Bovine Viral Diarrhea Virus Associated Malformations in a Holstein-Friesian Calf

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ABSTRACT

The aim of this study is to examine anatomical, pathological and virological findings of combined malformations in a 1 day old, female, Holstein Friesian Calf that naturally and congenitally infected with Bovine Viral Diarrhea Virus (BVDV). Anatomo-pathologic findings consisted of Arnold-Chiari malformation (ACM), partial corpus callosum agenesis, sacrococygeal agenesis, epitheliogenesis imperfecta, anchylotic spondylose and arthrogryposis. Microscopically, perivascular infiltration, edema, neuronal degeneration, necrosis and focal gliosis were observed in the central nervous system (CNS), especially in brain. BVDV antigen was detected by Enzyme Linked Immunosorbent Assay (ELISA) method in both mother and calf. At immunohistochemical examination, bovine viral diarrhea virus positive immunoreaction was detected in neurons, glial cells, purkinje cells and ependymal epithelial cells of CNS and pars nervosa cells of pituitary gland. Furthermore, immunopositive reactions were also observed in perivascularly infiltrated neutrophil leukocytes and lymphocytes. To the best of authors' knowledge, this is the first report of epitheliogenesis imperfecta and BVDV positive immunoreactions in pituitary cells in a calf with a congenital and natural infection.

Key Words: Arnold-Chiari Malformation, Anomalies, Bovine Viral Diarrhea Virus, Calf, Pathology.

INTRODUCTION

Infections with Bovine viral diarrhea virus (BVDV) 1 and 2 have been reported to cause abortions and congenital defects (1,2,3). The affinity of virus has been described in the reproductive, respiratory, gastrointestinal, circulatory, immune, lymphatic, musculoskeletal, integument and central nervous system (2,4). BVDV also causes thrombocytopenia, lymphopenia, leukopenia, brachygnathism, growth retardation, malformations of the brain and cranium, and rarely extracranial skeletal malformations in calves born to first-calf heifers (1). BVDV related anomalies have been reported in various animal species (5, 6). Also, BVDV lesions have been well defined in calves in Turkey (4).

The aim of this study was to examine serological and anatomo-pathological findings in a calf naturally infected by BVDV infection and to detect the localization of viral antigen in CNS by immunohistochemical methods.

MATERIAL AND METHODS

A 1 day old, female, Holstein Friesian calf from a private farm was included in this study. The arthrogrypotic calf was unable to stand and was admitted to the Department of Pathology, Faculty of Veterinary Medicine, University of Mehmet Akif Ersoy for diagnosis. After physical examination the calf was euthanized due to poor prognosis. Necropsy was performed and tissue samples were taken from all affected organs and

tissues and fixed in 10% buffered formalin. All specimens were blocked in paraffin and sectioned at 5 μm and stained with Haematoxylin Eosin (HE).

For antigen detection by ELISA, leukocyte extracts from whole blood samples were prepared as reported in the test procedure and these leukocyte samples were tested for BVDV antigen by using a commercial BVDV antigen ELISA kit (P0064/03 Institut Pourquier, France). The test was performed according to the procedure described by the manufacturer. For antibody detection by ELISA, BVDV in serum samples collected from the animals were tested by using commercial BVD/MD/BD P80-ELISA kit (Institut Pourquier, France). The test was performed according to the procedure described by the manufacturer.

