



First Detection of Hypomyelination Associated with Bovine Viral Diarrhoea Virus in an Aborted Calf in Gumushane Region

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Absract: In this study, hydranencephaly and mild cerebellar hypoplasia were reported macroscopically, microscopically and immunohistochemically in an aborted Holstein Friesian calf. A relationship between Bovine Viral Diarrhoea Virus (BVDV) infection and histopathology associated with the central nervous system (CNS) lesions were demonstrated. In the histopathology, perivascular haemorrhage, oedema, neuronal necrosis and degeneration were observed in the CNS. BVDV positive immunoreaction was detected in neurons, glial and endothelial cells of CNS by immunohistochemical examination. As far as the authors are concerned, this is the first report of BVDV present in an aborted Holstein Friesian Calf in Gumushane region. This finding may have important implications for the epidemiology and control of BVDV infection in the Gumushane region.

Keywords: Bovine Viral Diarrhoea, Cerebellar hypoplasia, Hydranencephaly, Hypomyelination.

Gümüşhane Bölgesinde Aborte Bir Buzağıda Sığır Viral Diyare Virüsü ile İlişkili Hipomyelinasyon'un İlk Tespiti

Öz: Bu çalışmada, Holstein Friesian ırkı bir buzağıda görülen hidranensefali ve orta şiddette serebellar hipoplazi olgusu makroskopik, mikroskopik ve immunohistokimyasal yünden incelenmiştir. Merkezi sinir sisteminde meydana gelen histopatolojiler ise Sığırın Virüsü İshali Virüsü (SVİV) ile ilişkili immunohistokimyasal olarak ortaya konuldu. Histopatolojik olarak, perivasküler kanama, ödem, nöronal nekroz ve dejenerasyonlar gösterildi. İmmünohistokimyasal olarak, SVİV antijenleri nöron, glial hücreler ve endotel hücrelerde gösterildi. Anılan vaka, Gümüşhane bölgesinde SVİV ile ilişkili olarak meydana gelen aborte bir Holstein Friesian buzağının ilk raporu olma özelliği taşımaktadır. Bu bulgu, Gümüşhane bölgesi için epidemiyoloji ve SVİV enfeksiyonlarının denetim gerekliliğinin ciddiyetini de gösterebilir.

Anahtar Kelimeler: Hidranensefali, Hipomyelinizasyon, Serebellar hipoplazi, Sığırın Virüsü İshali.

INTRODUCTION

Bovine Viral Diarrhoea is among reproductive diseases that cause significant economic losses as it results in aborted, stillbirths or birth of calf having very little change to live by cows (1,2). In addition to these, as it may cause anomalies in skeletal and central nervous systems (CNS), pathogenesis of *pestivirus* infections is of great importance and there are some related studies (3-5).

The severity of the pathology occurring after an infection is closely connected with the pregnancy period in which the infection occurs. When the pregnant animal is infected with the virus during the first trimester of pregnancy, the result will probably be abortion, mummification and resorption of the embryo. Besides the CNS anomalies such as cerebellar hypoplasia, porencephaly and hydranencephaly, scoliosis and arthrogryposis are commonly seen among the infections of this period (2,4,6-8). In the current study, the CNS of an aborted calf having hydranencephaly together with slight cerebellar hypoplasia was analysed for the detection of histopathologic and possible etiologic agent through the immunohistochemical methods.

In the current study, it was intended to make some contributions to pathogenesis research by investigating the histopathology and viral antigen localizations occurring in the CNS of a calf infected with natural BVDV. Moreover, as it is the report of the first incidence observed in Gümüşhane region, it is of great importance.

CASE REPORT

An aborted Holstein Friesian calf, female, from a private farm was included in this study. Necropsy was performed and brain tissue samples collected were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 48 h and then were thoroughly rinsed overnight under tap water. After performing the routine tissue preparation procedures of dehydration using graded alcohol and xylene, the tissue samples were embedded in paraffin blocks; 5 µm-thick paraffin sections were

then cut and mounted on glass slides. Hematoxylin-Eosin (H&E) and immunohistochemical tests were performed, and they were then analysed using a trinocular light microscope (Olympus BX51 and DP25 digital camera).

