

Short Communication / Kısa Bilimsel Çalışma

Central nervous system and skull malformations associated with Bovine Viral Diarrhea Virus in a calf

Hasan Tarık ATMACA¹, Güngör Çağdaş DİNÇEL¹, Ali KUMANDAŞ², Oğuz KUL¹, İsmail Önder ORHAN³

¹Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, ²Kırıkkale University, Faculty of Veterinary Medicine, Department of Surgery, Kırıkkale ³Ankara University, Faculty of Veterinary Medicine, Department of Anatomy, Ankara, Turkey.

Summary: In this study, cranial anomalies, hydranencephaly and cerebellar hypoplasia were reported macroscopically, microscopically and immunohistochemically in a calf. A relationship between Bovine Viral Diarrhea Virus infection and malformations associated with central nervous system lesions were demonstrated.

Key words: Bovine Viral Diarrhea Disease, Calf, Cerebellar hypoplasia, Cranial malformations, Hydranencephaly.

Bir buzağıda merkezi sinir sistemi ve kafatası malformasyonlarının Bovine Viral Diare virüsü ile ilişkisi

Özet: Bu çalışmada, bir buzağıda görülen baş anomalileri, hidranensefali ve serebellar hipoplazi olgusu anatomik, makroskopik, mikroskopik ve immunohistokimyasal yünden incelenmiştir. Anomaliler ve merkezi sinir sisteminde meydana gelen lezyonların Sığırların Virüsü İshali Virüsü ile ilişkisi immunohistokimyasal olarak ortaya konmuştur.

Anahtar sözcükler: Buzağı, Hidranensefali, Kafatası malformasyonları, Serebellar hipoplazi, Sığırların Virüsü İshali.

Bovine Viral Diarrhea (BVD) is recognized as one of the most important reproductive diseases in cattle. Infection causes significant economical losses by leading to abortion, stillbirth, central nervous system and skeletal anomalies, and birth of calves with a low survival rate (1, 2, 15). The severity of pathologic changes shows a positive correlation with the pregnancy period of infection. The animals exposed to Pestiviruses during the first trimester of their pregnancy show high rates of abortion, mummification, and embryo resorption as well as development of serious malformations in the calves such as cerebellar hypoplasia, porencephaly or hydranencephaly in the central nervous system (CNS). Moreover, important pathologic findings such as severe scoliosis of the skeletal system and arthrogryposis can accompany, as well (1, 6, 8, 9, 13).

In this study, cranial anomalies, hydranencephaly, and cerebellar hypoplasia in a calf with regard to anatomic, microscopic, and immunohistochemical aspects were investigated.

A Holstein-Simmental cross bred male calf weighing 20kg was brought to the clinic with a cystic mass of 20 cm over the frontal region, originating from

the occipital region and encompassing also the superior nasal area. The overall condition of the animal was poor as it was unable to stand up and suck its mother. The subsequent puncture of the cyst revealed a thin and transparent fluid content. Radiographic examination showed that skull was not fully formed. Following an anesthetic induction with 6 mg/kg i.v. propofol injection, the anesthesia was maintained by isoflurane (Forane Likid, ABBOTT Laboratories Ltd., England) 1.5% + 100% O₂ gas mixture. During the surgical intervention, the skull and cerebral hemispheres were not observed. After a skin incision, 2300 ml fluid was collected from the cyst. The calf became moribund 2 hours after operation and it was euthanized by delivery of intravenous tiyopental sodium (Pental sodyum 1 g I.E. ULAGAY Pharmaceutics Inc. Istanbul, Turkey). A systemic necropsy was performed and collected tissue samples were fixed in 10% neutral buffered formalin for 48 hours and they were washed under tap water overnight. Following routine tissue preparation procedures with graded alcohol and xylene series, tissue samples were embedded in paraffin blocks. Then paraffin sections at 5 µm thickness were obtained. Hematoxylin-

eosin staining was applied to the sections and they were analyzed histopathologically under binocular light microscope (Olympus BX51).

