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Short Paper

BVDV induced gastro-neuropathy outbreak in a feedlot calves around Tehran (Iran)

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Summary

An outbreak of a lethal disease was reported in 4–6-month-old Holstein calves in a feedlot around Tehran. The signs of central nervous system and gastrointestinal system (GI) involvement were observed in the diseased animals. Necropsy samples of GI, liver, kidney, spleen and lung from 3 died animals were prepared for histopathological examination. Blood and formalin-fixed ear notch samples of 6 calves were submitted for RT-PCR, antigen-capture ELISA (ACE) and immunohistochemistry (IHC) for the detection of BVDV. The results of ACE on buffy coats were negative but RT-PCR of all 6 cases and IHC of 4 cases were positive for BVDV infection. Based on the clinical signs and pathological findings in the GI system and brain, we strongly suggest that the BVDV may represent a gastro-neuropathogen strain. To the best of our knowledge, this is the first outbreak of gastro-neuropathogenic BVDV infection in Iran, which may be acquired postnatally.

Key words: BVDV, Gastro-neuropathogen strain, RT-PCR, Immunohistochemistry, Histopathology

Introduction

Bovine viral diarrhoea virus (BVDV) belongs to the *Pestivirus* genus, *Flaviviridae* family which is an important worldwide viral pathogen of cattle causing many of disease syndromes (Nettleton and Entrican, 1995; Brock, 2004; Hemmatzadeh *et al*., 2006). The virus has been described as affecting the reproductive, respiratory, gastrointestinal, circulatory, immune, lymphatic, musculoskeletal, integument and the central nervous systems (Brock, 2004). Prenatal infection of the BVDV can also result in homologous immunotolerance and persistent infection (PI) and its complications as mucosal disease (MD) in PI animals (Nettleton and Entrican, 1995; Brock, 2004).

Materials and Methods

A severe outbreak of a fatal disease was reported in 4–6-month-old Holstein calves in a feedlot around Tehran with a population of 800 calves. Of these, 270 were stabled in 3 separate yards. Animals at risk were transported from different dairy farms around Tehran in different party to the feedlot after weaning. The calves were fed on alfalfa, hay, corn silage, ground corn, ground oat, cotton seed meal, soybean meal and beet pulp. For dealing with disease at the first step, after history taking, at least 25 cases were examined clinically and 3 dead cases were necropsied. At the second step, and about 3 weeks later, blood and ear notch samples were collected from 6 calves with the same clinical and macroscopic signs.

Blood samples were collected from the jugular vein in 10 ml vacutainer tubes containing 1 ml of 3.85% sodium citrate and delivered to the laboratory on ice in the containers (4°C) maximum within 24 h.

The skin biopsies were collected from the distal part of the ear using an "ear notcher" to obtain a cylindrical notch, 1 cm in diameter, of the ear pinna. Notcher was washed with water and disinfected with 2% formalin solution between the collection of each sample. The samples were fixed in 10% buffered formalin solution.

Formalin-fixed specimens of palatine, oesophagus, rumen, abomasum, small and large intestines, brain, kidney, lung, liver and spleen of the 3 dead cases (as a result of disease) were prepared and referred to the pathology laboratory for sectioning and haematoxylin and eosin staining.

Antigen-capture ELISA (ACE)

BVDV antigen was detected in buffy coat cells using a commercially available kit (Pestivirus antigen detection kit, Moredun Scientific Limited, UK). According to the manufacturer's instructions, after sample preparation and antigen extraction, the assay was performed at 450 nm within 30 min and based on OD limits recommendations (C1>0.8, C2 = 0.4-0.6, C3 = 0.2-0.4 and C4<0.2).

Reverse transcription-PCR (RT-PCR)

Reverse transcription-PCR, as described (Vilcek *et al*., 1994) and modified previously (Moakhar *et al*., 2004) was performed on buffy coat samples from all examined calves.

TriPure isolation reagent (Roche diagnostic GmbH, Mannheim, Germany) was employed for RNA extraction. After this stage, cDNA was synthesized and then PCR procedure was carried out (Vilcek *et al*., 1994; Moakhar *et al*., 2004)

One primer set, forward (5'-ATGCCCTTAGTAGGACTAGCA-3') and reverse (5'-TCAACTCCATGTGCCATGTA C-3') specific for all of pestiviruses was used for RT-PCR according to Vilcek *et al*. (1994) which was modified by Moakhar *et al*. (2004). In this PCR process, one positive cDNA derived from NADL strain and one negative cDNA obtained from non-infected cell culture were used as positive and negative controls, respectively. The electrophoresis of the PCR products was run in 1.5% agarose gel using TAE buffer at 100 V for 45 min. DNA bands were stained with ethidium bromide and then visualized by a UV transilluminator and gel images were printed using a video camera and thermal printer.

