Sero-prevalence of peste des petits ruminants virus antibodies in sheep and goats from the Sudan, 2016–2017

Nussieba A. Osman¹ · Hashim M. A. Ibrahim^{1,2} · Alwia A. Osman³ ·
Rihab M. Alnour³ · Omiema A. Gamal Eldin³

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Abstract Recently, severe outbreaks of PPR among small ruminants were reported regularly in different parts of the country leading to significant economic losses. Between 2016 and 2017, a total of 320 sera were collected from sheep (258) and goats (62) from PPR suspected outbreaks from four different States in the Sudan. Screening of sera for the presence of PPRV antibodies by competitive ELISA revealed an overall antibodies sero-prevalence of 80.9% (259/320, 95% CI 20.5–28) among sheep and goats. On the species basis, sheep sera yielded the higher antibodies sero-prevalence of 84.5% (218/258, 95% CI 16.7–24.1) compared to a lower sero-prevalence of 66.1% (41/62, 95% CI 28.5–51.1) obtained from goats sera. Within Sudanese States where outbreaks occurred, the highest overall sero-prevalence of PPRV antibodies in sheep and goats was demonstrated in River Nile State 90.3% (159/176 sera, 95% CI 12.8–18.2) while the lowest incidence was present in Northern State 00.0% (0/2 sera, 95% CI 69.9–72.2). Of note, higher sero-prevalence values were achieved in this study than previously documented. Results of the present study indicated that PPR is currently circulating widely in the Sudan and still is a leading cause of disease outbreaks and higher fatalities in small ruminants. Therefore, the

effective PPR vaccine is recommended to cover all parts of the Sudan to prevent the occurrence of disease outbreaks.

Keywords Peste des petits ruminants (PPR) · Sudan · Sheep · Goats · PPRV antibodies · Sero-prevalence

Introduction

Peste des petits ruminants (PPR), goat plague, is an infectious, transboundary viral disease affecting mainly domestic small ruminants “sheep and goats” beside many wild small ruminant species and camels [19, 22]. PPR is considered as one of the notifiable diseases to the World Organization for Animal Health (OIE) [19]. Due to the higher rates of disease incidence, fatalities, and losses in small ruminants, PPR is generally considered a major constraint for small ruminant production in developing countries as the case in the Sudan [19].

PPR was firstly described in Cote d’Ivoire (The Ivory Coast) in West Africa in 1940th [12], subsequently, the disease continued to spread to the Middle and Eastern parts of Africa and into Asia. Currently, the disease is endemic covering almost most of Africa and Asia, the Arabian Peninsula, the Middle East, Turkey and recently reported in Georgia and Mongolia [4, 5, 19].

The disease is caused by peste des petits ruminants virus (PPRV), a member of the *Paramyxoviridae* family of the *Morbillivirus* genus which in addition contains the closely related Rinderpest virus (RPV), the causative agent of a typical clinical syndrome in cattle [13, 19].

PPR is characterized by pyrexia, ocular and nasal discharges, erosive and necrotic stomatitis, conjunctivitis, gastroenteritis, diarrhoea and pneumonia [17, 19]. PPR is primarily a disease of goats and sheep, however, goats are

✉ Nussieba A. Osman
nussieba@yahoo.com

¹ Department of Pathology, Parasitology and Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, P.O. Box 204, Kuku, Khartoum-North, Sudan

² Ministry of Agriculture and Animal Resources, Khartoum State, Kuku, Khartoum-North, Sudan

³ Ministry of Animal Resources, General Directorate of Animal Health and Epizootics Diseases Control, P.O. Box 293, Khartoum, Sudan

usually more severely affected than sheep [17, 19]. The virus is shed in all body secretions and excretions, thus transmission of PPR requires close contact between susceptible and infected animals and occurs mainly through inhalation of aerosols produced by sneezing and coughing. The disease usually appears after an introduction of new animals into a herd. Fomites such as bedding may also contribute to the onset of an outbreak [17, 19].

In the Sudan, PPR was initially reported in sheep and goats in Elgedarif in Eastern Sudan in 1971, then outbreaks of the disease occurred in Middle Sudan in Sinnar during 1971–1972 and in Mielig in 1972 [9]. At that time the disease was initially diagnosed as Rinderpest (RP) of sheep and goats and later PPR was declared responsible of these outbreaks [10]. Subsequently, outbreaks of the disease were reported during 1989–1990 in Elhilalia in Gezira State in central Sudan [8].