Bovine Viral Diarrhea Virus (BVDV) monoclonal antibody (VMRD D89, gp55, USA) and commercial indirect immunoperoxidase streptavidin/biotin immunoperoxidase kit (Novacastra, HRP, Catalog no: RE7 110-K, USA) were used for the detection of BVDV antigens in immunohistochemical analyses. All the procedures were performed as per standard protocols of the kits. Accordingly, the sections were deparaffinized for 5 minutes in each of 3 xylene series and rehydrated by being kept 5 minutes in absolute, 95%, and 70% alcohol, and distilled water. Enzymatic digestion was performed using 0.1% proteinase K (Vivantis, USA) at 37 °C. Thereafter, the sections were incubated with anti-BVDV monoclonal antibody (VMRD, USA) at 1/50 dilution for 60 minutes. After treating the sections with both biotin-marked secondary antibody for 30 minutes and streptavidin-peroxidase enzyme gain for 30 minutes, aminoethyl carbasole (AEC) chromogen (Zymed, Lot: 60682605, USA) was applied for 7 minutes for color reaction and Mayer's hematoxylin was applied for counterstaining for 1-2 minutes. Thereafter, they were mounted with water-based mounting medium.

The cranial anomaly was examined both macroanatomically by dissection methods and histologically over the structures formed below the palpebra superior within the right orbit. The photographs of the dissected anomalous structures were taken with Fujifilm C10 camera. The anatomic terminology was defined according to the Nomina Anatomica Veterinaria (2005).

Occipital, interparietal, and parietal bones constituting normal neurocranium were present, whereas rostral part of the frontal bone, which forms the roof of the skull, was curling upwards and continuing only with scalp after the disappearance of the osseous structure (Figure 1). This bony extension curling upwards was a thin structure with no lamina interna, lamina externa, sinus frontalis, and crista sagittalis interna, all of which constitute the normal anatomy of the frontal bone (Figure 2). Structures such as falx cerebri and tentorium cerebelli membraneus that enable the connection of the encephalon were observed to have an asymmetric form. Frontal bone forming the rostral part of neurocranium, maxilla, ethmoid bone, the entire part of nasal bone forming the splanchnocranium, and medial portions of the lacrimal and zygomatic bones were determined to be absent. Those areas were covered only with skin (Figure 2 and 3). Scalp was observed as attached to the incisive bone at planum nasolabiale level and cranial part of the concha nasalis dorsalis within the nasal cavity on the left side.

There was no bony structure between the nasal and cranial cavities except the periosteum (Figure 2).



Figure 1. View of the anomaly from lateraly.
Resim 1. Anomalinin lateral'den görünümü.

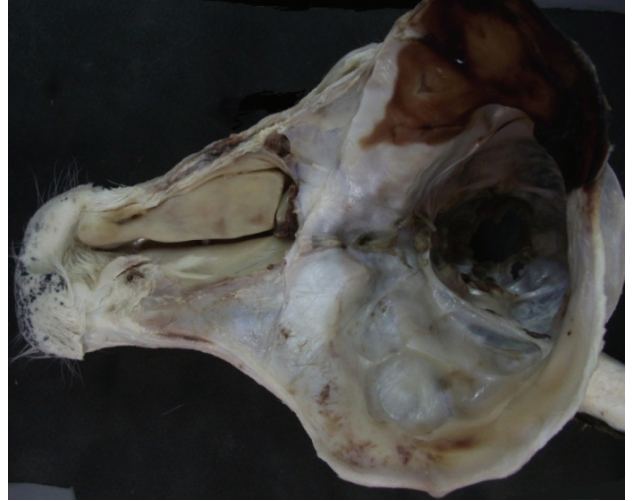


Figure 2. View of the cranium from dorsaly.
Resim 2. Cranium'un dorsal'den görünümü.

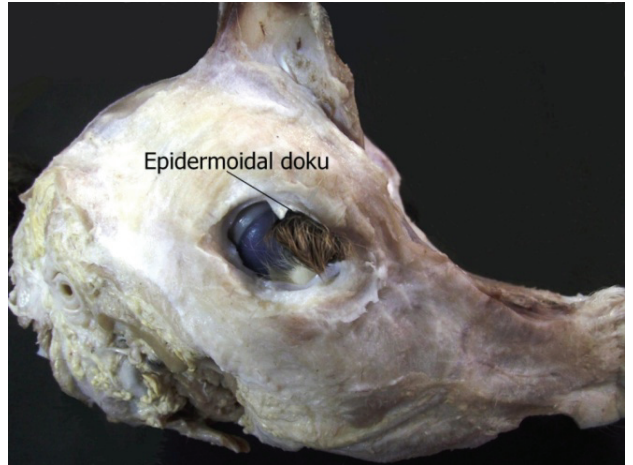


Figure 3. Lateral view of the orbita and cranium
Resim 3. Orbita ve Cranium'un lateral'den görünümü

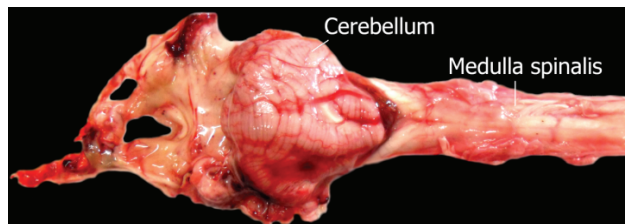


Figure 4. Dorsal view of the cerebellum and spinal cord.
Resim 4. Cerebellum ve Medulla spinalis'in dorsal'den görünümü

Foramen orbitotundum, fissura orbitalis, and foramen ovale had nerve tissue inside, whereas fossa hypophysialis, which normally houses formation of diaphragma sellae, was observed to contain hypophysis cerebri.