For immunohistochemical detection of the BVDV antigen in tissues, sections were attached to glass slides coated with poly-L-lysine. The slides were then dried overnight at 37°C to optimize adhesion. Sections were deparaffinized in multiple xylene baths, and tissues were rehydrated in sequentially graduated ethyl alcohol baths. UltraVision Detection System Anti-Polyvalent and ready-to use HRP/AEC (Lab Vision Co., Fremond, CA, USA) were used for immunohistochemical observations. To reduce nonspecific background staining due to endogenous peroxidases, slides were incubated in hydrogen peroxide for 10 minutes followed by two washes in PBS. The tissues were then boiled in 1:100 citrate buffer for 20 minutes and cooled for 20 minutes. The cooled tissues were washed in PBS four times prior to receiving blocking serum, for a further 5 minutes incubation. After incubation primary antibody specific to BVDV, (P0064/03 Institut Pourquier, France) was applied, and slides were incubated for 30 minutes at room temperature. After 4 washes in PBS, the biotinylated goat anti-polyvalent antibody (panspecific biotinylated secondary antibody that recognizes mouse, rat, rabbit, goat, sheep and cattle) (1:1) was added and slides were incubated for 10 minutes at room temperature. After washing in PBS three times, streptavidin peroxidase was applied for 10 minutes at room temperature, and then slides were rinsed in PBS four times. Tissues were further incubated for 20 minutes at room temperature in a solution of DAB (3-3'-diaminobenzidine tetrahydrochloride) chromogene substrate. After washing in PBS, tissues were counterstained with Mayer's haematoxylin, washed in water, and cover slips were applied with mounting media. A similar procedure was applied for the control tissues, but PBS was used instead of primary antibody. Immunopositivity was examined by Nikon E-600-trinocular microscope and UIII microphotography attachment was used for photography.

RESULTS

On physical examination, the calf exhibited marked tremor, incoordination, recumbency, arthrogryposis, epitheliogenesis imperfecta, sacrococcygeal agenesis and anchylotic spondylose especially between cervical vertebrates similar in structure to the cervical region of the hyena.

At postmortem examination, arthrogryposis was observed in both forelimbs and hindlimbs. Medial flexions were seen in distal joints of extremities (metacarpophalangeal and metatarsophalangeal). Both hindlimbs and the left forelimb were in a flexed position while the right forelimb was normal. The hindlimbs flexion was more severe than that of the forelimbs (Figure 1).

Epitheliogenesis imperfecta was diagnosed at the lumbosacral region. The defect was rounded in shape and 4x2 cm in diameter. Macroscopically the defect was hairless with a fluid-filled membrane attached to adjacent skin (Figure 2).

After opening the skull, the brain and skull were found to be elongated and flattened. The occipital lobe of brain was elongated to the transverse fissure and depressed to the cerebellar vermis which caused herniation of cerebellum through the foramen magnum (Figure 3). At the cut surface of the skull, thinning at the os frontale was observed. The splenium of corpus callosum was not developed and the lateral ventricle was compressed by the occipital lobe. At the dorsal inspection of sulci and gyri they were normal size and shape. The pineal gland was longer than normal and protruded between sulci and gyri. Herniation of uvula and nodulus were

