



## RESEARCH ARTICLE

### Seroprevalence and Risk Factors of *Peste des Petits Ruminants* in Sheep in Kassala and North Kordofan States of the Sudan

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#### ABSTRACT

A cross-sectional study was conducted from April to December 2011 to determine the seroprevalence of *Peste des Petits Ruminants* and associated risk factors in sheep in Kassala and North Kordofan States of the Sudan, using cELISA. The overall seroprevalence of PPR was found to be 70.2% (576/820). There was no statistically significant difference, ( $P \leq 0.05$ ), in seroprevalences of PPR estimated in the two States, between the surveyed localities, among breeds and among the different age groups. However, statistically significant difference, ( $P \leq 0.05$ ), was found between sexes. Significant risk factors associated with cELISA positive status for PPRV in the univariate analysis were found to be State, locality, breed, sex and number of males with a p-value  $\leq 0.05$ . Age, herd size, number of females in the herd, number of young animals in the herd, buying animals from outside, production system practiced, mixing herds at communal points and where herds get mixed were not significant risk factors. Factors found significantly associated ( $P \leq 0.05$ ) with increased odds of being cELISA positive in the multivariate analysis were localities (Jebrat Al-Shiekh, Barra, and Al-Girba) and sex (females), number of males ( $\leq 10$ ) and number of young animals ( $> 40$ ) in the herd, while the factors found not significantly associated ( $P \leq 0.05$ ) with increased odds of being cELISA positive were breeds, ages, and where herds get mixed. Based on the results of the study, PPR is prevailing in the two investigated States and risk factors associated with its occurrence are locality, breed, sex and number of males in the herd. Legislation enforcement to ensure that sheep movements are controlled through the implementation of a permit system is recommended. Also all sheep owners and herders should compulsorily vaccinate their animals annually.

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#### INTRODUCTION

The Sudan has a very huge wealth of animal resources that has been estimated to be around 143 million head of animals; of which sheep and goats are 51.8 and 43.2 million. The Western Sudan has the most livestock, followed by Southern Sudan and Central Sudan (Anonymous, 2008). The breeds of these animals are well adapted to the harsh environment of the country and are often trekked for long distances in search of feed and water. Moreover, the livestock sector in the Sudan is an

important contributor to the national economy, contributing 46% to the Gross Domestic Product (GDP), 27% to Foreign Exchange Earnings (FEE), and employing 40% of the country's population (Karrar *et al.*, 2006; Anonymous, 2008; ILRI, 2009; Fadlalla and Ahmed, 2010).

*Peste des Petits Ruminants* (PPR) is an acute, highly contagious, infectious and notifiable transboundary viral disease of domestic and wild small ruminants (Abubakar *et al.*, 2008; Balamurugan *et al.*, 2010; MARF, 2010; Khalafalla *et al.*, 2010; Luka *et al.*, 2011). *Peste des Petits*

*Ruminants virus* (PPRV), the causative agent, belongs to the genus *Morbillivirus* of the family *Paramyxoviridae* (Murphy *et al.*, 1999; Olivier *et al.*, 2011). Currently, PPR occurs in most African countries situated in the wide belt between the Sahara and the Equator (including the Sudan, Ethiopia, Kenya and Uganda), the Middle East, and the Indian subcontinent (Osama, 2010; Banyard *et al.*, 2010; Khalafalla *et al.*, 2010; Luka *et al.*, 2011). PPRV has also been reported in the European part of Turkey and in China (Ozkul *et al.*, 2002; DEFRA, 2008; Abubakar *et al.*, 2008; Wang *et al.*, 2009; Chauhan *et al.*, 2009; DEFRA, 2009; Banyard *et al.*, 2010). No seroevidence of PPR has so far been reported in Africa south of the Equator; however, uncontrolled movement of livestock between countries is a potential danger to the spread of the disease (Lughano and Dominic, 1996). Infection with PPR virus in the Sudan was observed for the first time in 1972 in Al-Gedarif by El Hag Ali (1973) and by El Hag Ali and Taylor (1984) (cited by Intisar *et al.*, 2009; Khalafalla *et al.*, 2010). Since then, continuous outbreaks occur in the country, affecting sheep and goats (Khalafalla *et al.*, 2010). Today the disease is thought to be endemic with prevalence varying from 58.1 to 93.8% in different States (Intisar *et al.*, 2009; MAREF, 2010; Banyard *et al.*, 2010). PPR can cause serious economic losses due to its high morbidity rates that range from 50 to 90% and case-fatality rates that reach 55 to 85% in goats, 10% in sheep, and 50% in camels (Radostits *et al.*, 2007; Abubakar *et al.*, 2008; Khalafalla *et al.*, 2010; Osama, 2010; Luka *et al.*, 2011). More important, PPR reduces the export of small ruminants and their products to international markets in North Africa, the Middle East, South East Asia, and Europe. As demands for food quality and safety assurance have been escalating and the importing countries are increasingly implementing sanitary and phyto-sanitary (SPS) regulations (ILRI, 2009).