Immunohistochemistry was performed to observe BVDV antigens in the 5 µm-thick paraffin sections of the tissues by using an indirect streptavidin/biotin immunoperoxidase kit (HRP, Thermo Scientific, USA), as previously described by Dinçel and Kul (4). Briefly, the sections were placed onto adhesive slides, deparaffinized for 5 min each in the 3-step xylene series, and rehydrated using a series of graded alcohol and distilled water. The antigens were retrieved by boiling the tissue sections on glass slides in citrate buffer (pH 6.0) (Thermo Scientific, USA) for 20 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide in absolute methanol for 7 min at room temperature (RT). The tissue sections were rinsed three times with PBS (pH 7.4) for 5 min, between each consecutive step. The sections were then incubated in a blocking serum for 5 min to prevent non-specific antibody binding. Thereafter, the sections were incubated with anti-BVDV monoclonal antibody (VMRD, USA) at 1/100 dilution for 60 min in a humidity chamber at the RT. After treating the sections with biotin-labelled secondary antibody for 15 min and streptavidin-peroxidase enzyme for 15 min at RT, the colour reaction was performed using aminoethylcarbasole (AEC) chromogen (Thermo Scientific, USA) for 5–10 min. Sections were counterstained with Mayer's hematoxylin for 1–2 min and suspended in water-based mounting medium (Thermo Scientific, USA).

The most remarkable macroscopic findings were hydranencephaly and mild cerebellar hypoplasia. Histopathologically, the most conspicuous finding was hypomyelinogenesis (Figure 1, Figure 2) in all parts of the brain characterized by perivascular haemorrhage, oedema in the grey matter, neuronal necrosis and degeneration in the CNS (Figure 3). Virchow Robin

spaces were enlarged due to oedema and infiltrations. Central chromatolysis and neuronal necrosis were also observed in the brain. In addition, immunohistochemical analyses showed strong BVDV antigen immunopositivity in glial cells, degenerative/necrotic neurons and endothelial cells

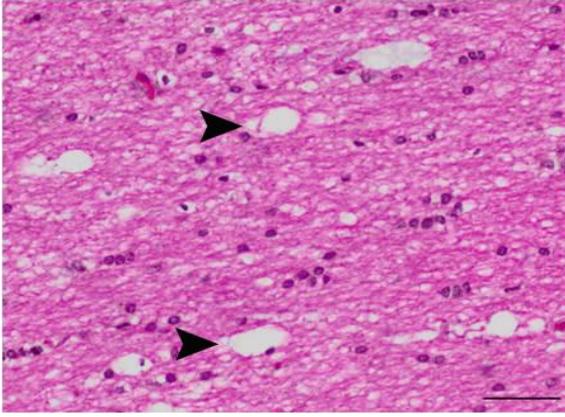


Figure 1. Severe myelin loss (arrowheads). H&E, Bar = 100 µm. Hematoxylin-Eosin (H&E).

Şekil 1. Şiddetli myelin kaybı (okbaşları). H&E, Bar = 100 µm. Hematoksilen-Eozin (H&E).

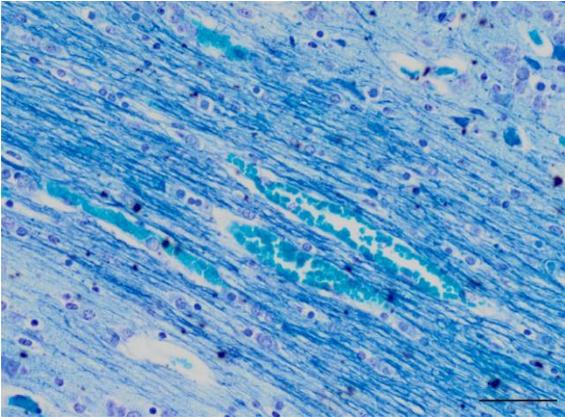


Figure 2. Severe hypomyelination and lack of staining over the myelin site. LFB. Bar = 100 µm. Luxol fast blue (LFB).

Şekil 2. Şiddetli hipomyelinasyon ve myelin alanlarda boyanma kaybı. LFB. Bar = 100 µm. Luxol fast blue (LFB).

in the CNS (Figure 4, Figure 5). The BVDV-infected animal showed markedly blanching areas of demyelination. LFB staining revealed marked demyelination in the white matter of the cerebrum and cerebellum (Figure 2).

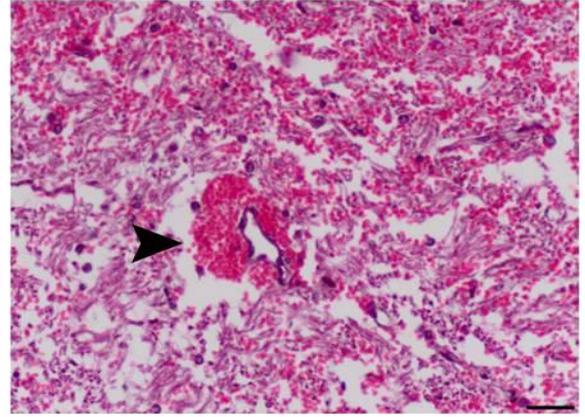


Figure 3. Perivascular erythrocyte diapedesis and oedema in grey matter (arrowhead). H&E, Bar = 100 µm.

