Original Article

Para influenza virus 3 infection in cattle and small ruminants in Sudan

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ABSTRACT

Objective: This study was aimed at elucidating the association between Para influenza virus 3 (PIV3) and respiratory infections in domestic ruminants in different areas of Sudan.

Materials and methods: During 2010-2013, five hundred sixty five lung samples with signs of pneumonia were collected from cattle (n=226), sheep (n=316) and goats (n=23) from slaughter houses in different areas in Sudan. The existence of PIV3 antigen was screened in the collected samples using ELISA and Fluorescent antibody technique. PIV3 genome was detected by PCR, and sequence analysis was conducted.

Results: Positive results were found in 29 (12.8%) cattle, 31 (9.8%) sheep and 11 (47.8%) goat samples. All the studied areas showed positive results. Highest prevalence (66.7%) was detected in the sheep and goats in Khartoum, followed by in goats in Nyala (33.3%) at western Sudan. Sequence analyses of PIV3 of different regions of Sudan indicated that these were similar in sequence and length. The BLAST analysis indicated that the test sequences were closely related to the available annotated sequences at the GenBank. All these sequences matched with Bovine parainfluenza virus 3 except two those were matching with Swine parainfluenza virus 3.

Conclusion: The results prove the existence of PIV3 infection in cattle, sheep and goats in the studied areas in Sudan and suggest its possible role in the respiratory infections. Genetic analysis indicate that the virus is mostly similar with bovine PIV3.

KEYWORDS

http://bdvets.org/javar/

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INTRODUCTION

Respiratory infections are one of the main problems facing animal production as a result of its adverse effect on young animals (Smith, 2000). Viruses and bacteria are the major causes of respiratory infections where viruses are suspected to be the initiators of the infection (Valarcher and Hägglund, 2006). The main viruses encountered in respiratory infections are BVDV, BHV-1, BRSV, PIV-3 and BAV (Hägglund et al., 2007).

Para influenza virus belongs to the family *Paramyxoviridae* which is subdivided into two subfamilies: *Paramyxovirinae* including the genera *Respirovirus, Rubulavirus* and *Morbillivirus* and; *Pneumovirinae* including the genera *Pneumovirus* and *Metapneumovirus* (Murphy et al., 1999). The genus *Respirovirus* of subfamily *Paramyxovirinae* includes: BPIV 3, Human parainfluenza virus 1, Human PIV 3, Sendai virus, Murine PIV 1 and Simian virus 10 (Anon, 2006).

The existence of PIV3 in cattle is documented worldwide through the detection of its antibodies, in Iran, antibodies to PIV3 were found in 84.4% of 642 tested cattle sera (Shirvani et al., 2012). In a study in Saudi Arabia, seroprevalence of PIV3 in non vaccinated cattle was found to be 69% (Yousef et al., 2013). Other reports documented the detection of PIV3 antibodies in cattle, in Mexico (Figueroa-Chávez et al., 2012), Canada (Chamorro et al., 2014) and its isolation in China (Zhu et al., 2011). Saad (2002) detected PIV3 antibodies in 72.1% of sheep and 73.6% of goats, in Egypt. PIV3 antibodies were detected in 13.2% of 388 sheep and goat sera in Turkey (Yeşilbağ and Güngör, 2009) and in 11.7% of 196 tested sera in Japan (Giangaspero et al., 2013).

In Sudan, 1443 serum samples were tested for PIV3 antibodies; the presence of PIV3 antibodies was seen in all species, 58% of cattle, 36% of sheep, 32% of goats and 7% of camels (Eisa et al., 1979). Since that time no study was conducted to explore the presence of PIV3 in cattle and small ruminants in Sudan; however Intisar et al (2010) detected PIV3 antibodies in 82.2% out of 495 camel sera. This study is to determin the presence of PIV3 in pneumonic lung lesions of domestic ruminants as indicator for its role in the respiratory infections through the detection of the virus antigen and genome.

MATERIAL AND METHODS

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

Ethical approval: The investigation was carried out according to the animal welfare code in Sudan.

Sample collection: Lung tissue samples were randomly collected during the routine post mortem examination in the slaughterhouses. 565 lung samples with pneumonia were taken from 226 cattle, 316 sheep and 23 goats in the slaughterhouses at the three selected areas. Samples were kept on ice till sent to the Veterinary Research Institute at Khartoum where it were kept at –20°C till examined.

Detection of PIV3 antigen using ELISA: Collected samples were examined for the detection of PIV3 antigen using ELISA Kits (BIO X Diagnostics, Jemelle, Belgium). Samples Preparation and the test were applied as instructed by the manufacturer.