It is a recognized principle that the probability of disease transmission is not uniform across national populations. There are often a number of risk factors that contribute to the overall risk of disease transmission in a particular community, production system or value chain (Elsawalhy *et al.*, 2010). These risk factors are often quite simple attributes of the sub-population such as the amount of movement, exchange of animals between households and flocks as a result of social practices and changes in economic conditions that exhibit seasonal patterns, distance from services, lack of large scale vaccination campaigns, altitude, season, and inter-species contact or interaction with wildlife (Radostits *et al.*, 2007; Waret-Szkuta *et al.*, 2008; Elsawalhy *et al.*, 2010). In addition, age, sex, species, and breed are very important individual risk factors (Radostits *et al.*, 2007; Waret-Szkuta *et al.*, 2008).

Control of PPR depends mainly on vaccination, isolation and quarantine of infected animals, restriction of movement, and disinfection of infected areas (OIE, 2010). In the Sudan, Rinder Pest Virus (RPV) vaccine has been used for PPRV control for many years in the past. However, RPV vaccination campaigns were stopped in the course of affirming African countries as RPV free. Concurrently with the rinder pest campaign, vaccination against PPRV using a homologous vaccine produced locally in the Sudan was established in 2002. Since then, a plan to control PPRV was launched, but organized

vaccination campaigns are not well performed (Intisar *et al.*, 2009; Osama, 2010; MAREF, 2010), in addition to that, the low number of the vaccinated animals against PPRV might not lead to the effective containment and control of PPRV considering the huge number of susceptible hosts in the Sudan. Only 6,184,435 animals were vaccinated from 2005 to 2008 (MAREF, 2009). Furthermore, during the first half of the year 2009, eleven outbreaks were reported in the Sudan (OIE, 2010), indicating that the measures applied in the country are not completely successful, consequently, the disease continues to spread in small ruminant populations, infecting new areas, and expanding its prevalence. Therefore, the aims of this study were to determine the seroprevalence and to investigate potential risk factors of PPR in sheep herds raised in the Eastern and Kordofan regions of the Sudan.

## MATERIALS AND METHODS

### Study Area

This study was conducted in the Eastern and Kordofan regions of the Sudan (Fig. 1). The Eastern region covers an area of 368,704 km<sup>2</sup> (UN, 2010). It falls within the Sudano-Sahelian climate zone of Africa. The annual rainfall is concentrated in a single relatively short summer season during June to September and amounts to around 680 mm per annum. Temperature ranges from a mean minimum of 17°C in January to a mean maximum of 40°C in April and May (Suliman and Buchroithner, 2006). The mixed crop-livestock system, the nomadic, and the semi-nomadic system predominate in the region. Dubassy, Gaash, and Watiesh desert sheep breeds are raised and produced in the region for both domestic and export markets (ILRI, 2009). The region has an estimated livestock population standing at around 13,370,764 animals of which 4,991,763 is sheep (Anonymous, 2008). Kordofan region borders the Northern State in the North, Northern and Southern Darfur States in the West, Warrab and Unity States in the South, and the Upper Nile State from the southeast and the East, together with the White Nile and Khartoum States. The region covers an area of 316,710 km<sup>2</sup> (UN, 2010). Annual rainfall is concentrated also in a single relatively short summer season during June to September and the region enjoys an annual rainfall of 0 to 500 mm. In the region a mixture of farming systems are practiced including nomadic, sedentary and semi-sedentary animal production systems (ILRI, 2009). The region has an estimated livestock population of 24,665,761 animals of which 10,131,693 are sheep (Anonymous, 2008).

### Study Population and Sample Size

The study population was sheep raised in the Eastern and Kordofan regions. The sample size for determining the prevalence of PPR in sheep in the Eastern and Kordofan regions was calculated based on the following parameters: 95% level of confidence, ±5% desired level of precision, the expected prevalence of PPR in sheep (Thrusfield, 2007). As the prevalence of PPR in sheep in different regions of the Sudan has not been substantially determined in previous studies, this study assumed an expected prevalence of PPR of 50% in sheep in the Eastern and Kordofan regions. By using the formula:

$$n = \frac{(1.96)^2 \times P_{exp} \times (1 - P_{exp})}{d^2} \text{ where,}$$

$n$  = Required Sample Size

$P_{exp}$  = Expected Prevalence Rate, 50%

$d$  = Desired Absolute Precision,  $\pm 5\%$

Accordingly, the required sample size was found to be 384 animals from each study region. This number was inflated 4-fold to account for the effect of randomness and representativeness in multistage sampling strategy with more than two levels (Thrusfield, 2007). Thus, total  $n$  was 3,072 serum samples from the Eastern region and from Kordofan region together.

