



## RESEARCH ARTICLE

### The Investigation of Bovine Viral Diarrhoea Virus Antigens with Immunofluorescence and Immunohistochemical Methods in Bovine Abortions

Ertan Oruc<sup>1\*</sup>, Yavuz Selim Saglam<sup>1</sup>, Ibrahim Sozdutmaz<sup>2</sup>, Kubra A. Terim Kapakin<sup>1</sup>, Ahmet Temur<sup>3</sup> and Serdar Altun<sup>1</sup>

<sup>1</sup>Ataturk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum, Turkey; <sup>2</sup>Erciyes University, Faculty of Veterinary Medicine, Department of Virology, Kayseri, Turkey; <sup>3</sup>Erzurum Veterinary Control Institute, Erzurum, Turkey

\*Corresponding author: [eoruc@atauni.edu.tr](mailto:eoruc@atauni.edu.tr), [ertanoruc@hotmail.com](mailto:ertanoruc@hotmail.com)

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#### ABSTRACT

The investigation of bovine viral diarrhoea virus (BVDV) in bovine abortions in Erzurum, Turkey was undertaken with immunofluorescence, immunohistochemistry and histopathological methods. For this purpose, tissue sections from 56 aborted fetuses were examined. At the end of the study, histopathological lesions were observed in 17(30.36%) samples. Hyperaemia in sinusoids and central veins, degeneration and necrosis of some hepatocytes and cellular infiltration in portal areas were main histopathological changes in liver. In brain sections, hyperaemia, degeneration and necrosis of neurons, spongiosis and vasculitis, especially in BVDV positive sections, were prominent. In addition, there was interstitial or catarrhal pneumonia in lung sections. Positive immunofluorescence staining was detected in 8(14.28%) foetal tissues by direct immunofluorescence method. In immunohistochemical staining, 6(10.71%) samples were found positive. Antigenic localisations were observed in the cytoplasm of neuronal cells, neuroglia and leptomeninges in brain; hepatocytes cytoplasm of the liver; apical portion of bronchiolar epithelium and alveolar macrophages of lung sections. It is concluded that BVDV is an effective factor for the cattle abortion in Erzurum, Turkey.

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#### INTRODUCTION

Bovine viral diarrhoea (BVD) is caused by bovine virus diarrhoea virus (BVDV), in *Pestivirus* genus within the *Flaviviridae*, and characterised by early embryonic death, mummification, abortion, congenital abnormalities and offspring with persistent infection. BVDV has 2 biotypes; cytopathogenic and non-cytopathogenic. The non-cytopathogenic biotype is common in cattle (Brownlie, 1990; Deregts and Loewen, 1995; Hamers *et al.*, 2001; Vilcek *et al.*, 2005; Collins *et al.*, 2009). Virus spreads through saliva, faeces, urine and uterine fluids and also embryo transfer, veterinary equipment, some types of flies and even live BVD vaccines (Kirkland *et al.*, 1991; Loken *et al.*, 1991; Tarry *et al.*, 1991; Houe, 1999; Brownlie *et al.*, 2000; Katholm and Houe, 2006). The prognosis of BVD varies depending on virus biotype and age of the foetus. Foetal immune system acts an important role in this situation. According to this, persistent infection, congenital abnormalities or seropositively

offspring births were observed in the disease. Because of viral affinity, vasculitis and cytolysis are seen in organogenesis period (Brown *et al.*, 2007). According to the serological study in Turkey, the percentage of anti-BVDV antibody varies between 50-94.1% (Ak *et al.*, 2002; Yildirim and Burgu, 2005; Tan *et al.*, 2006; Albayrak and Ozan, 2012). Contrary to serological studies, there are rare studies about antigenic determination of BVDV in foetal tissues in North-east Turkey. It was aimed that the investigation of BVDV antigens and some tissue localization in aborted foetal tissues of cattle with immunofluorescence and immunohistochemistry in Erzurum, Turkey.