Nasal bone forming the nasal cavity and nostrils was absent and cartilaginous skeleton forming the right nostril and planum nasolabiale was not formed, as well (Figure 2). Cartilago septi nasi on the median plane was not completely formed, whereas it was deviated towards the left nasal cavity so as to form the left nostril. While the left nostril was formed, it was occluded by an epidermoidal structure with the size of a chickpea over the roof of the nostril (Figure 2). Incisive bone, which normally forms the roof of the planum nasolabiale, was not formed completely, leading to the asymmetric formation of planum nasolabialis (Figure 2 and 3).

The most remarkable macroscopic findings were severe hydranencephaly and absence of the right nostril formation. Cerebral hemispheres were not fully developed and there was a mild cerebellar hypoplasia. Another notable macroscopic finding was the presence of a marked cavitation on the right side of the cerebellum (Figure 4).

Histopathologically, medulla oblongata had prominent congestion as well as erythrophagocytosis, erythrocyte diapedesis, hemosiderin-laden microglia, and perivascular edema in the grey matter (Figure 5). Skeletal muscles had patchy hyaline degenerations. Presence of hemosiderin-laden macrophages was also observed in the residual brain tissues. Histologic analysis revealed an additional eyelid within the right orbit (Figure 3) at the medial angle of eye along with an epidermoidal tissue formation from the floor of the orbit, occluding the anterior aspect of the cornea.

Immunohistochemical analyses showed strong BVDV antigen immunopositivity in glial cells and degenerative neurons throughout the medulla spinalis (Figure 6) and cerebellum as well as splenic sinusoidal macrophages.

In this case, according to immunohistochemical analysis and pathological findings, severe hydranencephaly and mild cerebellar hypoplasia associated with a Pestivirus infection was diagnosed. Recently, it has been focusing on the fact that Pestiviruses are not species-specific. Cattle can be infected with BVDV as well as border disease virus and classical swine plague virus, experimentally (14). In the reported case, severity of the macroscopic lesions suggested that the virus could be transmitted to the fetus during first and second trimester of the pregnancy when the organogenesis still continue.

Viral etiologies have a notable role in congenital CNS anomalies seen in newborn calves. Hydranencephaly, porencephaly, and cerebellar hypoplasia are the most common viral CNS anomalies. In addition the

Pestiviruses, the other viral infections causing CNS anomalies are: Akabane virus in Australia, Japan (7), and Israel (11); bluetongue disease in Northern Africa (12); Wesselsbron disease in Africa ; and Chuzan viruses in Japan (10). They have also been reported to be encountered, although experimentally, due to Aino virus. However, the most common CNS malformations develop due to Pestiviruses (8).

In conclusion, natural BVDV infection characterized by central nervous system anomalies and cranial malformations was described in a calf using detailed macroscopic and microscopic examinations. The microscopical findings, demonstrating the Pestivirus antigens in the lesioned tissues suggested that the virus affects the calf during the first or second trimester of the pregnancy.

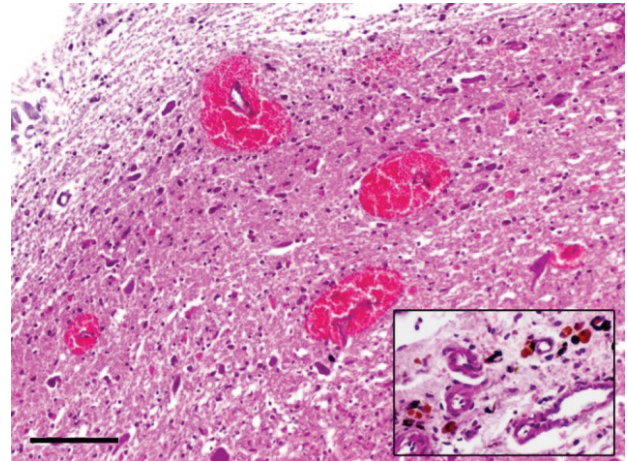


Figure 5. Perivascular erythrocyte diapedesis in gray matter. Erythrophagocytosis and hemosiderin-laden microglia (right lower image). Hematoxylin and Eosin. Bar= 100 µm.
Resim 5. Gri madde de perivasküler eritrosit diapedezi. Eritrofagositoz ve hemosiderin yüklü mikroglia hücreleri (alt resim). Hematoksilen ve Eozin. Bar= 100 µm.