### Study Design and Sampling Strategy

A cross-sectional epidemiological design was employed, from April to December 2011, with a multistage sampling strategy with three hierarchical levels of selection. The first level of selection was the region; whereby the Eastern and Kordofan regions were purposively selected due to their supply of animals to both export and domestic markets. The Eastern region has three states, namely the Red Sea, Kassala and El-Gadarif States; on the other hand, Kordofan region has two States which are North Kordofan and South Kordofan States. Within each selected region; one State was randomly selected (Kassala and North Kordofan States) and 50% of its localities were selected in the same manner (Fig. 2). Animals were sampled from unvaccinated herds in the selected localities either randomly or conveniently.

### Serum Samples

About 5 ml of blood sample was collected from the jugular veins of the selected animals using plain vacutainer tubes. The tubes were kept in a slant position and protected from direct sunlight until the blood clotted and thereafter the serum was separated. The separated serum was stored in cryovials at  $-20^{\circ}\text{C}$  until processed.

### Competitive ELISA (cELISA) for Detection of PPR Antibodies

PPRV antibody detection was carried out using PPR c-ELISA kits manufactured by the FAO Reference Laboratory (CIRAD EMVT; Montpellier, France), and obtained from BDSL, the distributing agent. The c-ELISA test was carried out according to the kit protocol and the manual provided with it. The test procedures were as followed: For coating of microplates, PPR antigen was diluted 1:100 in Phosphate Buffer Saline (BPS) and 50  $\mu\text{l}$  of diluted PPR antigen was added to each well of an ELISA plate. Then the plates were covered and incubated at  $+4^{\circ}\text{C}$  over night or placed on a shaker for one hour. Then the plates were washed three times with washing buffer, 40  $\mu\text{l}$  Blocking Buffer (BB), PBS 0.1% Tween 20+0.3% negative serums, were added to all wells and further 10  $\mu\text{l}$  was added to the monoclonal control wells (F1, F2, G1, G2) and 60  $\mu\text{l}$  to the conjugate control wells (A1, A2). Columns 1 and 2 were used as control, 10  $\mu\text{l}$  of test serum was added to test wells (vertical duplicates), 10  $\mu\text{l}$  of strong positive control serum to controls (B1, B2, C1, C2), 10  $\mu\text{l}$  of weak positive control serum to controls (D1, D2, E1, E2), 10  $\mu\text{l}$  of negative control serum to controls (H1, H2) were added. 50  $\mu\text{l}$  of MAb (1:100 in

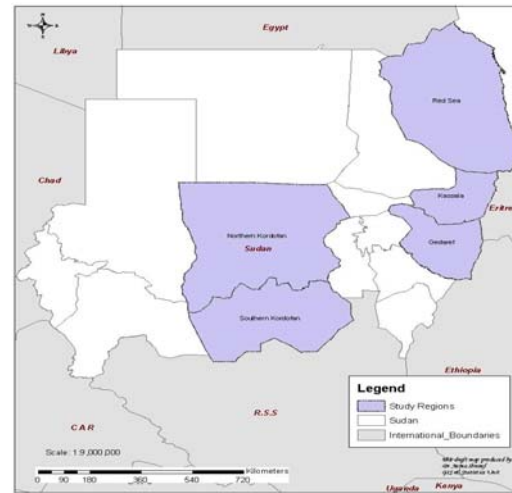


Fig. 1: Map of the study regions (MARE, 2011).

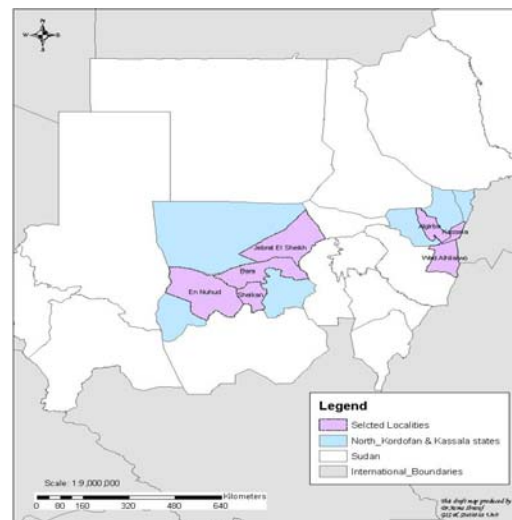


Fig. 2: Map of the selected States and localities in the study regions (MARE, 2011).

