



Malignant Catarrhal Fever caused by Ovine Herpesvirus-2 in a cow

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Abstract: Malignant catarrhal fever (MCF) has been defined as a viral disease of domestic cattle and wild ruminants. We report a case of MCF caused by Ovine herpesvirus-2 (OvHV-2) in a 4-year-old female Holstein cow. Macroscopically, eyelids and conjunctiva were edematous and all superficial and, visceral lymph nodes were swollen. Microscopic examination showed that fibrinoid necrotic vasculitis in all tissues and organs; inflammatory changes in the vessel walls of the brain (pons, cerebrum and meninges); ulcerative changes in the oral mucosa; mild inflammatory cell infiltrations around the glomeruli and interstitium in the cortex of the kidney; widespread edema between the muscle bundles in the heart. PCR was performed to determine the presence of Ovine herpesvirus-2 nucleic acids and 386 bp product was obtained by primer sequences that replicated the pol gene region of OvHV-2. This PCR finding confirmed our diagnosis as sheep-associated MCF (SA-MCF).

Key words: Malignant catarrhal fever, Ovine herpesvirus-2, pathology, PCR

Bir inekte Ovine Herpesvirüs-2'nin neden olduğu Sığır Gangrenli Nezlesi olgusu

Özet: Sığır gangrenli nezlesi (SGN) (malign kataral ateş), evcil sığır ve yabani geviş getirenlerin önemli viral bir hastalığıdır. Sunulan olguda, 4 yaşlı Holştayn ırkı bir inekte Ovine herpesvirüs-2'nin (OvHV-2) neden olduğu SGN enfeksiyonu patolojik ve PCR bulgularıyla birlikte tanımlanmıştır. Makroskopik olarak, göz kapakları ve konjunktiva ödemli; tüm viseral ve yüzeysel lenf düğümleri şişkindi. Mikroskopik incelemede, tüm doku ve organlarda fibrinoid nekrotik vaskülit, beyin damar duvarlarında yangısal değişiklikler (pons, serebrum ve meninkler), oral mukozada ülseratif değişiklikler, böbreğin korteksinde glomerüllerin çevresinde interstisyumda hafif yangısal hücre infiltrasyonları ve kalpteki kas demetleri arasında yaygın ödem görüldü. OvHV-2 nükleik asitlerinin varlığını belirlemek amacıyla PCR yöntemi uygulandı ve 386 bp ürünü, OvHV-2'nin pol gen bölgesini çoğaltan primer dizinleri ile elde edildi. Bu bulgular ile sunulan olguda koyunla ilişkili SGN tanısı doğrulandı.

Anahtar kelimeler: Ovine herpesvirüs-2, patoloji, PCR, sığır gangrenli nezlesi

Introduction

Malignant catarrhal fever (MCF) is a viral disease with a high mortality rate in cattle and other ruminants. The disease is usually sporadic, but occasionally causes outbreaks. Epidemiologically, two forms of the disease have been described. The first is the form associated with antelope named as Wildebeest-associated MCF (WA-MCF). The other form of the disease is thought to be sheep related and named as sheep-associated MCF (SA-MCF) (Dabak and Bulut 2003; Bedelian et al. 2007; Russell et al. 2009).

MCF is caused by many viruses in the Macavirus genus of the gammaherpesvirinae subfamily. There are at least 10 viruses in this genus; alcelaphine herpesvirus-1 (A1HV-1) and ovine herpesvirus-2 (OvHV-2) are among them which cause the

disease. WA-MCF is formed by A1HV-1 and SA-MCF is formed by OvHV-2 (Crawford et al. 2002; Li et al. 2005; Russell et al. 2009). SA-MCF has been reported from almost every region of the world such as North and South America, Europe, Middle East and New Zealand (Russell et al. 2009).

The aim of this case report was to analyze the clinicopathologic and molecular features of a case from an outbreak of MCF in a cattle farm in Turkey.

Materials and Methods

Systemic necropsy of a 4-year-old female Holstein cow which was brought to the Department of Pathology with a history of loss of appetite, stagnation, mouth and nose discharge, high fever, corneal dullness and redness (hyperemia-erythema) in conjunctiva was performed. In anamnesis; in a herd of

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120 animals, four animals were reported to have died shortly after showing similar clinical symptoms. It is also reported that there is a flock of sheep in the immediate vicinity of near of this farm.

Following necropsy, tissue samples were fixed in 10% buffered formalin solution, blocked in paraffin, sectioned at 4-5 µm, stained with hematoxylin and eosin (H&E) and evaluated under light microscope for microscopic examination (Olympus BX51).

