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Outbreaks of Peste des petits ruminants in two different localities in Sudan

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This report describes the details of outbreaks of Peste des petits ruminants (PPR) which occurred during 2008 in Khartoum and River Nile States in Sudan. An outbreak was reported in a sheep flock at the southern part of the State (Soba) in Khartoum, with 100% morbidity and mortality rates. In EIDamer city at River Nile State, the morbidity rate was 31.4% while mortality rate was 17.1% in the total animal population of 3,500. PPR antigen was detected in seven samples from both outbreaks using immunocapture enzyme-linked immunosorbent assay (IcELISA). The PPR ELISA results were confirmed by reverse transcription polymerase chain reaction (RT-PCR). Using cELISA, only one serum was sent from Khartoum and 6 out of 13 sera from EIDamer were positive for PPR antibodies.

Key words: Peste des petits ruminants (PPR), outbreaks, sheep, Sudan.

INTRODUCTION

Peste des petits ruminants (PPR) disease is caused by a virus that belongs to the genus Morbillivirus of the family paramyxoviridae (Murphy et al., 1999). The disease is characterized by onset of depression, fever, discharges from the eyes and nose, sores in the mouth, disturbed breathing, cough, foul smelling diarrhoea and death (Roeder and Obi, 1999). PPR was first reported in West Africa and is now known to be reported in different Asian countries including Oman (Furley et al., 1987), Lebanon (Lefevre et al., 1991) and India (Shaila et al., 1989).

In Sudan, El Hag Ali and Taylor (1984) reported the first outbreak of PPR. Since then many outbreaks of PPR in Sudan had been reported; in Darfur State (Rasheed, 1992), El Hilalia area of Central Sudan (Hassan et al., 1994),

in Khartoum (Zeidan, 1994). Intisar (2002) reported the antigenic prevalence of PPR in Khartoum, Gezira, River Nile, Kordofan and White Nile states. This report describes the occurrence of PPR outbreaks in Khartoum and River Nile State which was until recently considered as PPR free zone.

MATERIALS AND METHODS

Area of outbreaks

They include Soba city at Khartoum and EIDamer city at River Nile states, the former is the capital of Sudan and the second is bordering Khartoum to the North.

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Table 1. Detection of PPR antibodies in sheep sera collected during PPR outbreaks in Khartoum and River Nile States, Sudan during February 2008.

Area	Number tested	Number positive	Number negative	Percentage positive
Khartoum	1	1	0	100
River Nile (El Damer)	13	6	7	46.1
Total	14	7	7	50

Description of the outbreaks

In Soba area at the south of Khartoum State during the last week of January, 2008, a total of 20 sheep aged 9 to 15 months were brought to a farm for breeding, a week later, respiratory signs, diarrhoea and weakness appeared and within 2 weeks all the 20 animals died. On post mortem (PM), tissue samples (lung, spleen, lymph nodes) were collected and serum were collected from the last surviving ones immediately before death and sent to the Central Veterinary Research Laboratory for diagnosis. In ElDamer city at River Nile State during April, 2008, a flock of sheep (n = 3500) showed cases of diarrhoea, off food, respiratory signs and death. The affected ones were 1,098 (31.4% morbidity rate) and dead ones were 600 (17.1% mortality rate); the case fatality rate was 54.6%. Tissue samples (lung, spleen, lymph nodes of 6 animals) and 13 sera were collected and sent to the Central Veterinary Research Laboratory at Khartoum for examination. In both outbreaks, signs of pneumonia and gastroenteritis were observed on post-mortem examination. All animals in both outbreaks were not previously vaccinated against PPR.

Detection of PPR antigen

A total of 21 samples from both outbreaks were tested for PPR antigen using immunocapture enzyme-linked immunosorbent assay (IcELISA) as per the instructions of the manufacturer (CIRAD EMVT, Montpellier, France, distributed by BDSL, UK). It is a solid phase immunocapture ELISA (ICE) based on a technique described by Libeau et al. (1994).