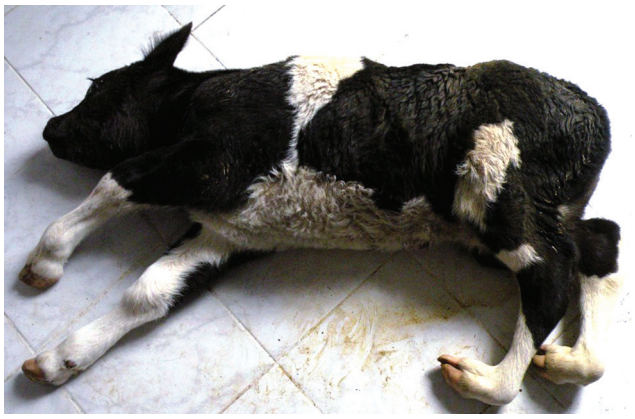

Figure 1: Articulatio, arthrogryposis in calf with BVDV

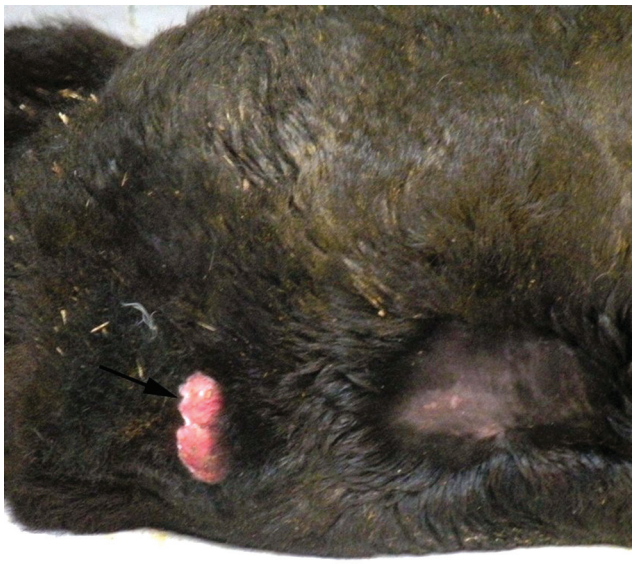

Figure 2: Skin, epitheliogenesis imperfecta in sacral region of calf (arrow)

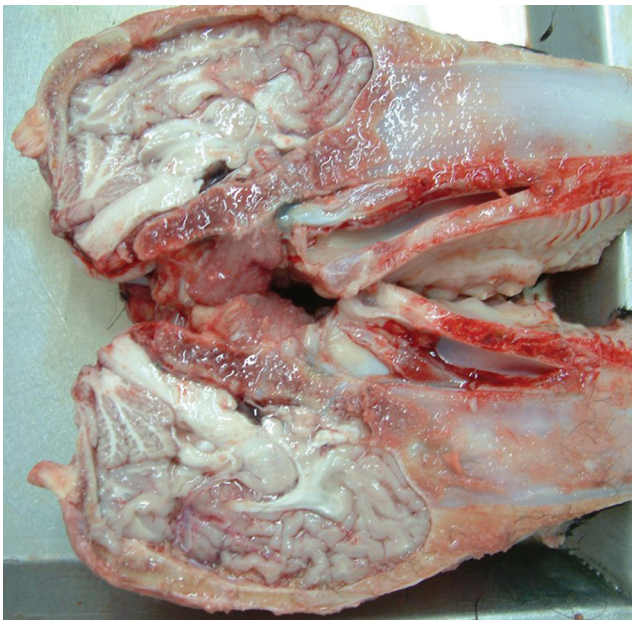

Figure 3: Central nervous system, Arnold-Chiari Malformation, os occipitale depressed to the cerebellar vermis with herniatoin of cerebellum to the foramen magnum.

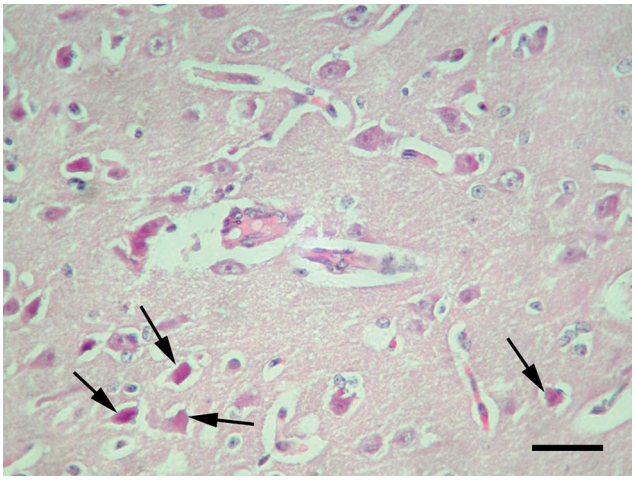

Figure 4: Brain, neuronal degeneration, necrosis (arrows) and edema. HE, bar=100 μm.