Tissue samples (kidney, lymph nodes, and brain) taken for virologic examination were tested by PCR method to investigate the presence of Bovine Viral Diarrhea Virus (BVDV), Bovine Herpesvirus-1 (BoHV-1), and Ovine Herpesvirus-2 (OvHV-2) nucleic acids. For this purpose; tissue samples were homogenized; DNA and RNA isolations were performed in accordance with following the procedure reported with the Quick-DNATM Universal kit (ZYMO Research, USA) and Quick-RNATM Mini Prep (ZYMO Research, USA) commercial kit, respectively. Primer sequences, product sizes and references used for PCR are presented in Table 1. The conver-

sion of viral RNAs obtained to investigate the presence of BVDV in nucleic acid was performed into complementary DNA (cDNA). For this purpose, the Revertaid First Strand cDNA kit (Fermentas, Lithuania) was used in accordance with the procedure. The obtained cDNAs were stored at -20 °C for use in conventional PCR. PCR was performed to determine the presence of BVDV, BoHV-1 and OvHV-2 nucleic acids. PCR was performed in a total reaction volume of 25 µl containing 2.5 µl of 10 x Hot Start Taq Buffer, 0.5 µl of Hot Start Taq DNA polymerase (5U/µl), 2.4 µl MgCl₂ (25 mM), 0.5 µl of each primers at a concentration of 10 pmol/µl and 3 µl of template DNA or cDNA. The amplification conditions for detection of BVDV, BoHV-1 and OvHV-2 nucleic acids used were 15 min at 95 °C; followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C and final extension step in 72 °C for 10min. PCR products were loaded onto 1,5% agarose gel electrophoresis and visualized with ethidium bromide (Sigma, USA). The amplicons, which were positively detected in conventional PCR in terms of OvHV-2 nucleic acid, were loaded into a 1% gel to evaluate gel images.

Table 1. Primer sequences, product sizes and references used for PCR.

| Gene | Primer sequences | Product length (bp) | Reference |
|----------------------|--|---------------------|---------------------|
| OvHV-2 (Pol gene) | F: 5'-GGC(CT)CA(CT)AA(CT)CTATGCTACTCCAC-3' R: 5'-ATT (AG)TCCACAAACTGTTTTGT-3' | 386 | Flach et al. 2002 |
| BoHV-1 | (PF: 5' CGGCCACGACGCTGACGA 3') (PR: 5' CGCCGCCGAGTACTACCC 3') | 572 | Esteves et al. 2008 |
| BVDV | F: P1 : 98-(5'-GAGGCTAGCCATGCCCTTAGT-3')-119 R: P3:371-(TCAACTCCATGTGCCATGTACAGCA-3')-395 | 297 | Boye et al. 1991 |

Results

Macroscopically, the body condition of the cow was cachectic. Eyelids and conjunctiva were edematous and conjunctival mucosa was congested. The opacity that started from the periphery of the eye cornea to the center was quite prominent. On the inner face of the lower lip and gingiva, numerous ulcer areas with an irregularly shaped hemorrhagic surface were covered with a dirty yellowish exudate. All superficial and visceral lymph nodes were large and edematous; their cross-sectional faces were generally congested with occasional hemorrhagic areas. Pharyngeal and laryngeal mucosa was edematous and hyperemic. The spleen was slightly enlarged and prominent lymph follicles were observed on the cross-sectional face of the spleen. Abomasum wall was edematous and ulcers with multiple irregular shaped hemorrhages were observed on the surface of abomasum mucosa. The kidney was multicolored, and there was a multifocal distribution of gray-white

colored foci of 2-4 mm in diameter, separated from the surface by hemorrhagic zone.

The most severe prominent microscopic finding was fibrinoid necrotic vasculitis in all organs, especially in the liver, kidney and central nervous system. In some vessels, endothelial cells were swollen and hyperplastic in appearance, and thrombi of varying size were seen in the lumens of some vessels. Ulcerative changes in the oral mucosa and fibrous connective tissue proliferations in areas close to these ulcers were evident (Figure 1). Severe necrotic changes in the parenchymal tissue were evident in areas where vascular lesions. In addition to vascular lesions in the liver, mononuclear cell infiltrations in the centrilobular area, dilatation of sinusoids, diffuse degeneration in hepatocytes, focal hemorrhages and necrosis were observed. There was widespread edema and hemorrhages between the muscle bundles in the heart. Edema was usually associated with hemorrhage and eosinophilic

fibrillary material (fibrin). Inflammatory cell infiltration and hyaline degeneration in muscle fibers near some areas of hemorrhage were detected. The most prominent changes in lymph nodes were dense lymphoid cell hyperplasia in the cortical and paracortical regions. Cortical and medullary sinuses were filled with lymphoid cells and macrophages. Thrombi were observed in some lesioned vessels. Also, areas of focal hemorrhage and necrosis were noted. In the spleen, lymphatic follicles were seen with marked hyperplasia and extensive hemorrhagic areas. Hemosiderin pigment was found in the cytoplasm of macrophages in the hemorrhagic areas.