#### MATERIALS AND METHODS

**Tissue samples and processing:** Tissue samples from liver, brain and lung tissues obtained from 56 aborted fetuses submitted to the Ataturk University Faculty of Veterinary Medicine Department of Pathology and

Erzurum Veterinary Control Institute were used as study materials. Tissue samples were fixed in 10% buffered formalin solution. After the routine histopathology process, microtome sections in 5µm were prepared and stained with haematoxylin and eosin (HE).

**Immunofluorescence process:** A commercial test kit (ANTI-BVDV, PESTI, monoclonal antibody labelled with fluorescein isothiocyanate BIO 316, BIO-X Diagnostics, Belgium) was used according to the procedure. For this reason, paraffin sections in 3-4 µm were placed on lysine-coated slides and fixed in Paraformaldehyde (2% in PBS) and acetone solution (9 volumes of acetone and 1 volume of water) for 15 minutes, rinsed with PBS and incubated in PBS-Blue Evans for 1 hour. After washing in PBS, they were dried and mounted with mounting medium (glycerol, 9 volumes; PBS 1 volume). All slides were examined in fluorescence microscope (Carl Zeiss axioskop A1 with Colibri 2 led fluorescence attachment).

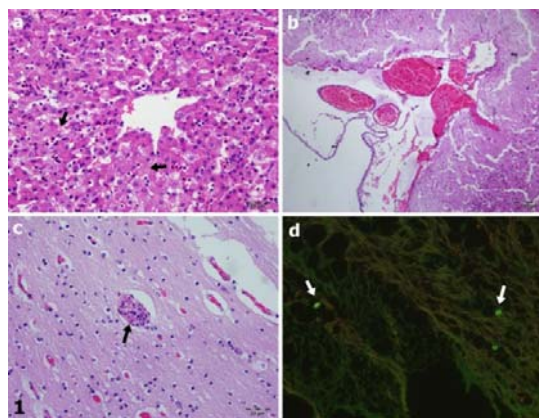
**Immunohistochemistry:** Paraffin sections in 3-4 µm were taken on to lysine-coated slides. After deparaffinization and dehydration, immunohistochemical staining was performed according to the test procedure (EnVision™ FLEX system, Dako). Bovine Viral Diarrhoea Virus 55kDA glycoprotein, VMRD-D89 Mab, was used as the primary antibody according to the manufacturer's protocol. Histopathological and immunohistochemical slides were examined under the light microscopy (Olympus BX52 with DP72 camera attachment).

## RESULTS

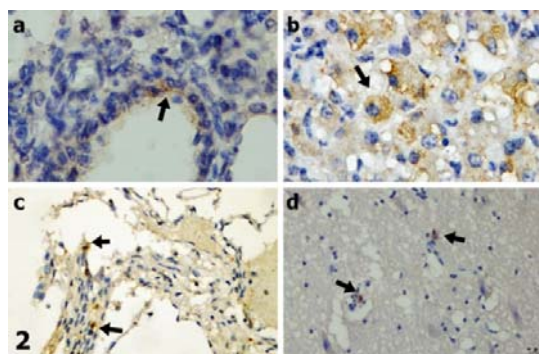
**Histopathology:** Histopathological changes were observed in 17 of 56 (30.36%) foetuses. Different lesions were observed in the liver (17 foetuses) brain (9 foetuses) and lung (7 foetuses) sections. Hyperaemia, hydropic degeneration and necrosis (especially in periacyary and mid-zonal regions), dissociation, mononuclear cellular infiltration and fibrosis in portal areas were observed in liver sections (Fig. 1a). Severe hyperaemia in cerebrum and cerebellum (more severe in meninges in some sections) (Fig. 1b), oedema, vasculitis (Fig. 1c) and neuronal degeneration were observed in cerebral tissues. Besides, gliosis and status spongiosis were detected in some sections. Interstitial and catarrhal pneumonia were seen in 2 and 5 samples of lung, respectively. The rate of histopathological lesions in the brain, liver and lung tissue are presented in Table 1.

