

First report of Bovine Viral Diarrhea Virus antigen from pneumonic cattle in Sudan

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ABSTRACT

To explore the expected role of Bovine Viral Diarrhea Virus (BVDV) in pneumonia in cattle, cattle lungs (n=242) showing signs of pneumonia were collected from slaughter houses of three different localities located at Northern, Central and Western Sudan during 2010–2013. The collected samples were tested for the presence of BVDV antigen using Enzyme-Linked Immunosorbent Assay (ELISA), and Fluorescent Antibody Test (FAT). Twenty six (10.7%) out of 242 samples were found to be positive for BVDV. Positive results were seen in all the three studied areas, with the highest prevalence (16.7%; n=4/24) at Gezira State in Central Sudan. BVDV genome could be detected in all ELISA positive samples. The results indicated the existence of BVDV infection in cattle in different areas in Sudan, and its possible association with respiratory infections in cattle. Analysis using BLAST indicated that the sequence was identical to the previously reported BVDV-1 (GenBank accession AF220247.1.); nucleotide A was found in our study at position 9 of our sequence, whereas T was present instead in the reference virus. This is the first report of detecting BVDV antigen, genome, and its sequence analysis collected from cattle lungs in Sudan.

Keywords

BVDV, NS2-3 protein, Respiratory infections, Sudan

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INTRODUCTION

Bovine Viral Diarrhea Virus (BVDV) is a member of the genus Pestivirus under Flaviviridae family, which also includes other two important animal viruses; classical swine fever virus (CSFV), and border disease virus (BDV). Bovine Viral Diarrhea (BVD) is a systemic disease that affects many body systems including respiratory tract (Murphy et al., 1999). BVDV is one of the most economically important pathogens of cattle, which have a serious effect on dairy as well as beef production. Acute infections may result in outbreaks of transient diarrhea or pneumonia (OIE, 2008). The association of BVDV with respiratory signs in cattle is well documented (Kabongo and Van Vuuren, 2004; Fulton et al., 2009; Bachofen et al., 2010).

The role of BVDV in causing reproductive and respiratory problems in cattle worldwide was previously studied. In Thailand, Kampa et al. (2004) examined 11 herds for antibodies to BVDV in milk, the level of exposure to BVDV was found to be 73%. Ghazi et al. (2008) detected 25% seroprevalence to BVDV in buffaloes with respiratory disorders in Egypt. An

increase of BVD magnitude has been reported in United Kingdom (UK) (Booth et al., 2013). Recently, BVDV was isolated from nasal swabs of cattle in China (Wang et al., 2014).

In Sudan, Ali and El-Amin (1982) reported the detection of BVDV antibodies in 71.9% of tested bovine sera. However, no study has been reported in Sudan investigating the existence of BVDV antigen in cattle, although recently BVDV antigen was detected in 7% of 186 camel lungs examined from different areas in Sudan (Intisar et al., 2010). This study was undertaken to investigate the possible role of BVDV in causing respiratory disorders in cattle through the detection of its antigen (BVDV type I NS2-3 protein) using Enzyme-Linked Immunosorbent Assay (ELISA) and Fluorescent Antibody Test (FAT) as well as its genome using Reverse Transcription (RT)-Polymerase Chain Reaction (PCR) in pneumonic cattle lungs.

MATERIALS AND METHODS

Study area: Three areas were selected for the study, Gezira State at Central, River Nile at Northern and North Kordofan at Western Sudan.

Sample collection: A total of 242 samples were collected from cattle lungs showing pneumonia in the slaughter houses at the three different areas. The samples were kept on ice, and sent to the Veterinary Research Institute at Khartoum where these were kept at - 20°C until examination.

Detection of BVDV antigen using ELISA: The collected samples were examined for the detection of BVDV antigen using ELISA Kits purchased from BIO X Diagnostics, Jemelle, Belgium. Preparation of samples, and the test was performed according to the instructions of the manufacturer.

