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Detection of Pestivirus in Pneumonic Sheep and Goats

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Detection of pestivirus antigen in pneumonic sheep and goats

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Abstract - The existence of pestivirus antigen in pneumonic sheep and goat lungs was investigated. A total of 382 pneumonic lung samples were collected from sheep (305) and goats (77) at slaughterhouses in five different areas in Sudan during 2010 – 2013. Collected samples were screened for pestivirus antigen using ELISA, 32 (10.5%) of sheep and 9 (11.7%) of goat samples were found to be positive. The highest prevalence (44.4%) was found in samples collected from Khartoum followed by Gezira in the center and Nyala in the west (20%). FAT was used to confirm the ELISA results. All ELISA positive samples were retested for pestivirus using PCR, with positive reaction. Sequence analysis indicated that the sequence of tested samples is identical to the Bovine viral diarrhea virus-1 (BVDV-1) sequence accession number (AF220247.1.), except a nucleotide substitution from A to T at position 9.The results confirmed the existence of BVDV-1 in pneumonic sheep and goat lungs reflecting a role played by this virus in pneumonia of small ruminants. This is the first report describing the detection of pestivirus antigen and nucleic acid and the sequence analysis of the circulating virus in small ruminants in Sudan.

Key words - Pestivirus, Antigen, Sheep, Goats, Pneumonia.

INTRODUCTION

Pestivirus genus is a member of Flaviviridae Family, it comprises the Bovine viral diarrhea virus 1 (BVDV-1), BVDV-2, Classical swine fever virus (CSFV), and Border disease virus (BDV) as well as a growing number of additional tentative pestivirus species isolated from domestic and wild ruminants and from pigs [1]. Pestiviruses are one of the economically important pathogens of animal production due to its direct effect on fertility as well as many other body systems. Clinical signs include barren ewes, abortions, stillbirths and the birth of small weak lambs. Affected lambs can show tremor, abnormal body conformation and hairy fleeces [2]. Pestivirus infection in sheep is distributed worldwide; in Turkey [3], [4], Spain [5], Iraq [6] and Japan [7]. The prevalence of pesrtivirus infection in goats was previously reported in Poland [8], Netherlands [9], Austria [10] and Egypt [11].

In Sudan, although clinical signs suggestive of pestivirus infection are reported annually [12] the disease was not

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considered in the diagnosis. We have recently presented the first report on evidence of pestivirus infection in small ruminants [13]. However no work was done to investigate the existence of pestivirus antigen in small ruminants either with pneumonia or infertility; this work is to elucidate the association of pestivirus with pneumonia cases in small ruminants using ELISA, FAT and RT/PCR.

MATERIALS AND METHODS

Collection of samples

A total of 382 pneumonic lung tissues were collected from sheep (305) and goats (77) at slaughterhouses in Atbara in the north, Khartoum, Gezira and While Nile in the center and Nyala in the west of Sudan during 2010 – 2013. Samples were kept at -20^{o} C till examined.

Detection of pestivirus antigen using ELISA

All collected lung samples (n=382) were screened for pestivirus antigen using ELISA Kits purchased from BIO X Diagnostics, Jemelle, Belgium. Preparation of samples and the test was applied according to the instructions of the manufacturer.

Detection of pestivirus antigen using Fluorescent antibody test (FAT)

All ELISA positive samples were tested for pestivirus antigen using FAT, BVDV specific conjugate was obtained from BIO X Diagnostics, Jemelle, Belgium.

Detection of BVDV using RT/PCR

All ELISA positive samples (n = 26) as well as five ELISA negative samples were examined for the detection of BVDV.

RNA extraction

RNA was extracted from 30 mg of pneumonic lung tissues, healthy sheep lung tissues (negative control), and from BVDV reference strain (positive control) using RNeasy Kit (QIAGEN).

RT/PCR

For the BVDV detection RT/PCR test was done using QIAGEN One step RT/PCR Kit using BVDV specific primer pair, P100 5'-CATGCCCWYAGTAGGACTAGC-3' and 5R 5'-AACTCCATGTGCCATGTACAG-3' [14]. A thermocycler (Techne, TC-512) with a heat lid was used. Briefly, to the kit reagents the forward and reverse primers (end concentration 0.6 µM) and 5 µl of the isolated RNA to a final volume of 25 µl was added and the following program was applied. Amplification conditions were, Reverse transcription in one step RT/PCR kit reagents at 50^{o}C for 30 minutes followed by 94^{o}C for 15 minutes this was followed by 40 cycles of PCR, denaturation at 94^{o}C for 30 seconds, annealing at 55^{o}C for 30 sec, and elongation of 72^{o}C for 30 sec, followed by final extension at 72^{o}C for 10 minutes.