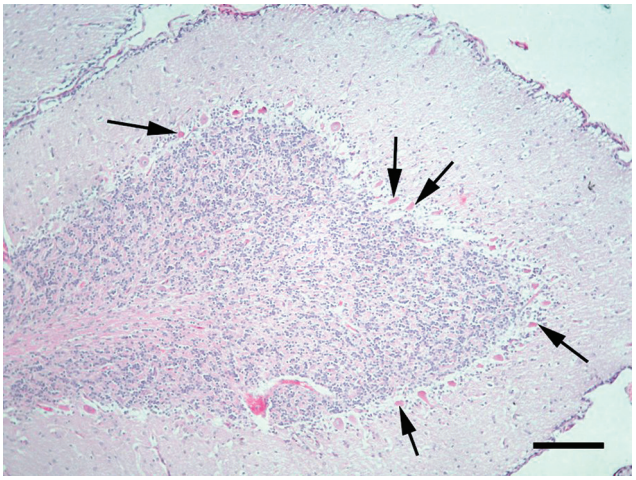

Figure 5: Cerebellum, degeneration and necrosis (arrows) in Purkinje cells. HE, bar=200 μm.

observed into the foramen magnum. The culmen, decli and tuber vermis were compressed by the occipital lobe. The occipital lobe was caudally elongated and compressed by the cerebellum. The foramen magnum and brain hemispheres were of normal localization and size, but the gyri were swollen and edematous. Meningeal blood vessels were hyperemic.

Histopathological examination of the brain revealed hyperemia, hemorrhage, edema and focal gliosis and/or glial proliferation, especially in mesencephalon. Prominent vasculitis was detected throughout the brain and meninges. Virchow Robin spaces were enlarged due to edema and infiltrations. Inflammatory cells were composed chiefly of lymphocytes, plasma cells and some neutrophils. A large number of degenerative and necrotic neurons were detected in the brain and cerebellum (Figure 4). Marked abiotrophy, degeneration and necrosis were observed in purkinje cell of the cerebellum (Figure 5).

Immunohistochemically, large number of BVDV immunopositive cells were detected in prosencephalon, mesen-

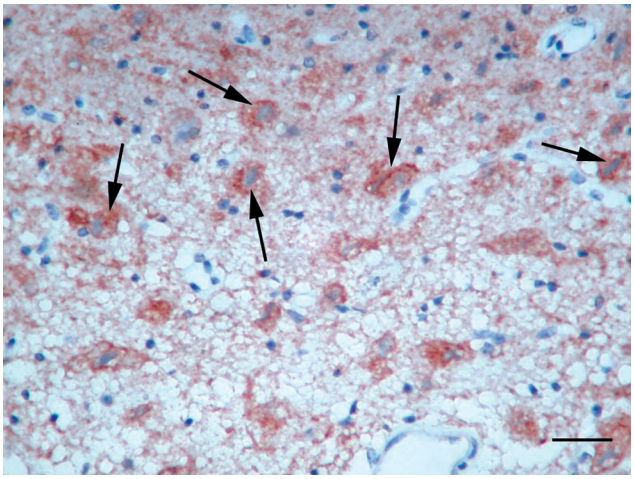

Figure 6: Brain, BVDV immunopositive reactions in neurons (arrows), avidin-biotin peroxidase complex method, bar=100 µm.

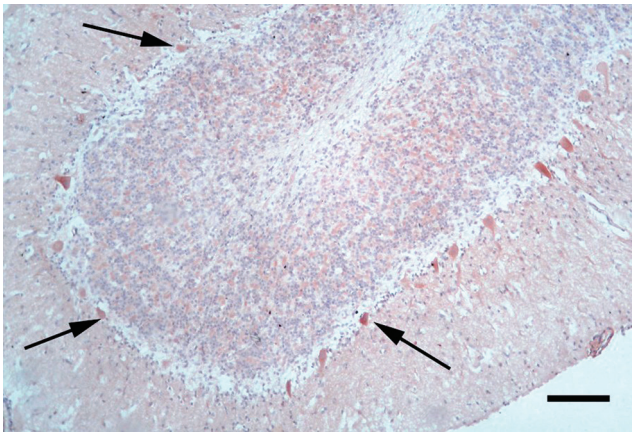

Figure 7: Cerebellum, immunopositive reactions in degenerative and necrotic purkinje cells (arrows), avidin-biotin peroxidase complex method, bar=200 µm.

