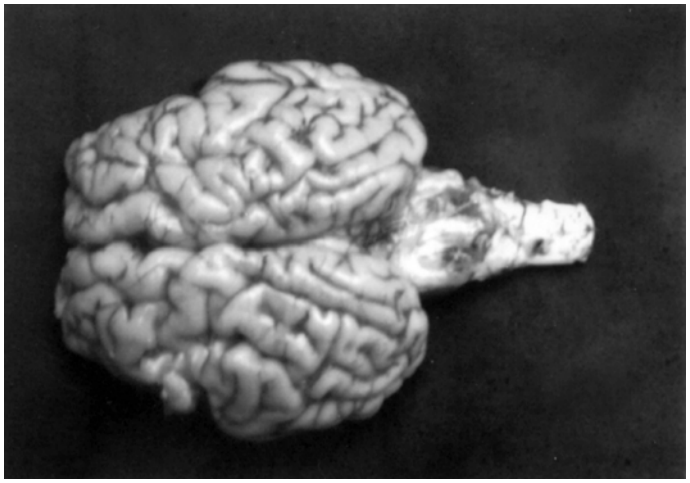

Fig. 1. Marked cerebellar hypoplasia. Cerebellum was not recognizable grossly.

Microscopic examination of the brain and spinal cord supported the gross post-mortem diagnosis. The main microscopic lesions were in the cerebellum. Folial changes varied from presumably partial formation to complete destruction of the cortical parts (Fig. 2a). Prominent Purkinje cell loss and focal to diffuse depletion of granule cells were evident. Purkinje cells were remarkably lost and granule cells clumped within a loosely arranged granular layer (Fig. 2b). The swollen axonal remnants of degenerate Purkinje cells were scattered throughout the granular layer. In some areas, where Purkinje cells existed, they were loosely arranged and still migrating granule cells were recognizable (Fig. 2c). The terminal and central portions of the cerebellum were almost completely replaced by an irregular cavity lined by a thin wall consisting of neuropil and meninges (Fig. 2b). A narrow presumably white matter, which was not stained greatly with HE and LFB, had linear streaks

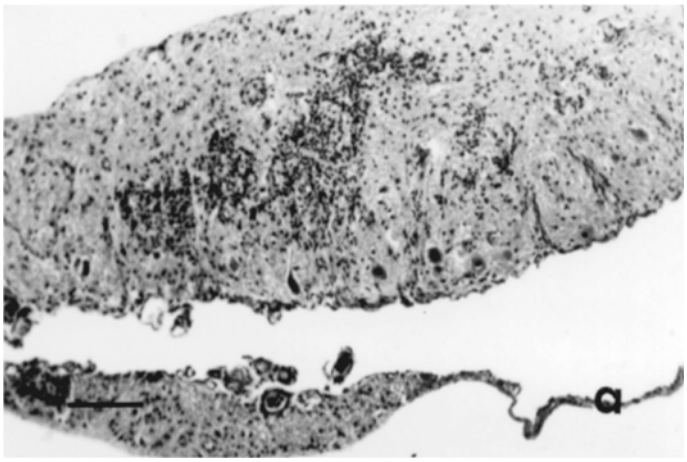

Fig. 2a. Disorganized folial formation. H&E; scale bar = 60 µm.

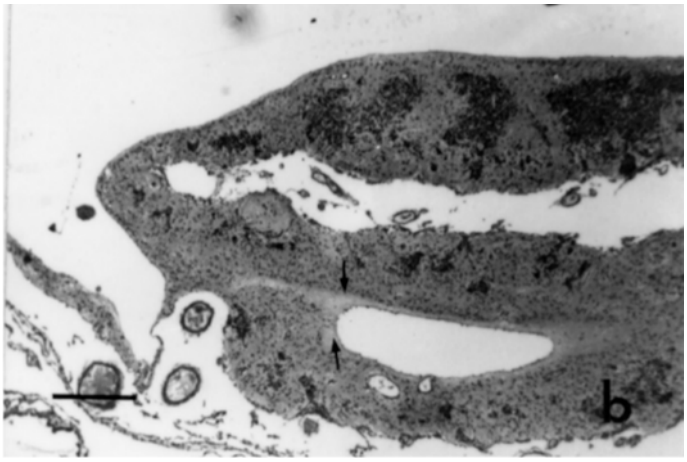

Fig. 2b. Purkinje cell loss and severe granule cell degeneration. An irregular cavity lined by a thin wall consisting of neuropil and meninges (arrow). H&E; scale bar = 110 µm

into the areas of cortical atrophy. In some portions of the folia there was marked depletion of the molecular layer and degeneration of granule cells, while the remaining granule cells showed a tendency to form clumps. A thin layer of presumably external granular layer was present. Ependymal cells covering the ventricular system were flattened, probably due to

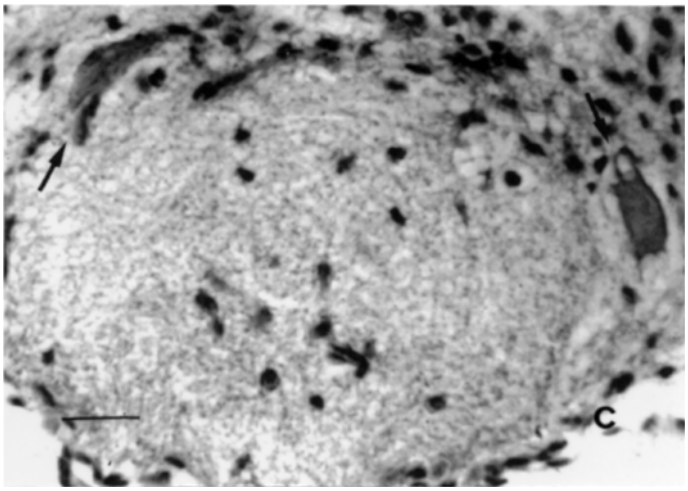

Fig. 2c. Loosely arranged Purkinje cells and migrating granule cells (arrows). H&E; scale bar = 25 µm