Detection of BVDV antigen using FAT: All the ELISA positive samples were tested for BVDV antigen using FAT. The conjugate was obtained from BIO X Diagnostics, Jemelle, Belgium.

Detection of BVDV genome using RT-PCR: All ELISA positive samples (n=26) as well as 5 ELISA negative samples were examined for the detection of BVDV genome.

RNA extraction and RT-PCR: RNA was extracted from 30 mg of lung tissue homogenate samples using QIAGEN RNeasy Kits (QIAGEN, USA), as instructed

by the manufacturer. RNA was extracted from BVDV reference strain and was used as a positive control. Negative control RNA was obtained from a healthy bovine lung specimen. For the detection of BVDV genome RT-PCR was done using QIAGEN One step RT-PCR Kit using BVDV specific primer pair, P100 5'-CATGCCCWYAGTAGGACTAGC-3' and 5R 5'-AACTCCATGTGCCATGTACAG-3', as described by Becher et al. (1998). A thermocycler (Techne, TC-512) with a heat lid was used. Briefly, to the kit reagents, the forward and reverse primers (final concentration 0.6 µM) and 5 µL of the isolated RNA to a final volume of 25 µL were added. Amplification conditions were, Reverse transcription in one step RT-PCR kit reagents at 50°C for 30 min followed by 94°C for 15 min, this was followed by 40 cycles of PCR, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 sec, and elongation of 72°C for 30 sec, followed by final extension at 72°C for 10 min.

Agarose gel electrophoresis and visualization: A minigel 8 cm long electrophoresis unit (MSMINI, Cleaver Scientific) was used for electrophoresis purpose. The reagents included 1.5% (w/v) agarose gel (Vivantis) in 0.5x Tris-Borate-EDTA (TBE) buffer, 1 µg/mL ethidium bromide (Vivantis) in ddH₂O, TBE running buffer, Blue/Orange 6X Loading Dye (Vivantis) and 100-bp DNA size markers (Vivantis). The separated DNA bands by electrophoresis were visualized and photographed using a gel documentation system (Ingenius, Syngene Bio Imaging).

Sequencing and analysis: The amplicon fragments were cut from the agarose gel, purified using gel purification kit (QIAGEN, USA), and were sent to Macrogen Incorporation for unidirectional sequencing. The sequences were analyzed by the BioEdit software package, and the Basic Local Alignment Search Tool (BLAST) (<http://blast.ncbi.nlm.nih.gov>).

RESULTS AND DISCUSSION

The BVD is one of the most economically significant diseases of cattle worldwide due its direct effect on fertility, immunity as well as its role in respiratory disorders especially in young animals (OIE, 2008). BVDV is one of the important pathogens causing bovine respiratory disease complex (BRDC) which exerts a serious effect on cattle industry (Caldow and Nettleton, 2000).

Table 1: Detection of BVDV antigen using ELISA in pneumonic cattle lung tissue samples collected from three localities in Sudan during 2010-2013.

Area	Total tested	No. positive (%)
River Nile (Atbara)	145	20 (13.8)
Gezira (Wad Medani)	24	4 (16.7)
Kordofan (AlObied)	73	2 (2.7)
Total	242	26 (10.7)

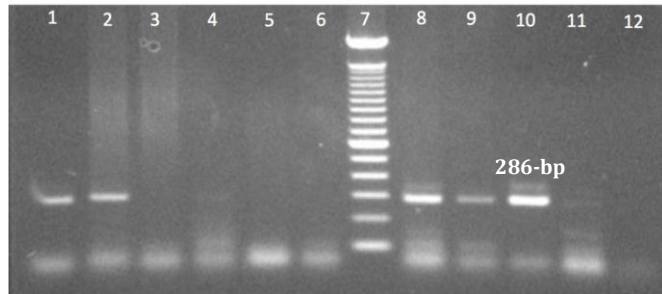


Figure 1: Gel electrophoresis of RT-PCR amplicon. Lanes 1, 2, 9 and 10, tested samples; Lanes 3-6, 11, ELISA negative samples; Lane 7, 100-bp DNA size marker, Lane 8, positive control; Lane 12, negative control.

