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An Outbreak of Pestivirus Infection in Sheep in West Kordofan, Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Author YHA designed the study, applied the serology laboratory work, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors IKS, applied the serology laboratory, RNA extraction and PCR work. Author AM collected samples, applied the bacteriological laboratory work. Author SBE applied the PCR work. Author FE managed the analyses of the sequencing data. All authors read and approved the final manuscript.

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ABSTRACT

During April- May 2010 an outbreak of a disease with obvious nervous and respiratory signs in sheep was reported in west Kordofan State in western Sudan. Four flocks at four different locations in the State were investigated. The deaths by the disease versus the total number of animals in the different areas were 18/150, 1/200, 3/280 and 17/300. The main clinical symptoms observed in adults and young lambs were central nervous signs and respiratory signs; most lambs were weak and emaciated with rough coats. Sera were collected from 11, 18, 19 and 4 of animals in the four

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flocks, respectively. Bacteriological examination on tissue samples did not reveal any positive results. Using competitive ELISA, antibodies against pestivirus were detected in 10/11, 7/18, 7/19 and 1/4, respectively of examined sheep sera in the different locations. RT/PCR using pestvirus specific primers to examine the lung and sera samples showed positive results for one lung and 17 sera samples, and sequence data indicated that the causative agent was Bovine viral diarrhea virus-1 (BVDV-1). This is the first report of BVDV-1 outbreak in sheep in Sudan.

Keywords: Pestivirus; outbreak; sheep; Sudan.

1. INTRODUCTION

Pestiviruses includes bovine viral diarrhea virus (BVDV) of cattle, border disease virus (BDV) of sheep, and classical swine fever virus (CSFV) of pigs. These viruses are typically isolated from primary host species, but are capable of infecting other species [1]. Under natural farming conditions there is no evidence so far of border disease viruses causing disease in cattle, but approximately 15% of outbreaks of BDV in the UK are caused by BVDV-1 viruses which probably originated from cattle [2].

BD was first reported in 1959 at the English/Welsh border. It is also called 'Hairy-shaker' or 'fuzzy-lamb' disease and has been recognized in most sheep-rearing areas of the world [3].

The disease is caused by BDV, which is closely related to CSFV and BVDV. It is a member of the genus Pestivirus in the family Flaviviridae [4].

Serological evidence of BDV in sheep sera (antigen and antibody) has been apparently studied worldwide, in Spain [5], Tunisia [6], Turkey [7] and Iran [8]. The first outbreak of Border disease in a sheep flock in Austria was described [9].

In Sudan, the sheep population is estimated to be 51.6 million [10]. Outbreaks of clinical disease with symptoms of border disease are continuously reported, however, no study had been conducted to investigate the existence of this disease in the country so far. In a recent study, we have detected pestivirus antigen using ELISA in 7 out of 98 samples from sheep lung collected from slaughter houses and in 5 out of 20 lungs of sheep collected from outbreaks of respiratory infections, abortion, diarrhea and nervous signs in Sudan (unpublished data). In a serological survey, we reported the first detection of antibodies to pestivirus in sheep (39.1%) and goats (14.8%) in different areas of Sudan [11].

Here we describe the first outbreak of pestivirus in sheep in Sudan.

2. MATERIALS AND METHODS

2.1 Area of the Outbreak

The outbreak was reported in four areas in West Kordofan State in western Sudan (Al Mafrya, Abu Hasheem, Huzeiran and Jabrat Elsheikh).

2.2 History of the Outbreak

During April-May 2010, which is the dry season where shortage in pasture and water is dominated in western Sudan, an outbreak of a disease with prominent nervous and respiratory signs had been reported to the veterinary authority from the fore mentioned areas. The total number of animals and the deaths of the disease in the different areas were 150 with 18 deaths, 200 with 1, 280 with 3 and 300 with 17, respectively. The main clinical signs observed in adults, especially in pregnant sheep and young lambs, were central nervous signs and respiratory signs; most of lambs were weak and looked emaciated with rough coats.

2.3 Collected Samples

The clinical signs (nervous and respiratory signs) were observed mainly in pregnant animals and young lambs. Most of samples were collected from clinically healthy animals with few sick ones. Samples were collected randomly from adult and young animals at 8 – 18 months of age.

A total of 52 serum samples were collected; 11 from Al Mafrya, 18 from Abu hasheem, 19 from Huzeiran and 4 from Jabrat Elsheikh.

