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# Existence of Pesti Des Petits Ruminants in Camels

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**Abstract**

PESTI DES PETITS Ruminants (PPR), a significant disease of sheep and goats was proved to be extended to camels. The present study is to elucidate the spread of the infection in camels in three different localities of Sudan through the detection of the viral antigen by Ic-ELISA and genome by Rt-PCR. During routine inspection, tissue samples collected from 200 camel lungs had showed pneumonic lesions at slaughter houses in Atbara at River Nile State, Northern Sudan, AlObied at Kordofan State, Western Sudan and Tampool at Gezira State at Central Sudan. Tissue samples were collected on ice in sterile containers, sent to Virology laboratory at Central Veterinary Research Laboratory, kept frozen till be tested. Using Ic-ELISA, ninety-six samples (48%) were positive for PPR antigen. The highest prevalence (58.2%) was seen in Tampool at Central State, then (40%) in Atbara at River Nile State and 23.3% in Kordofan State. All reactive subjects by Ic-ELISA were positive by RT-PCR. The results indicated the wide spread of PPR infection in camels in Sudan, thus a study to explore the role of camels in the transmission of PPR is highly recommended.

**Keywords:** Camel, PPR, Ic-ELISA, RT-PCR.

## Introduction

Peste Des Petits Ruminants (PPR) is one of most serious life threatening viral diseases of small ruminants, it is caused by PPR virus (PPRV), a morbillivirus genus of paramyxoviridae; causing an acute disease characterized by high fever, pneumonia, oral lesions and diarrhea in small ruminants [1]. PPR is mainly a disease of small ruminants, great losses in sheep and goats are reported in Africa and Asia [2]. PPR is existing in almost all countries bordering or near to Sudan, in sheep and goats in Chad [3], Kenya [4], Ethiopia [5], South Sudan [6] and Egypt [7]. In Asia PPR is also wide spread, where outbreaks were reported in India, during 1995 – 2019, 8168 outbreaks were reported, 3844 outbreaks in goats and 3473 in sheep while 851 outbreaks were in shared sheep and goat flocks [8]. In Sudan, PPR outbreaks occur annually in different areas [9, 10].

Serological and genetic evidence for the occurrence of PPR infections in large animals and camels has been documented [11, 12]. The first report of a highly contagious PPR outbreaks with respiratory signs in camels was in Ethiopia [13], in Sudan [14] and in Kenya as a disease in camels called "Camel Sudden Death Syndrome [15]. Recently, experimentally, PPR infection was found to occur in camels and it can transmit the disease to sheep and goats [1]. Most of the published work about the incidence of PPR in camels is based on serological evidence through detecting antibodies against the virus. This was seen in Ethiopia [16], Sudan [10, 17], Libya [18], Nigeria [19, 20], India [21, 22] and Saudi Arabia [23]. This study is to elucidate the existence of PPR in camels in Sudan through the detection of its antigen and genome.

## Material and Methods

*Ethical approvals:*

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This is an abattoir-based study, samples were collected during the routine meat inspection in slaughterhouses, and no direct contact with live animals was adopted. The study was approved by the Department of Virology and the Administration of the Central Veterinary Research Laboratory (ethical approval number, 15/10/2015).

*Collection of samples*

During the routine meat inspection process, 200 tissue samples from the lungs in slaughterhouses showing pneumonia lesions were collected in 2016-2019. The samples were collected from Tampool in Gezira State (n =115), Atbara in River Nile State (n = 55) and AlObied in Kordofan State (n =30) (Fig.1).

*PPR antigen detection using Ic-ELISA*

Lung tissue samples (n =200) were tested for detection of PPR antigen using immunocapture ELISA (Ic-ELISA) Kits kindly provided by Genevieve Libeau of CIRAD (Montpellier, France). Kits were carried out according to the instructions of the manufacturer.

*RT/PCR*

*Extraction of RNA*

The total RNA was purified using Qiagen RNeasy kits, according to the protocol provided by the producer. *Microbiological analysis*

*Amplification of the nucleic acid*

Some of the Ic-ELISA reactive samples (n =8) were tested for PPRV genome using Qiagen One-Step RT-PCR kit which join the cDNA and amplification steps with NP3 and NP4 primers to amplify a 352-bp fragment of the PPRV nucleoprotein gene as published by [24]. For the amplification, 5µl of RNA was added to a 45-µl reaction mix containing: 10µl of 5× Qiagen buffer, 2µl of dNTP mix, NP3 and NP4 primers (final concentration of 0.6µM), 10µl of Q-solution, and 2µl of One-Step RT-PCR mix. Thermal cycler conditions: reverse transcription for 30min at 50°C, initial PCR activation for 15min at 95°C, then 40 cycles of amplification corresponding to 30s at 94°C/30 s at 60°C/1 min at 72°C. and a final extension of 10 min at 72°C. Finally, 10µl of the amplicons were separated in 1.5% agarose gel in comparison with the control.