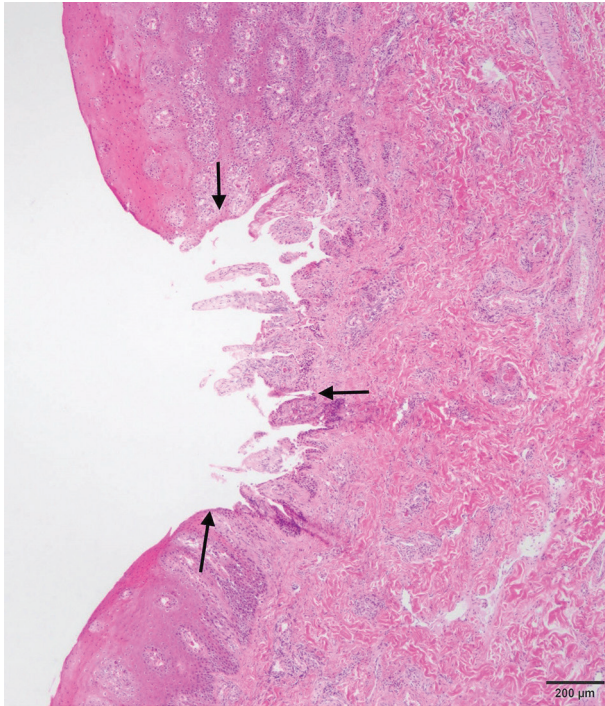


Figure 1. Histopathology of the oral mucosa. Ulcerative changes (arrows). H&E, bar = 200 µm.

Inflammatory changes in the vessel walls of the brain were usually seen associated with hemorrhage in the pons, cerebrum (Figure 2) and meninges. Degenerative changes in the brain parenchyma were also noted. In addition to these findings, diffuse mononuclear cell infiltration in the submucosa of the pharynx together with hemorrhage; diffuse hemorrhage and mononuclear cell infiltration around vessels of tonsils; mononuclear cell infiltration in the vessel wall at the junction of the cornea and sclera of the eye were also observed.

The most prominent changes in the kidney were mild inflammatory cell infiltrations of lymphoblastic and lymphocytic cells around the glomeruli

and interstitium in the cortex (Figure 3) and extensive hemorrhagic areas in the cortex and medulla. The degenerative changes in the epithelium of the tubules near the areas where hemorrhage was noted. The common areas of hemorrhage and necrosis were prominent in the adrenal cortex and medulla.

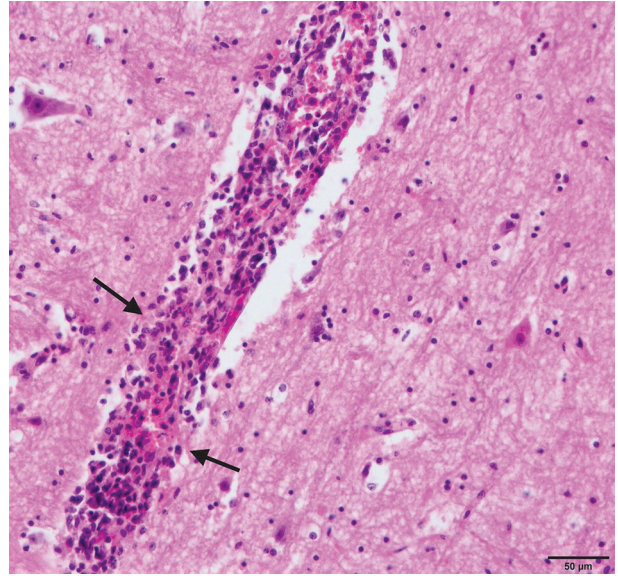


Figure 2. Histopathology of the cerebrum. Severe inflammatory changes in the vessel wall (arrows). H&E, bar = 50 µm.