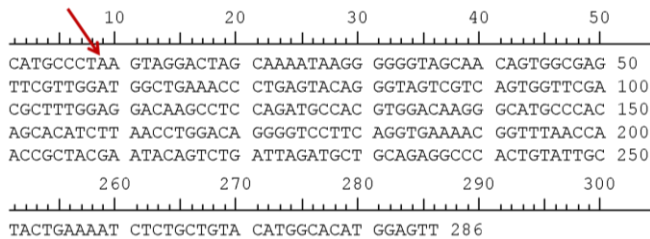


Figure 2: Nucleotide sequence of BDVD type 1 NS2-3 antigen originated from Sudan. All sequenced samples are identical to the Bovine viral diarrhea virus-1 (BVDV-1) sequence accession number (AF220247.1), except a nucleotide substitution at position 9; nucleotide A (arrow) was found in our study, instead, T was found in the reference virus.

In this study, the prevalence of BVDV in cattle was found to be 10.7% (n=26/242) (Table 1), reflecting the existence of this virus in cattle suffering from pneumonia; this could indicate the positive association of BVDV in respiratory infections in cattle. A comparable prevalence (6.2%) was detected in testing buffalo lungs in Iraq using ELISA (Khawlah and Saleem, 2012). Our study was based on slaughter houses survey to measure the magnitude of BVDV infections in cattle. This was inline with the findings of Galyean et al. (1999) who suggested that a valuable tool for monitoring bovine respiratory disease (BRD)

diagnosis and treatment would be the evaluation of lung lesions at slaughter. The highest prevalence (16.7%; n=4/24) was seen in the samples collected from Gezira state at Central Sudan; which was similar to the results of testing camel lungs (Intisar et al., 2010). It could be attributed to the location of this area at the center of Sudan where animals from different localities were passing either for pastures or trading leading to the spread of infections. The detected prevalence of BVDV antigen in this study was considered as moderate, although it was slightly higher than that of previous report in camels (7%), nevertheless it may indicate the wide spread distribution of this viral infection as clinically healthy animals were sampled in both studies. The role of BVDV in respiratory infections is well documented; Shahriar et al. (2002) demonstrated BVDV in 62.5% of examined heart and lung tissue collected from feedlot cattle with chronic pneumonia cases from Western Canadian feedlot cattle. Haines et al. (2004) detected BVDV in the tissues of 13 of 35 calves (37%) dying of pneumonia compared with 4 of 92 calves (4.3%) dying of myocarditis and in 0 of 45 calves (0%) dying of noninfectious causes.

FAT was used in this study to confirm ELISA results which correlated well with this known sensitive test. FAT is considered as one of the reliable techniques for the diagnosis of BVD; it was used to confirm ELISA results as well as virus isolation in cell culture (Collins et al., 2009, Zeitler and Rapp, 2013, Wang et al., 2014). This test was previously used to confirm ELISA results for detecting BVDV antigen in camel lungs (Intisar et al., 2010).

We used RT-PCR in this study for further confirmation of the serological tests used; the results correlated well with that of both serological tests used. All the 26 ELISA positive samples gave amplicon fragments of 286-bp (Figure 1). RT-PCR is known to be a highly sensitive tool for the diagnosis and characterization of BVD infections (Smith et al., 2008; Sharifzadeh et al., 2011; Booth et al., 2013). Sequence analysis indicated that the sequence is identical to the previously reported Bovine viral diarrhea virus-1 (BVDV-1) sequence accession (AF220247.1), except substituting a single nucleotide (Figure 2).

CONCLUSION

This work highlights the wide spread of BVDV infection in cattle in Sudan, and its possible association with respiratory infections. This is the first report of the detection of BVDV antigen, genome and its sequence analysis in cattle lungs in Sudan.

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CONFLICT OF INTEREST

All authors declare that none of them have any conflict of interest.

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