## Results

*PPR antigen detection*

Using Ic-ELISA, ninety-six samples (48%) were positive, the highest prevalence was found in Tampool (58.2%) (Table 1).

*Rt/PCR*

All ribonucleic acids (n = 8) under test gave positive result for the nucleoprotein gene (NP gene). Strong bands were detected in the ethidium bromide stained gel that correspond exactly to the expected band size (~352-bp) (Fig. 2).

## Discussion

PPR is one of the most devastating viral diseases of small ruminants. The disease is widely distributed in small ruminants in Africa and Asia. Confirmation of the disease existence is applied either through serological techniques for antigen and/or antibody detection and nucleic acid detection [25, 26, 27]. PPR viral RNA was detected in vaccinated animal tissues as well as in sera for the first time [28].

Many reports documented the confirmation of the disease through the detection of viral antigen in sheep and goats using ELISA, it was detected in 49% of sheep and goat tissues in India [29], Sudan [30]. More recently molecular techniques were used for confirmation of PPR infection, in Bangladesh [31], Kenya [15], West Africa [32] and Ethiopia [33].

In this study, the overall detected PPR antigen in camel lung tissues was 48%, this is close to the previously reported results (45%) in Sudan [34] but higher than the results obtained (34%) in other report [35]. Within localities, highest antigen prevalence (58%) was in Tampool at Central State which is close to results (60%) obtained [34] but far higher than those reported (19%) in the same area [35], however our results and the previous reports were lower than the recently obtained results in the same area (98%) using haem-agglutination (HA) test [36]. The second most obtained positivity (40%) was in Atbara at River Nile State, Northern Sudan, compared to the previous studies it is lower than the reported figures (49%) [35] and (57%), [34]. Lowest antigen positivity in our study (23.3%) was in Kordofan at Western Sudan, nevertheless it is much higher than the previously reported results, 10% [35] and only 3% [34].

Our results were far higher than that reported in camels in India [22], this could be attributed to the type of samples used (nasal and rectal swabs) where PPR antigen is more likely to be found in lung samples. The detected PPR antigen in camel lungs in this study as well as other reports, regardless to the variable positivity rates obtained indicates the wide spread of this infection in camels in Sudan which supports the same picture in other countries, Iran [37] and India [22].

Work documenting the confirmation of PPR infection through the detection of antibodies against the virus in camels were published. Variable seroprevalence rates were reported in camels, 39% of camel sera tested positive for morbillivirus antibodies in Nigeria [19], 23% in Libya [18], 19% in Pakistan [38], in Sudan 7% [17], 0.3% [14], 2% [35], India, 4.5% [21], and 8.5% [22]. Low PPR seroprevalence

(3%) was detected in camels in Saudi Arabia [23], Nigeria [20], Tanzania [39] and Ethiopia [16].

Recently, confirmation of PPR is applied using RT-PCR, Outbreak of PPR was confirmed using Ic-ELISA and RT-PCR in Iran in camels imported from Kuwait [37] and India [22]. PPR outbreak in camels in Kenya was confirmed using RT/PCR [15]. In this study RT/PCR was applied to confirm the existence of PPR in camel lung tissues, all Ic-ELISA positive samples were found to be positive, and this is in agreement with the previous reports [1, 3].

## Conclusion

It was concluded that PPR infection is of wide spread in camels, investigation of the disease in this species is of importance to aid in its control in small ruminants.

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*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

*Ethical of approval*

The study was approved by the Department of Virology and the Administration of the Central Veterinary Research Laboratory (ethical approval number, 15/10/2015).