Immunohistochemistry (IHC)

Serial sections were made from formalin-fixed ear notch samples referred to the pathology laboratory. Five-micrometer tissue sections were mounted on poly-L-lysine-coated slides and stained for BVDV by using a manually procedure adapted from a previously described technique (Haines *et al*., 1992). Anti-BVDV (pesti) monoclonal antibody labelled with fluorescein isothiocyanate (BIO 316, Bio-X diagnostics kit, Belgium) was used. Stained sections were examined by fluorescent microscopy. Positive IHC staining for BVDV was green fluorescent evidence within the cytoplasm of keratinocytes of epidermis and hair follicles, sebaceous epithelial cells, mononuclear cells in dermis, vascular smooth muscle cells and chondrocytes.

Results

Clinical findings

The most important clinical signs in the affected calves were fever (40-42°C), severe anorexia, hyperpnoea and coughing, mucopurulent nasal discharge, conjunctivitis, stomatitis with erosions in the palatine, gum, dorsal and ventral side of tongue, simple or bloody diarrhoea with or without melena and progressive weight loss and severe depression, recumbency and death. In addition, some calves showed incoordination and severe convulsion; all of the calves with these two latter signs died.

Macroscopic pathological findings

Besides the lesions mentioned in the clinical findings, single and multifocal erosions of esophagus, ruminitis with clumping of its villi, multifocal erosive abomasitis with dark content were observed

in macroscopic examination. Enteritis in small and large bowels with zebra line and sometimes melena in large intestine were obvious sings. Hyperaemia of the brain and cerebellum surface was another finding (Fig. 1A).

Microscopic pathological findings

Hyperaemia, widespread and severe hydropic degeneration of mucosal layer of mouth and oesophagus and oedema, hyperaemia of submucosal layer with infiltration of inflammatory cells in rumen villi were remarkable signs.

Severe oedema, hyperaemia with infiltration of inflammatory cells to submucosal layer and widespread necrosis and erosion of mucosal layer of abomasums were seen.

Severe oedema, hyperaemia with extention of inflammatory cells to submucosal layer and widespread necrosis and erosion of mucosal layer of small and large intestine with cystic degeneration of mucosal layer were obvious signs of the disease.

Hyperaemia of meninges, gliosis and mild neutrophilic lymphocytic perivascular cuffing (PVC) in the brain specimen (Fig. 1B-D) as well as hyperaemia of cerebellum were microscopic findings.

Abnormalities of lung specimen, including hyperaemia, atelectasia and emphysema, hyperplasia of pneumocytes and severe interstitial pneumonia were observed.

Interstitial nephritis with dilatation of tubules, squamous forming of tubular cells as a reason of hyperaemia and proteinuria (nephritic syndrome) were detected in the kidney samples.

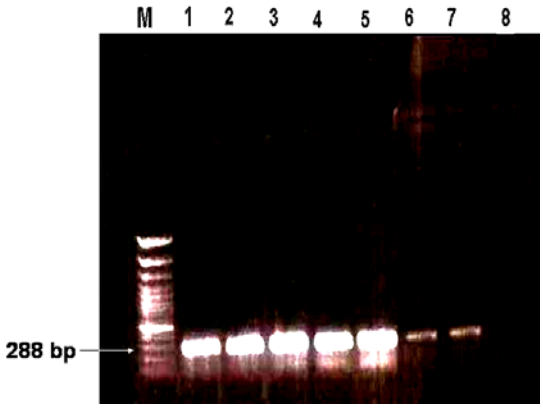

Fig. 2: Gel electrophoretic analysis of PCR products using a specific primer for pestiviruses. Lane 1: positive control, Lane 2-7: positive samples with 288 bp band, Lane 8: negative control and M: 100 bp marker