AGAROSE GEL ELECTROPHORESIS

A minigel 8 cm long electrophoresis unit (MSMINI, Cleaver Scientific) was used. The reagents included 1.5% (w/v) agarose gel (Vivantis) in 0.5x Tris-Borate-EDTA (TBE) buffer, 1 µg/ml ethidium bromide in ddH2O, TBE running buffer, Blue/Orange 6X Loading Dye (Vivantis) 100 bp DNA size markers (Bioneer).

VISUALIZATION OF RT/PCR PRODUCTS

The electrophoresis separated DNA bands, were visualized and photographed by a gel documentation system (Ingenius, Syngene Bio Imaging).

SEQUENCING AND SEQUENCE ANALYSIS

The amplicon fragments of about 300 b.p were cut from the agarose gel, purified using 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).

RESULTS

Detection of pestivirus antigen

Using ELISA, pestivirus antigen was detected in 41 out of 382 tested samples (10.7%); the prevalence in sheep was found to be 10.5% while in goats it was 11.7%. Within localities, the highest prevalence (44.4%) was seen in samples collected from Khartoum, then Gezira and Nyala (20%), the details are presented in Table 1.

Detection of pestivirus antigen using Fluorescent antibody test (FAT)

FAT was used to confirm ELISA results for the detection of pestivirus antigen, all ELISA positive samples were found to be positive using FAT.

Detection of pestivirus using RT/PCR

All examined ELISA positive samples yielded two amplicon fragments of about 200 and 300 bp (Figure 1).

SEQUENCE ANALYSIS

All samples are similar in nucleotide sequence and length (286 nucleotide) after analysis with the BioEdit software package. Figure (2) shows the sequence of one sample. Sequence analysis with the BLAST, indicated that the sequence is identical to the Bovine viral diarrhea virus-1 (BVDV-1) sequence accession number (**AF220247.1**.), except a nucleotide substitution from A to T at position 9.

DISCUSSION

Pestiviruses are a group of pathogens known to cause several clinical manifestations in animals including abortion, stillbirth, diarrhea and respiratory disorders [15]. Clinical presentation varies depending on strain of virus, species of host, immune
status, reproductive status and age of host, and concurrent infections with other pathogens [16]. In Sudan according to the observations raised by veterinary

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clinicians, clinical manifestations similar to those caused by pestiviruses are reported in sheep and goats continuously (personal communication). The main suspected causative agents for such manifestations are peste des petits ruminants and brucellosis, meanwhile pestiviruses are not focused [12] although antibodies to BVDV were detected in 71% of examined cattle in Sudan three decades before [17]. This work aimed to investigate the existence of pestivirus in sheep and goat lungs with pneumonic lesions. In the present work the overall detected pestivirus antigen in tested sheep and goat lungs was 10.7%; the prevalence in sheep was 10.5% while it was 11.7% in goats. Similar results (10.4%) were reported in aborted sheep lungs in Turkey [18]; while in other study in Turkey, higher prevalence (69.2%) was found in lungs of aborted lambs [19] and in diarrheic goat tissues (23.3%) in Japan [20]. However pestvirus was detected only in 1.6% of Korean goats [21].

The prevalence of pestivirus detected in this report is slightly higher than that reported in camels in Sudan [22]. The infection in camels is most probably transmitted from sheep as both are mainly raised in close contact in most areas of Sudan.

Variable prevalence rates were noticed in different localities, the highest one (44.4%) was seen in samples collected from Khartoum state. This could be attributed to the continuous flow of large numbers of sheep and goats from different areas for marketing beside the extensive introduction of foreign breeds in the last two decades. The second highest prevalence was found in Gezira and Nyala, similar results were observed in camel lungs in Gezira, while in Nyala lower prevalence was observed [22]. This is expected as Gezira is located at the center of Sudan where large number of animals are reared beside animals from different areas are passing through it for trading and export as well as for natural pastures during rainy seasons. FAT was used in the present study to confirm ELISA results, pestivirus antigen was detected in all ELISA positive samples; FAT is known to be a sensitive technique and was used to confirm ELISA results for pestivirus [22], [23].