Şekil 3. Beyaz maddede perivasküler kanama alanları ve ödem (okbaşı). H&E, Bar = 100 µm.

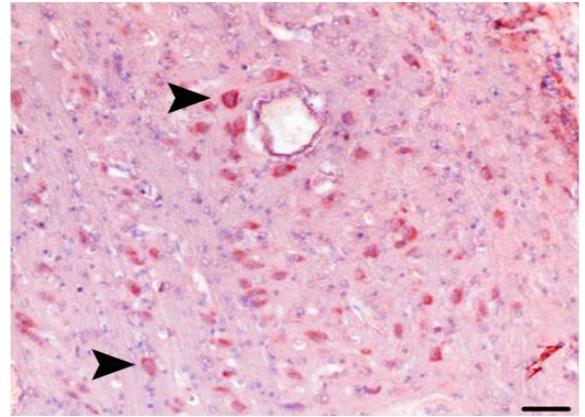


Figure 4. Immunohistochemical detection of intense BVDV antigen. Note the positive immunolabelling (red pigment) in vascular endotheliums, degenerative/necrotic neuronal cytoplasm and glial cells (arrowheads). ABC technique (anti-BVDV), Mayer's hematoxylin counterstain, Bar = 100 µm. Avidin-biotin-peroxidase complex (ABC).

Şekil 4. Yoğun BVDV antijenlerinin immünohistokimyasal tespiti. Glial hücrelerde, nekroze/dejeneratif nöronlarda ve endotel hücrelerde immün boyanmalar (kırmızı pigmentler) (okbaşları). ABK teknik (anti-BVDV), Mayer's hematoxylin arkaplan boyaması, Bar = 100 µm. Avidin-biotin-peroksidaz kompleks (ABC).

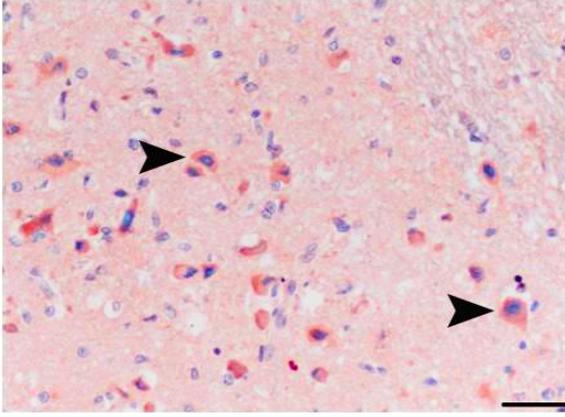


Figure 5. Immunohistochemical detection of intense BVDV antigen. Note the positive immunolabelling (red pigment) in vascular endotheliums, degenerative/necrotic neuronal cytoplasm and glial cells (arrowheads). ABC technique (anti-BVDV), Mayer's hematoxylin counterstain, Bar = 100 μ m.

Şekil 5. Yoğun BVDV antijenlerinin immünohistokimyasal tespiti. Glial hücrelerde, nekroze/dejeneratif nöronlarda ve endotel hücrelerde immün boyanmalar (kırmızı pigmentler) (okbaşları). ABC teknik (anti-BVDV), Mayer's hematoxylin arkaplan boyaması, Bar = 100 μ m

DISCUSSION and CONCLUSION

Viral aetiologies have an important role in causing CNS anomalies in newborn calves. Hydranencephaly, porencephaly and cerebellar hypoplasia are among the most commonly seen CNS anomalies. Besides pestiviruses, akabane (9), bluetongue (10) and wesselsbron (11) are viral diseases causing CNS anomalies. However, in small ruminants and cows, the cause of the most common CNS anomalies is seen to be *pestiviruses*.

In the current study, the first report showing through histopathologic and immunohistochemical analyses that hydranencephaly together with slight cerebellar hypoplasia occurring in an aborted calf is connected with *pestivirus* infections is presented. Besides hypomyelination and neuronal necrosis, almost every part of the brain was affected from the virus. When the findings of the study are evaluated, the severity of macroscopic lesions and histopathology led us to think that the infection occurred in the first or second trimester of the pregnancy. Since the present study is the first to detect a BVD incidence in Gümüşhane region, which

is one of the *pestivirus*-free regions where *pestivirus* infections cause heavy economic losses, it is of great importance for the region.

As a result, this case shows that in calves living in Gümüşhane region, there might be *pestivirus* infections but they could go unnoticed. Cows delivering such aborted births should be sacrificed and regular monitoring should be conducted so that the region could be protected. Moreover, the attempts to detect other possibly infected animals should be started immediately and the infected cows should be sacrificed. Research conducted in recent years demonstrated that there are incidences of interspecies transmission (12). Thus, small ruminants kept in the same place with the cows or close to them should be checked to prevent the spread of *pestiviruses*.

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