**TABLE 1. PPR antigen detection in camel lung tissues using Ic-ELISA**

| Area | No. Tested | No. +Ve | % |
|---|---|---|---|
| Atbara | 55 | 22 | 40 |
| Kordofan | 30 | 7 | 23.3 |
| Tampool | 115 | 67 | 58.2 |
| Total | 200 | 96 | 48 |

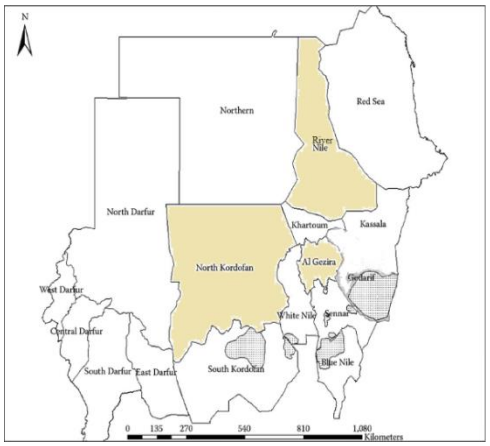


**Fig.1. Map of the Sudan showing the location of the study including River Nile state, North Kordofan and Gazira.**

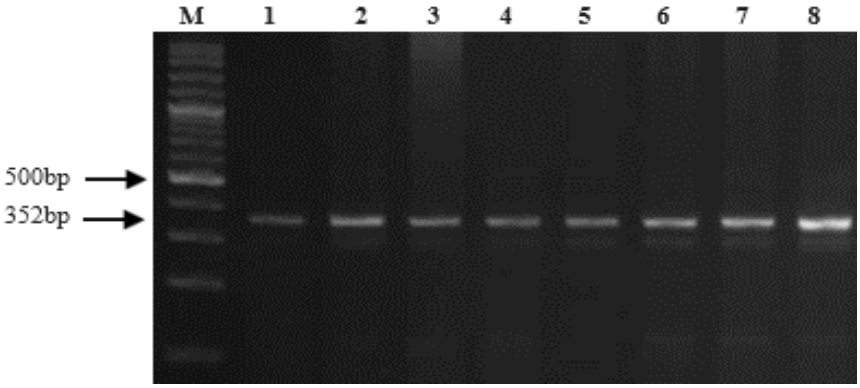


**Fig. 2. Ethidium bormide stained agarose gel (1.5%). RT-PCR was carried out on cDNA samples extracted from camel lung tissues. Lane M: 100bp ladder, Lane 1-3: Tissues from Atbara, Lane 4-5: Tissues from Kordofan, Lane 6-7-: Tissues from Tampool, Lane 8: Control positive cDNA.**

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## وجود طاعون المجترات الصغيرة في الابل

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**الملخص**

طاعون المجترات الصغيرة مرض مؤثر في الضان و الماعز و قد ثبت امتدادة الي الابل. تم تصميم الدراسة الحالية لاستكشاف مدي انتشار المرض في الابل في ثلاثة مناطق مختلفة في السودان عن طريق الكشف عن وجود مستضد الفايروس باستخدام اختبار الاليزا و تقنية الاحياء الجزيئية باختبار البلمرة التسلسلي. اثناء عمليات فحص اللحوم الروتينية في المسالخ من عدد 200 من الابل تم جمع عينات انسجة من الرئة التي يظهر فيها علامات الالتهاب الرئوي في المسالخ بمدينة عطبرة بولاية نهر النيل بشمال السودان و مدينة الابيض بولاية شمال كردفان بغرب السودان و مدينة تمبول بولاية الجزيرة بوسط السودان. تم جمع عينات الانسجة في حافظات معقمة و وضعت في الثلج و نقلت للمعمل المركزي للبحوث البيطرية في الخرطوم و تم حفظها مجمدة لحين فحصها. باستخدان اختبار الاليزا تم الكشف عن وجود مستضد الفايروس في عدد 96 (48%) من العينات و وجد اعلي معدل حدوث (58.2%) في العينات من مدينة تمبول بوسط السودان تليها مدينة عطبرة (40%) بولاية نهر النيل ثم مدينة الابيض بولاية شمال كردفان (23.3%). جميع العينات الايجابية باختبار الاليزا و جدت ايجابية باختبار البلمرة التسلسلي. اثبتت النتائج االانتشار الواسع لعدوي مرض طاعون المجترات الصغيرة في الابل في السودان. توصي الدراسة بضرورة اجراء دراسة لاستكشاف دور الابل في نقل مرض المجترات الصغيرة.

**الكلمات الدالة:** الابل، طاعون المجترات الصغيرة، الاليزا، اختبار البلمرة التسلسلي.