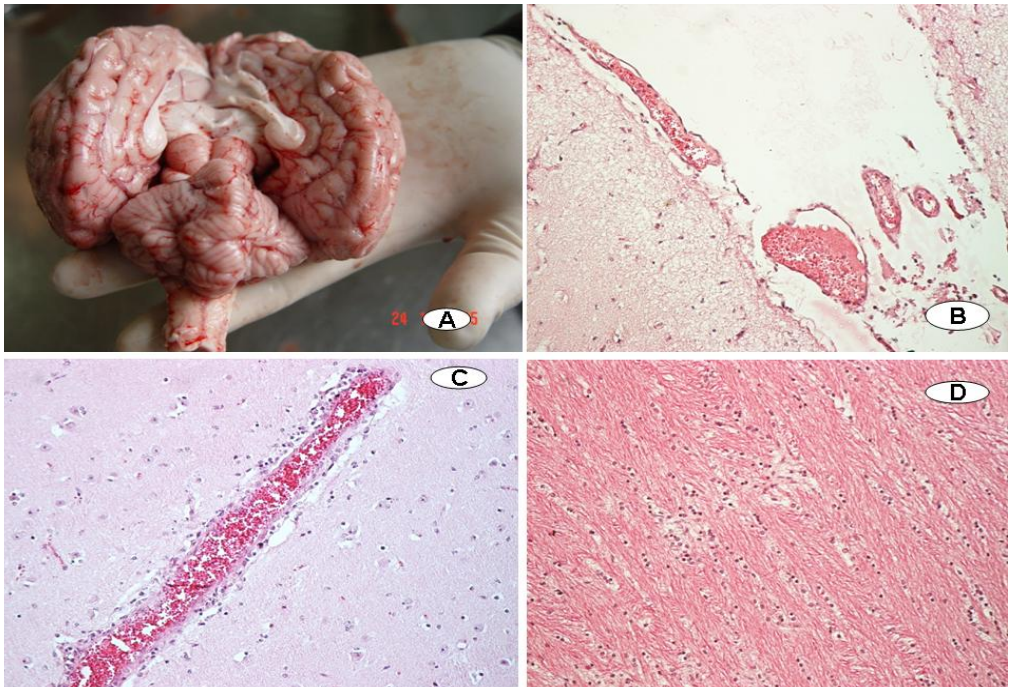

Fig. 1: Hyperaemia of the brain surface (A), meninges (B), Hyperaemia and mild neutrophilic lymphocytic PVC (C), gliosis in the brain specimen (D), (H&E staining, 10 × 20)