Detection of PPR using RT-PCR

To confirm the results detected by IcELISA, clear PPR positive samples from both outbreaks (n = 7) were examined for molecular based confirmation by using RT-PCR. RNA was extracted using QIAGEN RNA extraction Kit, the procedure was applied as per the procedure supplied by the manufacturer. The PPRV nucleic acid was detected in the clinical tissue material by RT-PCR using two sets of primers following the method described by Kwiatek et al. (2007). Reagents used for PCR were obtained from Invitrogen, Germany. First the Pan-morbillovirus primers located in the middle of the gene (Nad1 and Nad2) allowed the amplification of the nucleoprotein (N) gene of all morbilliviruses and gave a product of 222 bases. The second pair of primers (NP3 and NP4) designed by Couacy-Hyamann et al. (2002) on highly conserved sequence of PPRV N gene gave a 351 bp product amplification. Amplification was done on a thermal cycler following the described conditions: reverse transcription 30 min at 50°C, initial PCR activation during 15 min at 95°C then 40 cycles of amplification corresponding to 30 s at 94°C/30 s at 60°C/1 min at 72°C and final extension during 10 min at 72°C.

Detection of PPR antibody

Sera collected during both outbreaks (n = 14) were tested for PPR antibodies using cELISA manufactured by CIRAD EMVT, Montpellier, France, distributed by BDSL, UK. The test is based on the competition between antibodies in sera and monoclonal antibody (MAb) to bind to the antigen (Libeau et al., 1995).

RESULTS

Detection of PPR antigen

PPR antigen was detected using IcELISA in 7 samples (lungs) of the two outbreaks.

Detection of PPR by molecular assay RT PCR

PPR was confirmed by RT PCR assay in all the seven lung samples positive for PPR antigen during the outbreaks (Figure 1).

Detection of PPR antibodies

Using cELISA, PPR antibodies were detected in 7 of 14 sera, the details are presented in Table 1.

DISCUSSION

PPR is known to be one of the most serious viral diseases affecting small ruminants; continuous outbreaks of the disease are reported in many countries. PPR has been existing in Sudan since the first reported outbreak in 1971 (El Hag, 1973) which was first diagnosed as rinderpest and later confirmed to be PPR (El Hag Ali and Taylor, 1984). During the last two decades, PPR was reported in different localities in Sudan; in sheep in Western Sudan (Rasheed, 1992); in sheep and goats in Central Sudan (Hassan et al., 1994) and in sheep and goats in Khartoum State (Zeidan, 1994; El Amin and Hassan, 1998). Until recently, River Nile State was known to be a PPR free area, however the disease was detected and the virus was isolated from River Nile State as well as from

Figure 1. Detection of PPRV using PCR. Amplification is generated by primers NP3 and NP4 designed by Couacy Hymann et al. (2002) on highly conserved sequence of PPRV N gene giving a PCR product of 351 bp.

Lanes 1 and 12: DNA molecular weight marker (#100 bp ladder, Eurobio); Lanes 2, 10 and 11: Positive control (PPR 75-1 vaccine strain); Lane 3: Negative control; Lane 4: sample No 1 (Khartoum); Lanes 5 to 9, samples No 2 to 6 (ElDamer).

from different parts of Sudan [Gezira, White Nile (Central), Kordofan (Western), Khartoum] between 2000 and 2002 (Intisar, 2002).

PPR which was restricted to African countries have spread to Asia. Kwiatek et al. (2007) described an outbreak of PPR in 3 districts in Tajikistan. Similarly, Ahmad et al. (2005) reported an outbreak of PPR in goat flock in Pakistan. By using cELISA they found 35 sera tested for PPR antibodies to be positive. A severe outbreak of PPR was reported in 70 adult sheep and goats in Al-Hasa province of Saudi Arabia. Thirty out of seventy animals in the herd were affected (43% morbidity rate). The case mortality rate was 100% (Housawi et al., 2004); this picture is close to that seen in River Nile State in this report but less severe than that noticed in Khartoum. In Africa, During August, 2008 an outbreak of PPR was reported in Morocco; the outbreak has largely affected sheep, with 133 outbreaks in 29 provinces (FAO, 2008). In Ethiopia, PPR antibody seroprevalence was 3% in camels, 9% in cattle, 9% in goats and 13% in sheep (Abraham et al., 2005), although Ethiopia is bordering Sudan, this is considered a very low prevalence compared to our results.

The reported outbreaks in this study were the most severe ones in Sudan, especially in Khartoum, in which the morbidity and mortality rates were 100%, this is far higher than the previous reports in Sudan and is comparable to the previous report by Nussieba et al. (2008) who detected PPR antigen in 92.5% of 40 tissue

samples of sheep in the Sudan during an active outbreaks, and most of samples ($n = 32$) were from the same flock at Khartoum. This report points to the existence of virulent PPR virus in Khartoum and River Nile States; the characterization and sequence analysis of PPR virus of the two outbreaks revealed the existence of Lineage IV PPR for the first time in Sudan (Kwiatek et al., 2011).

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Conflict of Interest

The authors declare that they have no conflict of interests.

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