Pestivirus nucleic acid was detected in this study in all ELISA positive samples using RT/PCR; the technique was widely used for the diagnosis as well as for the typing of the virus [4], [24], [25]. Our results confirmed the existence of pestivirus in sheep and goats which will aid in the further molecular characterization to specify the type of the virus circulating in the country. Sequence analysis indicated that the detected viruses are identical to the Bovine viral diarrhea virus-1 (BVDV-1) sequence accession number (**AF220247.1**).This is the first report of the detection of pestivirus antigen and genome as well as its sequence analysis in small ruminants with pneumonia in Sudan. Serological, virological and molecular investigations on pestiviruses in small ruminants in Sudan are highly recommended and the diseases it causes should be considered in routine diagnosis of fertility, respiratory and gastrointestinal disorders.

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CONFLICT OF INTEREST

All authors declare that they do not have any conflict of interest.

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Table 1: Detection of pestivirus antigen using ELISA in pneumonic sheep and goat lung tissue samples collected from five areas in Sudan during 2010-2013

Area	Sheep			Goat			Total		
	Tested	+ve	%	Tested	+ve	%	Tested	+ve	%
River Nile (Atbara)	170	11	6.5	34	1	2.9	204	12	5.9
Gezira (Wad Medani)	40	9	22.5	10	1	10	50	10	20
White Nile (Rabak)	80	6	7.5	10	1	10	90	7	7.8
Khartoum	10	4	40	8	4	50	18	8	44.4
Nyala	5	2	40	15	2	13.3	20	4	20
Total	305	32	10.5	77	9	11.7	382	41	10.7

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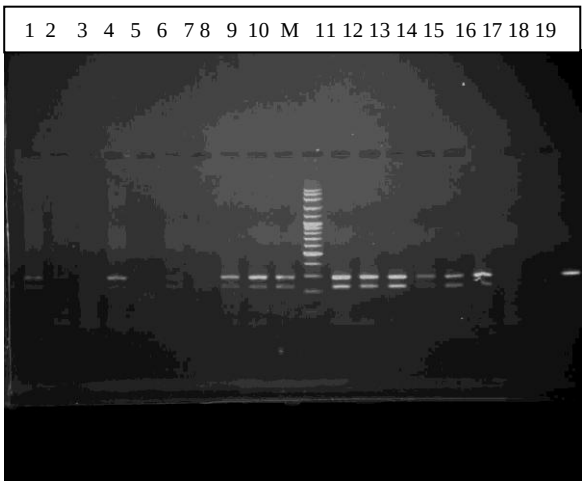

Figure 1: Gel electrophoresis of RT/PCR for pestivirus nucleic acid determination, Lane 1, 3, 4, 6- 10, 12 – 16, 19 tested positive samples, Lane 2– 5, 18, tested negative samples, M, 100 bp marker, Lane 11, positive control, Lane 17, negative control.

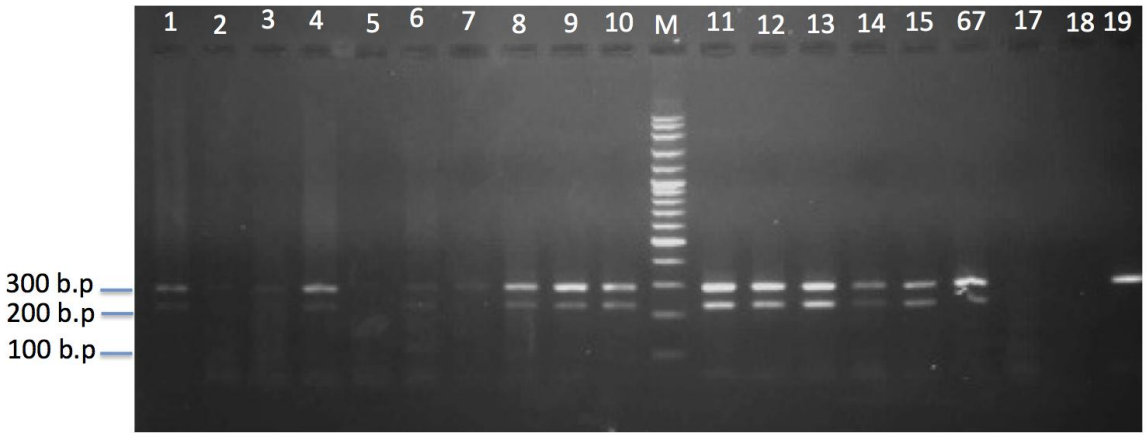

Figure 1: Gel electrophoresis for the RT/PCR amplicons. Lane 1, 3, 4, 6- 10, 12 – 16 and 19 tested positive samples; Lane 2– 5 and 18, tested negative samples; M, 100 b.p marker; Lane 11, positive control; Lane 17, negative control.

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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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Figure 2: The sequence of one sample. 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, from A to T.

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