Detection of PIV3 antigen using Fluorescent antibody test (FAT): All samples tested positive by ELISA were tested for PIV3 antigen using FAT, the conjugate was obtained from BIO X Diagnostics, Jemelle, Belgium.

Detection of PIV3 genome using RT/PCR: All ELISA positive samples (n=71) as well as five ELISA negative samples were examined for the detection of PIV3 genome.

RNA extraction: RNA was extracted from 30 mg of lung tissue homogenate samples, PIV3 reference strain (National Veterinary Laboratories Service – USA, SF-4) as a positive control and healthy bovine lung specimen as negative control using QIAGEN RNeasy Kits.

RT/PCR: Using Qiagen one step RT-PCR Kit, a pair of primers targeting HA gene were used, the sequence of each was: PI3 A 5´-TGTGCATGGTGAGTTCGCA-3´, PI3 BR 5´-ATTCAGCATCACGTGCCACTG-3´, the expected amplicon size is 164-bp (Noori et al., 2014). 5 µL RNA Template was added into 45 µL mastermix in a 0.2 mL eppendorf tube. Eppendorf tubes were placed into thermocycler machine. The amplification conditions were, Reverse transcription in one step RT/PCR kit reagents at 50°C for 30 min then 94°C for 15 min. Following this, 40 cycles of PCR, denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and elongation of 72°C for 30 sec, final extension at 72°C for 10 min were done.

Agarose Gel Electrophoresis: Agarose gel in TBE1.5% (w/v); buffer, 1 µg/mL ethidium bromide, TBE running buffer, Loading Dye and 100-bp DNA size markers were used.

Visualization of RT/PCR amplicon fragments: Visualization and photography of the separated DNA bands by electrophoresis were done by a gel documentation system (Ingenius, Syngene Bio Imaging).

Sequence analysis: Some of PCR products (n=22), includes 8 camel, 7 sheep, 5 cattle, 2 goat samples were sent for sequence analysis. After cutting the amplicon fragments from the agarose gel, it was purified using gel purification kit (QIAGEN, USA), 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

Prevalence of PIV3 antigen in cattle: Using ELISA, out of 226 cattle lung samples, 29 (12.8%) were tested positive for PIV3 antigen, 16.7% (highest prevalence) was seen in samples collected from Gezira State (Table 1). Out of 316 sheep lungs tested, 31 (9.8%) and 11 of 23 (47.8%) of goat samples tested positive with the highest overall prevalence of 66.7% in samples collected from Khartoum State (**Table 2**).

Table 1: Detection of PIV3 antigen using ELISA in pneumonic cattle lung tissue samples collected from three localities in Sudan during 2010-2013.

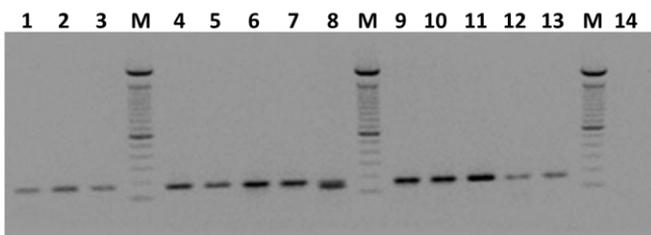| Area	Total tested	No. positive	% +ve
River Nile (Atbara)	145	20	13.8
Gezira (Wad Medani)	24	4	16.7
Kordofan (AlObied)	57	5	8.8
Total	226	29	12.8

Figure 1: Gel electrophoresis for the RT-PCR amplicons. Lane M: 100-bp DNA size marker. Lanes 1-5: sheep samples; Lanes 6–8 and 10: cattle samples, Lane 9: positive control; Lane 11-13: goat samples; Lane 14: negative control. The amplicon sizes were in the range between 150 and 200-bp.

Detection of PIV3 antigen using Fluorescent antibody test (FAT): To confirm ELISA results, all positive (n=71) samples for PIV3 antigen examined positive using FAT.

Detection of PIV3 genome using RT/PCR: On applying RT/PCR on ELISA and FAT positive samples (n=66), the PCR-amplicon size on agarose gel was in the range between 150 and 200. **Figure 1** shows some of these samples.

Sequence analysis: Analysis of the sequences using BioEdit software package indicated that they are similar in sequence and length (~164-bp). The BLAST analysis indicated that the test sequences are closely related to the available annotated sequences at the GenBank including KJ647288.1, EU439428.2, DQ166823.1, AB770485.1, AB770484.1, Y00114.1, D84095.1, KP757872.1, KJ647289.1 and JQ063064.1. All these sequences are from Bovine parainfluenza virus 3 except EU439428.2 and DQ166823.1 which are from Swine parainfluenza virus 3. **Table 3** illustrates the alignment results.