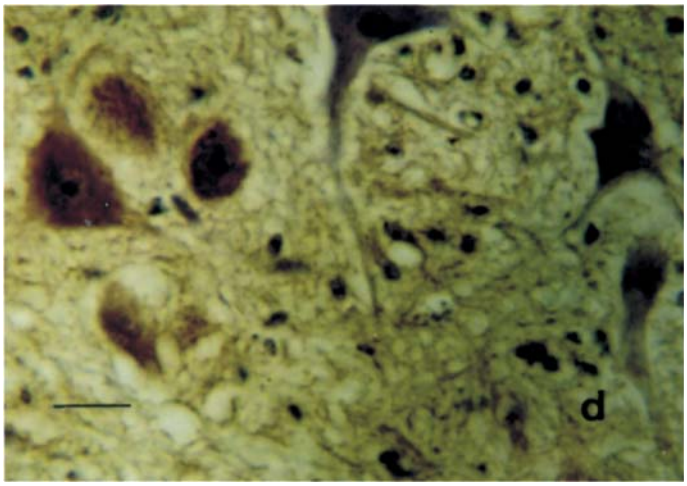

Fig. 2d. Immunohistochemical demonstration of BVD-MD virus infected cells in the nucleus cuneatus. ABC-P H&E; scale bar = 25 µm.

the compression of cerebro spinal fluid. Grossly unobserved central canal in the last part of the rhombencephalon and spinal cord was detected microscopically. However, the diameter of the central canal was narrow and the ependymal cells were dysplastic.

The retinas of both eyes of the calf had lost their laminated organization, although some ganglion cells were recognizable within the optic fibre layer. There was, to some degree, pigmentary migration into the fibrosed retina. The pigment-cell layer was hypertrophic in some regions. There were few focal areas in which the retina was disrupted. In these areas, diminishing of the rods and cones was observed. There was also a decreased number of cells in the nuclear and outer reticular layers. The optic nerve was narrowed throughout its course and the connective tissue within the nerve bundle was prominent. The connective tissue also appeared disorganized. There was no inflammatory reaction in both eyes and optic nerves.

In immunohistochemical staining using antibodies against the BVD-MD virus, a positive reaction was observed mainly in the nucleus commissura posterioris of thalamus, gyrus dentatus, nucleus cuneatus and rudimenter lingula of the cerebellum (Fig. 2d) No positive reaction was detected in the retinas.

Discussion

In the present observation, a recumbent and blind calf was necropsied and severe cerebellar hypoplasia was observed. The most significant changes in the cerebellum were complete destruction of the cortex and the presence of irregular cavities in the terminal portions. Positive antibody reactions against the BVD-MD virus were observed in the CNS, but not in the eyes and optic nerves.

Serum specimen collected from the calf before it has ingested colostrum is important for the diagnosis of BVD-MD infection (KAHRS et al., 1970b; HAZIROGLU, 2000). In this case the antibody against BVD-MD virus was found in serum specimen collected after ingestion of colostrum from the calf and serum specimen from her mother. In this condition, determination of the antibody against BVD-MD in the serum was not significant for the diagnosis. Establishing a clear relationship between cerebellar hypoplasia and BVD-MD virus infection depends on the virus isolation and/or demonstration of the BVD-MD virus antigenic structures on the CNS and ocular tissues. In the current case, viral antigenic structures have been successfully demonstrated in the CNS. However, such antigenic structures could not be demonstrated in the retinas.

It is well known that cerebellar hypoplasia is a characteristic abnormality of BVD-MD virus infection (HAZIROGLU, 2000). It was demonstrated (HEWICKER-TRAUTWEIN et al., 1995) that cerebellar hypoplasia was the most frequent outcome of transplacental BVD-MD virus infection. They also recorded hydranencephaly, internal hydrocephalus, microencephaly or porencephaly as other common lesions. During pregnancy the virus can cause cerebellar hypoplasia and ocular defect in newborn calves. However, the virus must infect the cow at the critical stage of pregnancy and the calf must be susceptible to infection at the time of exposure. This time must be between the 100th and 200th days

of gestation (WARD, 1969; KAHRS et al., 1970b; BROWN et al., 1973; SCOTT et al., 1973; HAZIROGLU, 2000). In the present case, a severe cerebellar hypoplasia and slight internal hydrocephalus were observed. Therefore, the observed malformations of the CNS might be due to intrauterine exposure to the virus.

In conclusion, in a calf naturally infected with BVD-MD virus, macroscopic and microscopic CNS lesions characterized by cerebellar hypoplasia and hydrocephalus were determined, and the association between the viral infection and the lesions was investigated.

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SAŽETAK

Opisane su patomorfološke i imunohistokemijske značajke prirođene hipoplazije malog mozga povezane s prirodnom infekcijom virusom virusnog proljeva/bolesti sluznica u holštajn-frizijskog teleta. Osebujan makroskopski nalaz očitovao se u obliku cerebelarne hipoplazije s hidrocefalusom i gotovo nestalim malim mozgom. Dokazane su promjene u obliku kortikalne razgradnje, destruktivnih promjena granula i Purkinjeovih stanica i nepravilna oblikovanja šupljine folije. Pozitivan nalaz za virus virusnog proljeva/bolesti sluznica goveda dokazan je u središnjem živčanom sustavu, ali ne i u oku i perifernim živcima.

Ključne riječi: cerebelarna hipoplazija, virusni proljev/bolest sluznica, tele