**Immunofluorescence:** Positive staining was observed in 8(14.28%) foetuses. All brain samples of 8 foetuses were positive. Positive staining was also observed in 3 livers of these 8 foetuses. Antigenic localization was scattered in these samples (Fig. 1d).

**Immunohistochemistry:** There was 10.71% positivity (in 6 foetuses) in immunohistochemical staining. All brain samples of 6 foetuses were positive. Similar to immunofluorescence staining, positive staining was also observed in 3 livers of these 6 foetuses. Antigenic localisations were observed in the cytoplasm of neuronal



**Fig. 1:** a) Degenerated and necrotic hepatocytes in periacyary and mid-zonal regions (arrow) and sinusoidal dilatations, b) Hyperaemia in cerebral meninges, c) Vasculitis in cerebral tissue (arrow) and d) Immunofluorescence positivity in paraffin sections of cerebrum (arrows). ANTI-BVDV, PESTI, monoclonal antibody labelled with fluorescein isothiocyanate; a-c: H & E staining. Original magnifications: X200 (a and c), X100 (b) and X400 (d).



**Fig. 2:** Antigenic localization of BVDV. a) Bronchiolar epithelium in lung (arrow) b) hepatocytes cytoplasm (arrow), c) meninges (arrows) and d) neural cells (arrows). BVDV 55kDA glycoprotein (VMRD-D89 Mab), Envision™ FLEX system. Magnifications: X400 (a,b) and X200 (c,d).

cells, neuroglia and leptomeninges in brain sections (Fig. 2c and 2d). Besides, antigenic localisation was detected in the cytoplasm of hepatocytes in liver tissue (Fig 2b) and apical portion of bronchiolar epithelium and alveolar macrophages of lung sections (Fig. 2a).

## DISCUSSION

The age of the foetus and virus biotype is two main factors for the course of BVD. Abortion, congenital abnormalities and immunocompetent calf births are seen depending on these factors (Brownlie *et al.*, 2000). Congenitally abnormality was not observed in the present study. Submission of foetuses in the last trimester to our laboratories may be the reason for this situation.

The rate of antibody seropositivity in Turkey was reported as between 50-94 % (Ak *et al.*, 2002; Okur *et al.*, 2007). Yildirim and Burgu (2005) found antibody positivity as 76-82% in Erzurum province. In the antigen screening studies with ELISA or PCR in BVDV in Turkey, variable results were seen. Ak *et al.* (2002) found the percentage of antigen positivity as 13.46%. Tan *et al.* (2006) reported the percentage of antigen positivity as 4.9% in Aydin province. Similarly, Erturk *et al.* (2006)

**Table 1:** Frequency of histopathological lesions in various organs of foetuses found aborted due of bovine viral diarrhoea virus

Lesion	Number of all aborted foetuses (n=56)	Percentage of all aborted foetuses % (n=56)	Number of foetuses infected by BVDV (n=8)*	Percentage of foetuses infected by BVDV % (n=8)*
Cerebrum and cerebellum				
Meningeal hyperaemia	9	16.07	4	50
Cerebral hyperaemia	9	16.07	4	50
Haemorrhage	1	1.78	1	12.5
Vasculitis	4	7.14	4	50
Perivascular cellular infiltration	2	3.57	1	12.5
Gliosis	3	5.36	1	12.5
Neuronal degeneration and necrosis	3	5.36	3	37.5
Status spongiosis	3	5.36	2	25
Liver				
Sinusoidal hyperaemia	14	25	6	75
Hydropic degeneration and necrosis of hepatocytes	8	14.29	4	50
Periportal mononuclear cell infiltration	3	5.36	1	12.5
Periportal fibrosis	1	1.78	0	0
Lung				
Hyperaemia	5	8.93	3	37.5
Catarrhal bronchopneumonia	5	8.93	0	0
Interstitial pneumonia	2	3.57	0	0

\*According to Immunofluorescence positivity.

reported this percentage as 3.61% in swap, defibrinated blood and lung samples. Albayrak and Ozan (2012) found nucleic acids of pestivirus as 73.6% and 91.9% of aborted calves and lambs, respectively. When compared to the previous bovine abortion study conducted in different provinces, the percentage of BVDV antigen positivity was found to be lower in our study. Animal movements, herd differences and probably test methods (serologic, molecular or immunohistochemistry) may be effective for these different results.