Lung and brain tissues samples were collected from a recently dead young animal at Jabrat Elsheikh.

2.4 Screening of Collected Samples

2.4.1 Screening for PPR

PPR is the main causative agent of respiratory infections in sheep. peste des petits ruminants (PPR) antigen and antibodies were screened in collected samples using PPR specific ELISA kits (CIRAD, Montpellier, France).

2.4.2 Bacteriological examination

Collected tissue samples were screened for bacterial growth in different media.

2.4.3 Investigation of pestivirus antibodies

Collected sera were examined for pestivirus antibodies using competitive ELISA kits (BIO X Diagnostics, Jemelle, Belgium). The test was performed according to the instructions of the manufacturer.

2.5 Pestivirus Genome Determination

2.5.1 RNA extraction

RNA was extracted from serum samples using QIAamp and from brain and lung samples using RNeasy. Both kits were purchased from Qiagen, Germany.

2.5.2 RT/PCR

The extracted RNA samples were subjected to RT/PCR using One step RT/PCR Kits (Qiagen), and a pair of primers; 5'-CATGCCCWYAGTAGGACTAGC-3' and 5'-AACTCCATGTGCCATGTACAG-3' [12]. synthesized by Bioneer Corporation. The reverse transcription and thermocycling were carried out using a TC-512 (Techne) thermocycler. The conditions were, reverse transcription at 50°C for 30 minutes followed by 94°C for 15 minutes; thermocycling for 40 cycles at 94°C for 30 seconds, 55°C for 30 sec and 72°C for 30 sec; one cycle at 72°C for 10 minutes.

2.6 Analysis of RT/PCR Amplicon

2.6.1 Agarose gel electrophoresis

The amplified fragments were separated on a 1.5% agarose (Vivantis) gel using a horizontal mini-gel electrophoresis system (MSMINI, Cleaver Scientific). DNA bands were visualized by a gel documentation system (Ingenius, Syngene Bio Imaging).

2.6.2 Sequencing and sequence analysis

The amplicon fragments were isolated from the agarose gel, purified by gel purification kit (QIAGEN) and sent to Macrogene 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).

3. RESULTS

3.1 Screening for PPR

Neither antigen nor antibodies of PPR were detected in any tested samples.

3.2 Bacteriological Examination

Screening of collected tissue samples for bacterial growth in different media did not reveal any positive result.

3.3 Detection of Pestivirus Antibodies

Pestivirus antibodies were detected in 10/11, 7/18, 7/19, 1/4 of examined sheep sera in the different locations (Table 1).

Table 1. Detection of pestivirus antibodies using cELISA in sheep sera collected during pestivirus outbreak in Kordofan at western Sudan (2010)

Area	Total No. in the flock	No. tested	No. Positive	% positive	% positive in each flock
AlMafrya	150	11	10	90.9	6.7
Abuhasheem	200	18	7	38.9	3.5
Huzeiran	280	19	7	36.8	2.5
Jabrat Elsheikh	300	4	1	25	0.3
Total	930	52	25	48	

3.4 Pestivirus Genome Determination

3.4.1 Electrophoresis

One lung sample and 17 sera samples gave amplicon fragments of about 300 bp. The positive results for each area were, 7/19, 5/11, 4/18 and 1/4; bands are shown for the lung sample and four selected sera samples (Fig. 1).

3.4.2 Sequence analysis

All samples were identical in nucleotide sequence and length (286 nucleotide) after analysis with the BioEdit software package as shown for one sample (Fig. 2). Sequence analysis with the BLAST, indicated that the sequence is identical to the BVDV-1 (sequence accession number AF220247.1.), except for a single nucleotide substitution from A to T at position 9.