Competitive ELISA (C-ELISA) is the most commonly used method for serological diagnosis of PPR in the Sudan due to its highly sensitivity (94.5%) and specificity (99.4%) compared to VNT assay that can detect PPRV antibodies in the sera of affected sheep and goats after the first week of infection [2, 18, 24, 26], however, it is unable to differentiate vaccinated from infected animals. Serological surveillance confirmed the presence of the disease in different regions of the country namely Gezira, White Nile, Blue Nile, Khartoum, River Nile, Northern, Red Sea, Kordofan and Darfur States [1, 7, 14, 21, 23]. PPR infection in camels was firstly described in Kassala State in Eastern Sudan in 2004 as a peracute fatal respiratory disease of camels [15]. Since 1989 and up to date, the PPRV Nigeria 75/1 live-attenuated cell culture vaccine [6] has been continuously used in the Sudan for the control of PPR in small ruminants in the field. When applied in the field, the vaccine is very effective for protection of small ruminants against natural PPRV infection [11, 25].

Recently, despite all control measures and the application of PPR effective vaccination, severe outbreaks of PPR among small ruminants were reported regularly in different parts of the country leading to significant economic losses. The present study aimed to assess the sero-prevalence of PPRV in sheep and goats in different areas of the country.

Materials and methods

Study area

A total of 320 serum samples were collected from recently suspected PPR outbreaks reported in different regions of the Sudan during 2016 and 2017. These sera were collected from 258 sheep and 62 goats of different ages and breeds (Table 1). Of these, sera which were collected during 2016

include: 2 sheep serum samples from Dongola, River Nile State in Northern Sudan; 64 sera (48 sheep and 16 goats) were from Kab Elgidad, Gezira State in the middle of the Sudan; 64 sera (42 sheep and 22 goats) were collected from Raboob—a region in East Nile-, Khartoum State; 72 sheep sera were collected from Garie, River Nile, Northern Sudan. During 2017, sera which were collected from suspected PPR outbreaks include: 104 sera (80 sheep and 24 goats) were collected from Hajar Elasal region, River Nile State in addition to 14 sheep sera were collected from Elgadid Elthaora, Gezira State (Table 1, Fig. 1).

Collection and preparation of samples

Blood samples for sera preparation were collected, in vacutainers without anticoagulants. After delivery to the laboratory, blood samples were left at room temperature for 1–2 h then were kept overnight in a refrigerator at 4 °C. On the following day serum was separated from blood by centrifugation and stored frozen at – 20 °C till used. Sera were collected from herds of sheep and goat from suspected PPR outbreaks from four different States of the country.

In order to confirm that PPRV was the main cause of the recent suspected outbreaks, about 5 whole blood samples were collected, in vacutainers containing anticoagulants “EDTA”, from each herd for PPRV antigen detection.

Whole blood samples were taken from infected animals only but sera were collected from both infected and healthy animals in the herds.

Immunocapture ELISA assay (IC-ELISA)

Whole blood samples were subsequently tested for detection of PPRV antigen by an Immunocapture ELISA assay using an immunocapture ELISA (IcELISA) kit (CIRAD EMVT, Montpellier, France) following the manufacturer’s instructions.

Competitive-enzyme linked immunosorbent assay (C-ELISA)

For detection of PPRV antibodies in the suspected sera, a competitive ELISA (C-ELISA) assay using monoclonal antibodies directed against PPRV nucleoprotein gene was performed using a competitive screening ELISA kit “ID Screen® PPR Competition kit for the detection of antibodies against PPR in sheep and goats serum and plasma” (IDVet Innovative Diagnostics, France) following the manufacturer’s instructions.

Table 1 Sheep and goat sera collected from PPR outbreaks in the Sudan during 2016–2017

Date of collection	Place of collection	No. of sera		Total no. of sera
		Sheep	Goats	
September, 2016	Dongola, Northern State	2	–	2
October, 2016	Kab Elgidad, Gezira State	48	16	64
November, 2016	Raboob, East Nile, Khartoum State	42	22	64
December, 2016	Garie, River Nile State	72	–	72
February, 2017	Hajar Elasal, River Nile State	80	24	104
May, 2017	Elgadid Elthaora, Gezira State	14	–	14
Total		258	62	320

Fig. 1 Locations of areas of PPR outbreaks occurred in the Sudan during 2016–2017

Results

Description of areas of PPR outbreaks

During 2016, suspected PPR outbreaks among sheep and goats occurred in different locations of the Sudan namely: Dongola in Northern State and Garie in River Nile State (northern Sudan); Kab Elgidad in Gezira State and Raboob in East Nile region in Khartoum State (middle Sudan)

(Fig. 1). Additionally, suspected PPR outbreaks occurred during 2017 were in Hajar Elasal in River Nile State (Northern Sudan) and Elgadid Elthaora in Gezira State (middle Sudan) (Fig. 1).