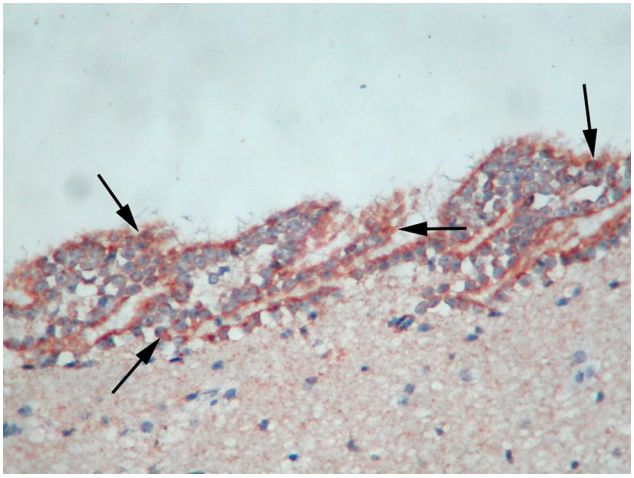

Figure 8: Brain, BVDV positive reactions in ependymal cells (arrows), avidin-biotin peroxidase complex method, bar=100 µm.

cephalon and rhombencephalon. In addition to these, positive reactions were observed in neutrophils, lymphocytes, localized perivascular areas, degenerative and necrotic neurons, astrocytes, glial cells, ependymal cells and in the meningeal and purkinje cells of the central nervous system (Figures 6-8) and the pars nervosa cells of the pituitary gland. However, immunopositive reactions were more prominent in the pons, medulla oblongata and thalamus as compared to the rest of the CNS.

At the serological examination of the mother and calf sera, BVDV positive reaction was detected by ELISA both in the mother and calf.

DISCUSSION

BVDV may cause several disorders and syndromes in cattle (2, 10). It can particularly contribute to viral abortions (7, 8). The virus has been reported to result in congenital malformations including, hydrocephalus, hydranencephaly, cerebellar hypoplasia, growth retardation and mandibular brachygnathia, multiple craniofacial skeletal, brain, and eyes (cataracts and/or retinal lesions) and defects occurring during different stage of gestation (1, 2). Exposure to BVDV may result in persistent infection and homologous immunotolerance (1, 4, 9, 10). The malformations due to BVDV generally occus along with spina bifida and meningomyelocele and it is a common cause of congenital hydrocephalus (11). Hemivertebrae and other abnormalities of the vertebral bodies may also be present (12). However, in the present study, Arnold-Chiari malformation (ACM) with epitheliogenesis imperfecta, sacrocoxygeal agenesis and arthrogryposis was observed in a BVDV positive calf. This combination of abnormalities has been not described in veterinary literature. The joint bending has been described in several congenital diseases including BVDV; our finding in this case was similar.

Sacrococcygeal agenesis is described as the absence of all coccygeal vertebrae and muscles, deformation of sacral vertebrae and is considered a rare spinal anomaly in the calf. Sacrococcygeal agenesis occurs in association with spina bifida in Manx cats, dogs, and sheep (13). In the present study, the viral agent of BVD was considered to play a role in the formation of sacrococcygeal agenesis.