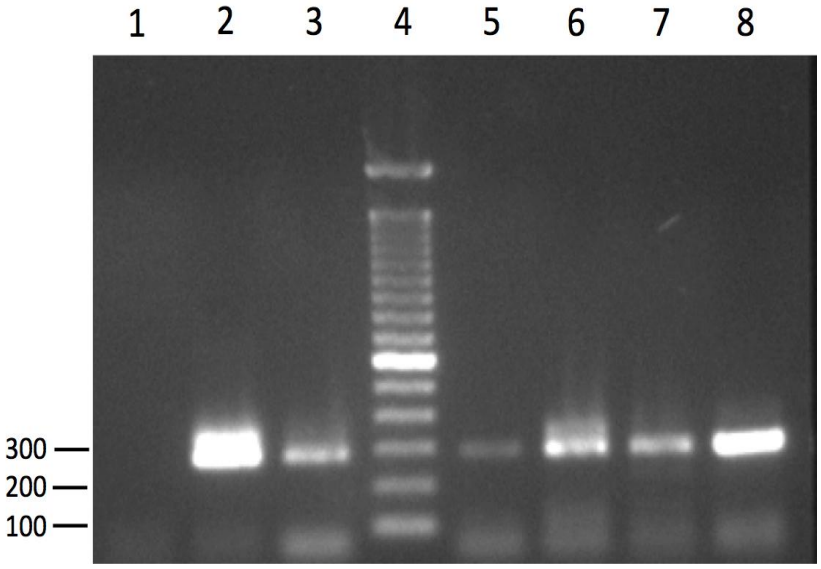

Fig. 1. Electrophoresis of RT/PCR amplified band

The amplicon yield is about 300 bp. in size. Lane 1: Negative control, Lane 2: positive control, Lane 3: lung sample, Lane 4: 100 bp DNA marker, Lane 5- 8: sera samples

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         10         20         30         40         50
CATGCCCTAA GTAGGACTAG CAAAATAAGG GGGGTAGCAA CAGTGGCGAG 50
TTCGTTGGAT GGCTGAAACC CTGAGTACAG GGTAGTCGTC AGTGGTTCGA 100
CGCTTTGGAG GACAAGCCTC CAGATGCCAC GTGGACAAGG GCATGCCCAC 150
AGCACATCTT AACCTGGACA GGGGTCCTTC AGGTGAAAAC GGTTTAACCA 200
ACCGCTACGA ATACAGTCTG ATTAGATGCT GCAGAGGCCC ACTGTATTGC 250
        260        270        280        290        300
TACTGAAAAT CTCTGCTGTA CATGGCACAT GGAGTT 286
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Fig. 2. The sequence of one sample

All sequenced samples were identical to BVDV-1 (sequence accession number AF220247.1) except for a single nucleotide substitution from A to T at position 9

4. DISCUSSION

In Sudan, viral infections are the major constrains for sheep production, due to their wide spread PPR and sheep pox draw the most attention, however, pestivirus causing similar clinical signs to PPR has been neglected. In this study, the determination of pestivirus as the causative agent for an outbreak of a disease with nervous and respiratory signs in sheep in western Sudan is documented. Screening for PPR and bacterial pathogens revealed negative results which supports the suggestion of pestivirus as causative agent for the outbreak. The observed clinical signs (nervous signs in lambs, weak lamb) were as previously described signs of pestivirus infection in sheep and goats [3]. The overall detected seroprevalence of pestivirus in affected sheep in this study was 48% with variable prevalence within the four studied areas (25–90%) which reflects the role of pestivirus in the investigated outbreak. This level is comparable to our previous results (41.6%) in Kordofan with an overall seroprevalence of 39.1% for pestivirus in Sudan [11]. Both studies highlighted the existence of pestivirus infection in sheep in Sudan, which was not considered before although high seroprevalence of pestivirus in camels in Sudan was reported previously [13].

Our results are matching with the global distribution of pestivirus infection; pestivirus antibodies have been detected in 46% of 42 sheep flocks examined in Ireland [14], 75.9% in 465 sheep in Turkey [15], 62.9% in Austria [16], 23.4% of 1777 sheep in India [17].

The detection of the pestivirus genome during outbreaks using RT/PCR is indicative for the existence of the virus and its role in the outbreak [18-23].

In this study, pestivirus genome could be detected using RT/PCR in 32.7% of 52 sera tested, as well as in lung tissue of newly died sheep; this confirms the role of pestivirus in causing the described outbreak. Sequence analysis of the PCR products indicated that the infection is due to BVDV-1. This is the first report of an outbreak of BVDV-1 infection in sheep in Sudan. This viral infection should be considered in investigations of epidemics as well as infertility diseases of sheep in Sudan.

4. CONCLUSION

This study investigated an outbreak of nervous and respiratory signs in sheep in West Kordofan State of Sudan. The causative agent of the outbreak was found to be BVDV-1. This is the first report of pestivirus outbreak in sheep and its sequence analysis in Sudan

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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