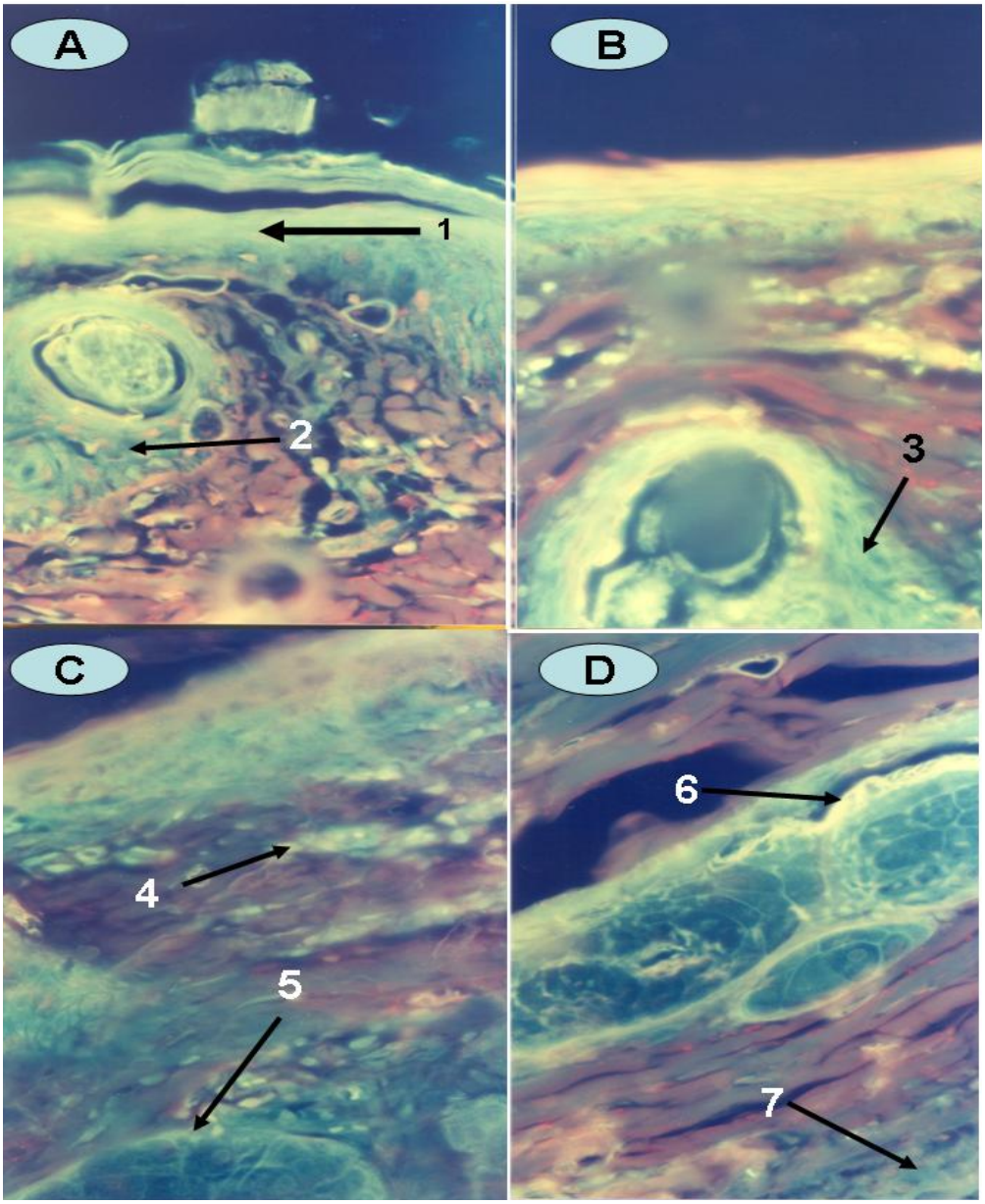

Fig. 3: Positive IHC staining for BVDV. Green fluorescent evidence within the cytoplasm of keratinocytes (A1, A2, B3), sebaceous epithelial cells (C5, D6), mononuclear cells of dermis (C4) and chondrocytes (D7)

ACE, RT-PCR and IHC

Results of ACE test were negative in all of 6 cases, all cases were positive in RT-PCR (Fig. 2) but 4 cases were positive in IHC (Fig. 3).

Discussion

According to the history, clinical sings, pathological findings and severity of the outbreak, the disease is highly resembled BVDV infection.

It is likely that the four IHC positive calves were undergoing an acute BVDV infection and were viraemic at the time of testing. Detection of BVDV in the skin by IHC following acute or persistent infection has been reported to be inconsistent (Haines *et al*., 1992; Baszler *et al*., 1995; Thur *et al*., 1996; Brock *et al*., 1998; Fredriksen *et al*., 1999; Njaa *et al*., 2000; Grooms and Keilen, 2002; Ridpath *et al*., 2002; Brodersen, 2004; Cornish *et al*., 2005; Luzzago *et al*., 2006). Principally, interpretation of IHC sections is highly objective and this problem will be worsening when these sections are prepared

badly (Ellis *et al*., 1995); more than 2 weeks formalin fixation (Luzzago *et al*., 2006), lack of sufficient virus antigen (Njaa *et al*., 2000) and presence of virus-antibody complex in the examined skin sections (Baszler *et al*., 1995) are the likely reasons for these results.

RT-PCR can detect the genome of neutralized and semi-neutralized viruses; in contrast, neutralizing antibodies can mask viruses and make negative ACE results (Brock *et al*., 1998; Brock, 2004; Saliki and DuBois, 2004; Cornish *et al*., 2005; Sagar and Ridpath, 2005; Sandvik, 2005; Houe *et al*., 2006), and this might be the reason for different results of RT-PCR and ACE. In contrast, viral antigen may be detectable in tissues for an extended time after the virus has been cleared from the blood (Grooms and Keilen, 2002; Cornish *et al*., 2005; Sagar and Ridpath, 2005) and is not affected by maternal (Grooms and Keilen, 2002) or humoral antibodies (Brodersen, 2004).

It is speculated that cold weather, high velocity wind, remarkable difference between night and day temperature, stress and immunosuppressant effect of BVDV, accompanied by decline in maternal immunity might play role in this high incidence of the disease. Different types of GI tract lesions from mouth to terminal intestine are the pathological effect of BVDV (Nettleton and Entrican, 1995; Brock, 2004). Furthermore, BVDV type 2-induced meningoencephalitis in a 15-month-old female Angus heifer, with a 48-h history of central nervous system involvement has been reported (Blas-Machado *et al*., 2004). To the best of our knowledge, this article is the second report of neuropathy of BVDV infection, and the first outbreak of gastro-neuropathy due to this virus infection acquired postnatally in Iran.

In general, little is known about the mechanisms of neuropathy and neurovirulence of BVDV in cattle. The most commonly reported brain lesion in calves with transplacental infection of BVDV is cerebellar hypoplasia often associated with hydranencephaly, hydrocephaly, micro-encephaly and or porencephaly. The acute neurological signs observed in these cases were consistent with the microscopic and laboratory findings described before and the severity of the lesions indicates that these animals died as a result of an acute meningoencephalitis associated with BVDV infection (Blas-Machado *et al*., 2004).

According to the first report on BVDV by Blas-Machado *et al*. (2004) and based on the findings of the present study, we propose that the BVDV detected in these cases may represent a neurovirulent strain of the virus. Further studies are needed to corroborate such proposed mechanisms of BVDV type 2 neurovirulence and neuropathogenicity and also to determine the genotype and biotype of virus and confirm such neuropathogenicity mechanisms of BVDV in cattle.

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