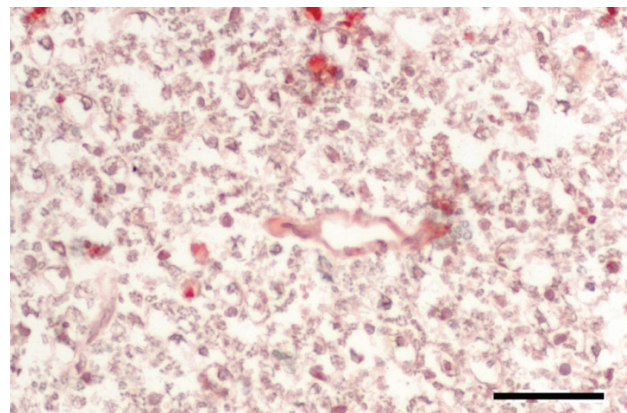


Figure 6. BVDV positive cells in medulla spinalis. ABC method (anti-BVDV), counter stain Mayer's hematoxylin. Bar= 50 µm.
Resim 6. Medulla spinalisteki BVDV pozitif hücreler. ABC metod (anti-BVDV), Mayer's Hematoksilen karşıt boyama. Bar= 50 µm.

References

1. **Baker JC** (1987): *Bovine viral diarrhoea virus: A review*. J Am Vet Med Assoc, **190**, 1449-1458.
2. **Barlow RM, Nettleton PF, Gardiner AC, Greig A, Campell JR, Broon JM** (1986): *Persistent bovine viral diarrhoea virus infection in a bull*. Vet Rec, **118**, 321-324.
3. **Haziroglu, R.** (2000): *Sinir Sistemi*. In: *Veteriner Patoloji* 2nci ed., Cilt. 1. (Milli U. H., Haziroglu R.) Medipres, Ankara pp. 241-340.
4. **Hewicker-Trautwein M, Liess B ve Trautwein G** (1995): *Brain Lesions in Calves following Transplacental Infection with Bovine-virus Diarrhoea Virus*. J Vet Med B, **42**, 65-77.
5. **Konno S, Nakagawa M** (1982): *Akabane disease in cattle: congenital abnormalities caused by viral infection*. Vet Pathol, **19**, 267-79.
6. **Kul O, Kabakçı N, Özkul A, Kalender H, Atmaca H T** (2008): *Concurrent peste des petits ruminants virus and pestivirus infection in stillborn twin lambs*. Vet Pathol, **45**, 191-196.
7. **Liess B** (1990): *Bovine viral Diarrhoea virus*. In "Virus Infections of Ruminant" Ed. Dinter Z, B Morein, Elsevier, The Netherlands.
8. **Miura Y, Kubo M, Goto Y, Kono Y** (1990): *Hydranencephaly-cerebellar hypoplasia in a newborn calf after infection of its dam with Chuzan virus*. Nippon Juigaku Zasshi, **52**, 689-94.
9. **Markusfeld O, Mayer E** (1971): *An arthrogryposis and hydranencephaly syndrome in calves in Israel, 1969/70*. Epidemiological and clinical aspects. Refuah Vet, **28**, 51-61
10. **MacLachlan NJ, Osburn BI** (1988): *Congenital bluetongue virus infection*. Prog Clin Biol Res, **281**, 33-47.
11. **Orhan İÖ, Haziroğlu RM, Kutsal O** (2001): *Bir buzağda aksesuar dil ve diğer konjenital malformasyonlar*. Turk J Vet Anim Sci, **25**, 863-866.
12. **Paton DJ** (1995): *Pestivirus diversity*. J Comp Pathol, **112**, 215-236
13. **Radostits OM ve Littlejohns IR** (1988): *New Concepts in the Pathogenesis, Diagnosis and Control of Diseases Caused by the Bovine Viral Diarrhoea Virus*. Can Vet J, **29**, 513-528.

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Adress for correspondence:

Prof.Dr.İsmail Önder Orhan
AnkaraUniversity, Faculty of Veterinary Medicine,
Department of Anatomy, 06110, Ankara
e-mail: oorhan2010@hotmail.com