Description of PPR clinical disease in sheep and goats

Sheep and goats from PPR suspected outbreaks were recognized mostly by the appearance of the typical clinical signs of the disease including pyrexia, anorexia, profuse serous nasal and ocular discharges while some animals showed mucopurulent discharges, dry muzzle, respiratory signs and diarrhea. The cases were associated with a higher morbidity and mortality rates particularly among young animals.

Animal owners declared the complete absence of any vaccination against PPR in sheep and goats herds in these areas.

Detection of PPRV antigen using an immunocapture ELISA (IC-ELISA) assay

PPRV antigen was detected in whole blood samples, from sheep and goats, which were analyzed by an Immunocapture ELISA Assay (IC-ELISA) (data not shown) confirming that PPR is the main cause of these outbreaks.

Detection of PPRV antibodies using a competitive ELISA (C-ELISA) assay

Examination of the 320 sheep and goats sera by Competitive ELISA (C-ELISA) for the presence of PPRV antibodies indicated that 259 (80.9%, 95% CI 20.5–28) sera were positive while 61 (19.1%) sera were negative.

Sero-prevalence of PPRV antibodies in sheep and goats sera

Results of the C-ELISA yielded an overall antibodies sero-prevalence of 80.9% (259/320, 95% CI 20.5–28) among sheep and goats in the Sudan (Table 2).

On the species basis, of the 258 sheep sera analyzed, 218/258 sera were positive with an overall antibodies sero-prevalence of 84.5% (95% CI 16.7–24.1) whereas only 40/258 (15.5%) sera were found negative. Additionally, of the 62 goats sera tested by the C-ELISA, 41/62 sera were positive with an overall antibodies sero-prevalence of 66.1% (95% CI 28.5–51.1) whereas 21/62 (33.9%) sera were found negative (Table 2).

Generally, sheep sera yielded the higher antibodies sero-prevalence of 84.5% compared to goats sera which yielded a lower antibodies sero-prevalence of 66.1% (Table 2).

Sero-prevalence of PPRV antibodies in sheep and goats sera in different States of the Sudan

The overall antibodies sero-prevalence of PPR in sheep and goats in the Sudan as demonstrated by C-ELISA assay is 80.9% (95% CI 20.5–28) (Tables 2, 3). The highest overall sero-prevalence of PPRV antibodies was demonstrated in River Nile State 90.3% (159/176 sera, 95% CI 12.8–18.2), followed by Gezira State 88.5% (69/78 sera, 95% CI 14.4–25.5), then Khartoum State 48.4% (31/64 sera, 95% CI 41.4–63) and finally the lowest incidence was present in Northern State 00.0% (0/2 sera, 95% CI 69.9–72.2) (Table 3).

Sero-prevalence of PPRV antibodies in sera in the four different States of the Sudan considering species level are demonstrated in Table 4. Within sheep, the highest sero-prevalence of PPRV was demonstrated in Gezira State 90.3% (56/62 sera, 95% CI 12.4–22.8), followed by River Nile State 88.8% (135/152, 95% CI 12.3–18.2), then Khartoum State 64.3% (27/42 sera, 95% CI 29.1–57.1) and finally the lowest incidence was present in Northern State 00.0% (0/2 sera, 95% CI 69.9–72.2) (Table 4).

Within goats, the highest sero-prevalence of PPRV antibody was demonstrated in River Nile State 100% (24/24, 95% CI 11.1–23.4) followed by Gezira State 81.2% (13/16 sera, 95% CI 11.3–46.5) and finally the lowest incidence was present in Khartoum State 18.2% (4/22 sera, 95% CI 55.5–83.7) (Table 4).

Discussion

Peste des petits ruminants (PPR), is an infectious and fatal viral disease affecting mainly sheep and goats. PPR was firstly recognized in the Sudan in early 1970th [9], now it is considered endemic in Africa and particularly in the Sudan [16, 19]. In the Sudan, sheep and goats are the major known hosts of PPR, however, lately PPR was known to cause a fatal respiratory syndrome in camels as well [9, 10, 14–16, 20]. Outbreaks of the disease continued to occur over the years covering a huge part and are described in almost all parts of the Sudan.