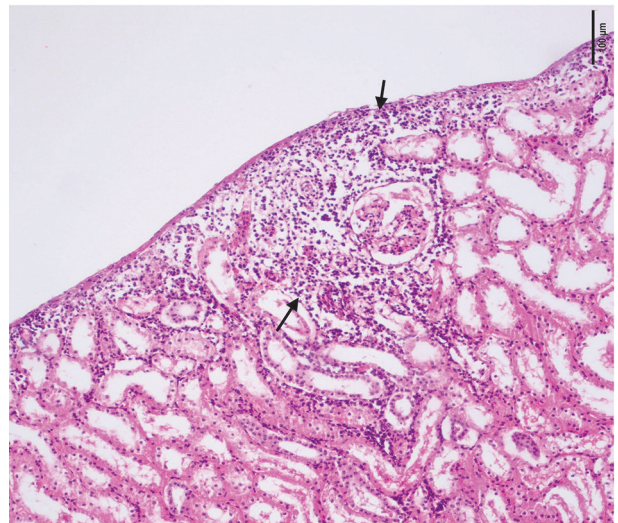


Figure 3. Histopathology of the kidney. Inflammatory cell infiltrations around the glomeruli and interstitium in the cortex (arrows). H&E, bar = 100 µm.

In virological analysis conducted to determine the cause of the disease; while tissue samples were not positive in terms of BVDV and BoHV-1, 386 bp

product was obtained as a result of PCR analysis performed by primer sequences that amplified the pol gene region of OvHV-2 (Figure 4).

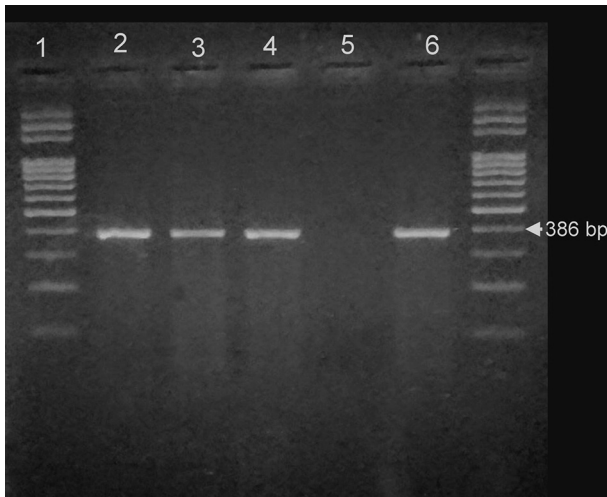


Figure 4. Agarose gel electrophoresis of PCR products amplified from different DNA samples with primers specific for OHV-2. Lane 1, 100-bp DNA ladder; lanes 2 to 4, DNA extracted from lymph nodes, brain and liver samples, respectively; lane 5, no-DNA control; lane 6, positive control.

Discussion and Conclusion

MCF was identified in Turkey for the first time in 2003 (Dabak and Bulut 2003). Since then, sporadic cases or outbreaks of MCF have been encountered from time to time (Yıldırım et al. 2012; Kırbaşı et al. 2013). In these cases, the disease is reported to occur in cattle housed in the same environment of sheep. In the presented case, the sheep herd was reported to be very close to the cattle herd with MCF disease. It was also shown that the disease was formed by OvHV-2 by PCR method. However, the lack of direct contact between cattle and sheeps suggested that aerosol transmission could also play a role in the transmission of the disease.

The diagnosis of MCF is based on clinical symptoms together with pathological findings. Especially, histopathological changes are accepted as the gold standard in the definitive diagnosis of the disease by some authors (Baxter et al. 1993; Russell et al. 2009). In postmortem examinations, petechial hemorrhages and ulcers on the tongue, buccal mucosa, in the gastrointestinal tract, respiratory tract and urinary bladder, raised pale foci on the surfaces of the kidneys, general enlargement of lymph nodes are noted. Microscopically, MCF is characterized by the accumulation of lymphocytes in different tis-

ues sometimes associated with fibrinoid necrotic vasculitis and necrotic lesions (Russell et al. 2009). In the present case, Macroscopically, all superficial and visceral lymph nodes were large and edematous; pharyngeal and laryngeal mucosa was edematous and hyperemic; the kidney was multicolored with a multifocal distribution of gray-white colored foci of 2-4 mm in diameter. Histologically, changes in the vessel wall in many organs are very important in the preliminary diagnosis of MCF. Fibrinoid necrotic vasculitis in all tissues and organs, especially in the liver and central nervous system was prominent and determinative histopathological finding. Additionally, the PCR method, which is based on the amplification of viral DNA, has been widely used in the diagnosis of diseases in recent years. Use of PCR method enables sensitive affirmation of the presence of MCF viruses in infected animals. Conventional and real-time PCR assays have been used to detection of OvHV-2 and AIHV-1 viral DNA (Baxter et al. 1993; Flach et al. 2002; Russell et al. 2009). In this case, conventional PCR assay was performed to confirm the causative agent of disease. According to these assays, 386 bp product was obtained by primer sequences that amplified the pol gene region of OvHV-2. This finding confirmed our diagnosis as SA-MCF.

In conclusion, our experience with the case described in this report has led us to perform PCR method to establish a reliable diagnosis and develop the disease control strategies in MCF.

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