Histopathological lesion was detected in 17 foetuses of 56(30.36%) in our study. Hyperaemia within the sinusoids and central veins, degeneration and necrosis of some hepatocytes and cellular infiltrations in portal areas were the main histopathological changes in the liver. In brain sections, hyperaemia, degeneration and necrosis of neurons, spongiosis and vasculitis, especially in BVDV positive sections, were prominent. Besides, there was interstitial or catarrhal pneumonia in lung sections. Liver and lung findings have been accepted as nonspecific lesions and generally seen in aborted foetuses (Tuzcu *et al.*, 2010; Tuzcu *et al.*, 2011). As shown in table 1, liver lesions (hyperaemia within the sinusoids and central veins, degeneration and necrosis of some hepatocytes) were more detectable changes in all aborted foetuses. Besides, cerebral tissue changes (hyperaemia, degeneration and necrosis of neurons, and vasculitis) were more prominent in BVDV infected foetuses. According to our observations cerebral lesions such as vasculitis and neuronal degenerations were thought to be more specific lesions for BVD infection in the present study. The central nervous system, especially the cerebellum, has been known as the primary localization for BVDV (Montgomery *et al.*, 2008; Atmaca *et al.*, 2012). In our study, viral antigen was not demonstrated on the other 9 samples which were detected some histopathologic lesion. Other abortion factors may have been effective in this situation. Viral antigens were demonstrated in different tissues in many BVD studies. Fredriksen *et al.* (1999a) demonstrated BVDV antigens in foetal tissues as early as the 14th day after post-inoculation of heifers at day 85-86 of gestation and investigated with genital organs of dams at days 7, 10,

14, 18 or 22 post-inoculation. They detected BVDV antigens in the mesenchymal cells and immature megakaryocytic-like cells. In another study (Fredriksen *et al.*, 1999b), antigen localisation reported in peribronchial cells of lung, red pulp of spleen and crypt epithelium of intestine. Viral antigens were demonstrated thymus, heart, pancreas, placenta, ovarium, uterine, skin, sinusoids of spleen and mucosa of the digestive system in many studies (Odeon *et al.*, 1999; Taniyama *et al.*, 1999; Fredriksen *et al.*, 1999a; Lam *et al.*, 2007). Brain tissues have been reported as one of the main localization of BVDV antigens (Alkan *et al.*, 2001). Montgomery (2007) demonstrated the BVDV antigen in neuron, astrocyte, oligodendrocyte, pericyte, macrophage and leptomeninges in each of the persistently infected eight calves. Similarly, Montgomery *et al.*, (2008) detected viral antigen in the brain tissues of calves at 75 day of gestation after intranasally infection of some healthy heifers at 75 and 175 days of gestation. Finally, Atmaca *et al.* (2012) showed BVDV antigens in degenerated neuron and neuroglia cells of cerebellum and spinal cord. In our study, BVDV antigen was mostly demonstrated in tissues of the foetal brain (14.28 and 10.71% by Immunofluorescence and immunohistochemistry, respectively).

**Conclusion:** Positive staining was detected as 14.28 and 10.71% by direct immunofluorescence and immunohistochemistry, respectively. Antigenic localisations were observed in the cytoplasm of neuronal cells, neuroglia and leptomeninges in brain; hepatocytes cytoplasm of the liver; apical portion of bronchiolar epithelium and alveolar macrophages of lung sections. Cerebral tissue changes such as hyperaemia, degeneration and necrosis of neurons, and vasculitis and liver lesions such as hyperaemia, degeneration and necrosis of hepatocytes were more prominent in BVDV infected foetuses.

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