Table 2 The overall sero-prevalence of PPRV antibodies in sheep, goats, both sheep and goats sera screened by C-ELISA assay

	Sheep sera (%)	Goats sera (%)	Total no. tested sera (%)
Positive sera	218 (84.5)	41 (66.1)	259 (80.9)
Negative sera	40 (15.5)	21 (33.9)	61 (19.1)
Total no. tested sera	258 (100)	62 (100)	320 (100)

Table 3 The overall sero-prevalence of PPRV antibodies in both sheep and goats sera in different States of the Sudan

Location	Sheep and goats sera		
	Total no. tested (%)	No. +ve (%)	No. -ve (%)
Northern State	2 (100)	0 (00.0)	2 (100)
River Nile State	176 (152 sheep + 24 goats) (100)	159 (135 sheep + 24 goats) (90.3)	17 (17 sheep + 0 goats) (9.7)
Khartoum State	64 (42 sheep + 22 goats) (100)	31 (27 sheep + 4 goats) (48.4)	33 (15 sheep + 18 goats) (51.6)
Gezira State	78 (62 sheep + 16 goats) (100)	69 (56 sheep + 13 goats) (88.5)	9 (6 sheep + 3 goats) (11.5)
Total	320 (258 sheep + 62 goats) (100)	259 (218 sheep + 41 goats) (80.9)	61 (40 sheep + 21 goats) (19.1)

Table 4 Sero-prevalence of PPRV antibodies in sheep and goats sera in different States of the Sudan

Location	Sheep sera			Goats sera		
	No. tested (%)	No. +ve (%)	No. -ve (%)	No. tested (%)	No. +ve (%)	No. -ve (%)
Northern State	2 (100)	0 (00.0)	2 (0.100)	0 (00.0)	0 (00.0)	0 (00.0)
River Nile State	152 (100)	135 (88.8)	17 (11.2)	24 (100)	24 (100)	0 (0)
Khartoum State	42 (100)	27 (64.3)	15 (35.7)	22 (100)	4 (18.2)	18 (81.8)
Gezira State	62 (100)	56 (90.3)	6 (9.7)	16 (100)	13 (81.2)	3 (18.8)
Total	258 (100)	218 (84.5)	40 (15.5)	62 (100)	41 (66.1)	21 (33.9)

During the years 2016–2017, several suspected PPR outbreaks, involved mainly sheep and to lesser extent goats, were reported in different parts of the country including Northern, River Nile, Gezira and Khartoum States. Screening of sheep and goats sera by competitive ELISA (C-ELISA) for the presence of PPRV antibodies, demonstrated a higher overall antibodies sero-prevalence of 80.9% (95% CI 20.5–28) among both sheep and goats. In contrast, a much lower overall antibodies sero-prevalence of 50.67% among both sheep and goats was previously demonstrated in the Sudan [21].

On the species basis, indeed sheep sera yielded the higher antibodies sero-prevalence of 84.5% (95% CI 16.7–24.1) compared to goats sera which yielded a lower antibodies sero-prevalence of 66.1% (95% CI 28.5–51.1). In contrast, a much lower PPRV antibodies sero-prevalence of 51.9% and 56.2% in sheep and goats, respectively, were reported earlier in the country [14]. A recently performed sero-prevalence study revealed lower PPRV antibodies sero-prevalence of 67.1% and 48.2% among sheep and goats, respectively, in the Sudan [23]. Of note, higher sero-prevalence values were achieved in this study than previously documented. These findings revealed higher sero-prevalence percentages in sheep compared to goats which are in accordance with previous comparable studies performed in the country [14, 23]. The results indicated the widespread of PPR in the country and that it is responsible

of the outbreaks and the reported clinical cases knowing that infected animals were never being vaccinated before against PPR as declared by the animal owners.

In the four States under study, where sera were tested, results revealed sero-prevalence of PPRV antibody in River Nile State, Gezira State, and Khartoum State while no incidence at all in Northern State. Indeed, previous studies for sero-surveillance of PPR in the country indicated the distribution of PPR in most of the country including the States under study [21, 23]. Concerning areas where outbreaks occurred, the higher incidence represents areas where there is a huge number of animals and where animal movement occurs regularly and could lead to the exposure of naïve animals to PPRV causing infection and disease. In the Northern State of the Sudan, the animal movements are somehow restricted due to the location of the area in the far north of the Sudan which is occupied mostly by the desert.

The findings indicated that PPR is currently circulating widely in the Sudan and still is a leading cause of a clinical disease and higher fatalities. The continual presence of PPR outbreaks, despite the application of PPRV vaccine, might be due to that the vaccination program is not reaching herds of animals in some areas. Therefore, the effective PPR vaccine is recommended to be used with a plan for covering all parts of the Sudan. Other control measures, i.e. restriction of animal movement and quarantine of infected animals, should be extensively

implemented to prevent the occurrence of the disease and the appearance of outbreaks.

C-ELISA is unable to differentiate PPRV vaccinated from infected animals as other available recognized diagnostic tests. Currently there are no available DIVA diagnostic tests for PPR, this arise the need for the development of DIVA diagnostics or marker vaccines to differentiate between antibodies induced due to the wild-type and vaccine strain PPR viruses [3].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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