Epitheliogenesis imperfecta, or aplasia cutis, is described as the widespread absence of areas of squamous epithelium of the skin and mucous membrane and is a rare anomaly of calves, piglets, foals, lambs puppies, and kittens (9, 14, 15). The condition may occur as an autosomal recessive trait and it is seen most often in extensive inbreeding in herds (9, 14). Macroscopically, a sharply demarcated, variably sized defect occurs in the epidermis or mucosa, resulting in exposure of a glistening, red moist, hairless dermis or oral or esophageal submucosa (14, 15). The lesions are frequently seen in extremities, although, any portion of the body can be affected such as squamous epithelium of muzzle, lips and oral cavity (9, 14). The malformation occurs with brachygnathia and atresia ani in some calves. Histopathologically, epithelium agenesis and lack of adnexal structure in dermis layer are known as epitheliogenesis imperfecta. Also in this malformation there are rare rudimentary hair follicles devoid of apocrine and sebaceous glands (14, 15). Microscopically, the normal epithelium terminates abruptly at the affected site (9). In the present study epitheliogenesis imperfecta was determined in a BVDV positive calf and to the best of authors' knowledge this is the first report of the combination of BVDV and epitheliogenesis imperfecta.

Arthrogryposis is also known as a crooked joint or fixation of the joint (9, 16). Congenital articular rigidity and arthrogryposis multiplex congenital is seen in lambs, calves, piglets, and foals, and less frequently in kittens and puppies (16, 17). Arthrogryposis is caused by viral infections and toxins. It is assumed that the critical event resulting in this condition may occur early in pregnancy (16). Genetic causes are postulated in calves, sheep, and piglets, but the establishment of such a relationship does not require a different pathogenetic mechanism since the gene effects would apparently be directed to the neural component (16, 17). Syndrome in the Charolias, Friesian, Swedish, and Red Danish breeds of cattle, sometimes associated with cleft palate, are consistent with a simple recessive or modified recessive characteristic (9, 16). Environmental toxins and viruses may result in a similar pattern. The viruses of Akabane disease, Cache Valley fever, bluetongue, and border disease cause outbreaks of arthrogryposis in cattle and sheep. Arthrogryposis in cattle can be associated with other lesions such as scoliosis, torticolis and cleft palate (9, 16, 17). In the present study, arthrogryposis, epitheliogensis imperfecta, sacrococcygeal agenesis and ACM were diagnosed in a 1 day old, Holstein Friesian Calf.

BVD virus is present in leukocytes (buffy coat), especially lymphocytes and monocytes, and in plasma (18). Viral antigen of BVD has also been detected in cytoplasm of neurons of the myenteric ganglion in experimental and natural infections (19). Immunohistochemically, BVDV antigen has been demonstrated in keratinocyte, hair follicle epithelium, hair matrix of hair bulb and dermal papillae in persistent infection (4). Meningoencephalitis has been reported in neuronal infection with BVDV-2 (18, 20). Viral antigen has been detected by immunohistochemistry in variety of cells in several organs. Furthermore, the viral antigen may be detected by immunohistochemistry before the lesions appear in BVDV infections (18). In the present study, immunohistochemically, perivascularly infiltrated cells were composed of lymphocyte and neutrophils showing positive reactions in the CNS. Thus, BVD viral antigens were demonstrated in mononuclear cells and degenerative, necrotic neurons, astrocytes, glial cells, meningeal and purkinje cells in CNS. These result suggested that positive inflammatory cells may react to the viral agent on reaching the CNS. Possible causes of the immunopositive ependymal cells may be explained by the spread of virus via cerebrospinal fluid. This is the first report that BVDV positive reaction by immunohistochemistry method in pituitary cells.

The findings described in this calf may serve to help diagnose BVDV cases in calves. Moreover, this study revealed that BVDV can causes anomalies such as sacroccocygeal agenesis, ACM, and epitheliogenesis imperfecta. At the same time, this is the first report of the combination of these anomalies in a BVDV positive calf. Serologically, BVDV antigen and antibody were diagnosed by ELISA method. The immunopositive reaction that was seen in mononuclear cells may be important in elucidating the pathogenesis of BVDV.

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