DISCUSSION

Respiratory infections have a major economic importance in animal industry. In a study in Ethiopia, pneumonia was reported as the most important disease affecting calves and heifers (32.5%) while respiratory problems were diagnosed in 63.5% of sheep and goats (Moges and Bogale, 2012). Co infection with other respiratory viruses and bacteria often complicate BPIV infection, which is supposed to be one of the causes of enzootic pneumonia in calves and bovine respiratory disease complex in feedlot cattle (Ellis, 2010). PIV3 infections were reported in cattle, humans, sheep (Lyon et al., 1997), goats (Yener et al., 2005) and buffalo (Maidana et al., 2012). In this work, PIV3 antigen was detected in 12.8% of tested cattle, 9.8% of sheep and 47.8% of goat lung samples. The results were in line with a report describing the detection of PIV-3 viral antigens in 44.4% of goat lungs using FAT (Çeribasi et al., 2012), in 25% of buffalo's lungs (Zaher et al., 2014) and in 30.3% of cattle lungs using ELISA (AL-abudy and Alrodhan, 2014).

The detection of PIV3 antigen in different species is expected due to the close contact between animals during pasture and watering in most areas of Sudan which increases the rate of infection. It was noticed from this study that PIV3 is more prevalent in goats than in sheep; similar observation was previously reported (Yeşilbağ and Güngör, 2009), this could be attributed to the management system of goats where it is kept freely

Table 2: Detection of PIV3 antigen using ELISA in pneumonic sheep and goat lung tissue samples collected from three localities in Sudan during 2010-2013.

Area	Sheep			Goat			Total		
	Tested	+ve	%+ve	Tested	+ve	%+ve	Tested	+ve	%+ve
River Nile (Atbara)	208	13	6.3	0	0	0	208	13	6.3
Gezira (Wad Medani)	51	8	15.7	0	0	0	51	8	15.7
White Nile (Rabak)	53	8	15.1	0	0	0	53	8	15.1
Khartoum	4	2	50	8	6	75	12	8	66.7
Nyala	0	0	0	15	5	33.3	15	5	33.3
Total	316	31	9.8	23	11	47.8	339	42	12.4

Table 3: BLAST analysis of the test sequences and their similarity to annotated GenBank sequences.

Accession*	Identities	Gaps
KJ647288.1	155/164(95%)	0/164(0%)
EU439428.2	154/164(94%)	0/164(0%)
DQ166823.1	154/164(94%)	0/164(0%)
AB770485.1	153/164(93%)	0/164(0%)
AB770484.1	153/164(93%)	0/164(0%)
Y00114.1	153/164(93%)	0/164(0%)
D84095.1	153/164(93%)	0/164(0%)
KP757872.1	151/164(92%)	0/164(0%)
KJ647289.1	151/164(92%)	0/164(0%)
JQ063064.1	151/164(92%)	0/164(0%)

All sequences are from Bovine parainfluenza virus 3 except, EU439428.2 and DQ166823.1 which are from Swine parainfluenza virus 3.

outdoors in small numbers and usually are collected in large groups to search for pasture which increase the possibility for the spread of infection. The prevalence of PIV3 antigen was the highest in goats, then cattle, although higher prevalence (25%) was previously detected in cattle in Sudan (Noori et al., 2014); both are higher than that previously reported (2.1%) in camel lungs in Sudan (Intisar et al., 2010). However, these results are comparable to that reported in Sudan by Eisa et al (1979). The results of this work reflect the existence of this viral infection in different studied areas; highest prevalence was seen in Gezira at central Sudan. This is not unusual as this area is rich in animal resources and it has a relatively high rainfall, hot weather in summer and slightly cold in winter during which the rate of respiratory infections are known to increase (Valarcher and Hägglund, 2006).

In this study, the detection of PIV3 in the different three species was confirmed using PCR which was documented as a more sensitive technique (Zaher et al., 2014, Veljovac et al., 2014).

Sequence analysis of the PCR products revealed that the sequences have identity to the Bovine parainfluenza virus 3 ranging from 95-93% and to 94% of Swine parainfluenza virus 3. These results could be attributed to the fact that, RNA viruses have high mutation and recombination rates (Murphy et al., 1999). The existence of bPIV3 of different genotypes was documented by sequencing in many countries (Wen et al., 2012; Oem et al., 2013).

CONCLUSION

It was concluded from this study that PIV3 infection widely exists in ruminants in the studied areas of Sudan and it is causing economic losses due to its associated respiratory infection. Further study for sequencing of complete PIV3 genome circulating in different species in Sudan is recommended.

CONFLICT OF INTEREST

None of authors have any conflict of interest.

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