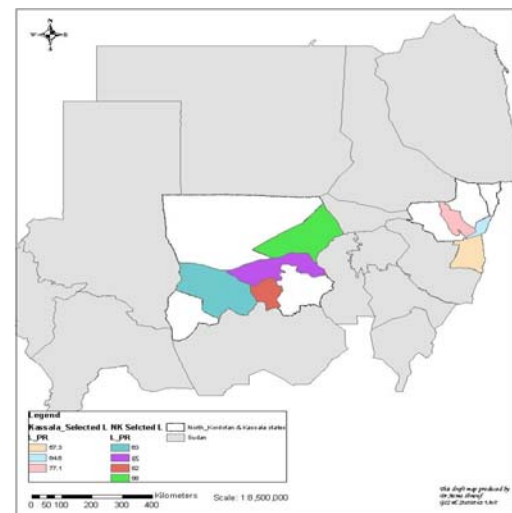


Fig. 3: Map of locality mean sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011) (MARE, 2011).

BB) was added to each well except A1 and A2 (conjugate controls wells). The plates were covered and incubated at 37°C for one hour in an orbital shaker, washed three times with washing buffer and blotted to dry. Then 50 µl of anti mouse HRPO conjugate (1:100 in BB) was added to each well and incubated at 37°C for one hour in an orbital shaker. The plates were washed three times with washing buffer and blotted to dry. 50 µl of chromogen/substrate (4 µl of H<sub>2</sub>O<sub>2</sub> added to each ml of OPD) were added to all wells. The plates were incubated at room temperature without shaking and avoiding direct light for 10 minutes. The reaction was stopped by the addition of 50 µl of sulphuric acid 1M to each well. OPD/H<sub>2</sub>O<sub>2</sub> + H<sub>2</sub>SO<sub>4</sub> in one column were used as blank. Optical Density (OD) values were read at 492 nm with an ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems, Finland). The absorbance was converted to Percentage Inhibition (PI) using the formula below with the help of the ELISA Data Interchanges (EDI) software manufactured by FAO/IAEA.

$$PI = \frac{\text{Absorbance of the test wells}}{\text{Absorbance of the MAb control wells}} \times 100$$

**Interpretation of cELISA Results:** any sample with an average Percentage Inhibition (PI) of <50% was considered as negative, 51-80% considered as weak positive (WP), >81% considered as strong positive (SP).

#### Questionnaire Survey

Questionnaires were administered and discussed with owners and herders of sheep. Questions included herd size, number of young animals, males and females within the herd, measures taken when introducing new animals into the herd, mixing different species of livestock, mixing herds with each other at pasture or watering points, and the type farming system practiced. Other questions like frequency of PPR outbreaks, and the attitude of the owners and herders of sheep toward vaccination and the effect of animal movements on disease spread were also included in the questionnaire.

#### Data Management and Statistical Analyses

All collected data were entered, coded, and stored electronically in a Microsoft® Excel for Windows® 2007 data base. The Statistical Package for Social Sciences (SPSS) for Windows® version 18.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained. For each variable (age, sex, breed, and locations), frequencies (number of observations within variable) and prevalences by cross-tabbing (number of positive valid samples/number of individuals sampled in the variable) were obtained. Hypotheses of differences of age group, breed, sex, and locations between test-positive and test-negative animals were first tested by univariate analysis by means of the 2-tailed chi-square test. In a second step, a logistic regression model was used to assess the association between the potential risk factors sex, breed, state, and locality and the outcome variable PPR serological status. Age and other potential risk factors with  $P \leq 0.20$  in the univariate analysis were entered into the regression model. Associations in the logistic regression model were deemed significant when  $P \leq 0.05$ .

Opinions, perceptions, and data collected from sheep herders and owners were entered, coded, and stored electronically in the Microsoft® Excel for Windows® 2007 data base as well. Uni-variable frequencies (number of observations within variable) and multiple responses were calculated. Hypothesized associations between some risk factors collected in the questionnaire survey and positive or negative animals were firstly tested by a 2-tailed chi-square test. In a second step, as with the herd demographic data above, a logistic regression model was developed.

Chloropleth maps were produced using ArcGIS version 9.1 (ESRI, Redlands, California) to show i) the study regions ii) selected study States within each region iii) selected study localities within each State iv) seroprevalences of PPR by locality.

## RESULTS

A total of 2,642 serum samples were collected from sheep in Kassala and North Kordofan States. From them, due to financial and technical constraints, 820 serum samples were randomly selected, 400 samples from North Kordofan State and 420 samples from Kassala State, to estimate the seroprevalence of PPR by using cELISA. As presented in Table 1, the overall seroprevalence was 70.20% (576/820) with 95% CI from 67.07 to 73.33. The seroprevalences estimated in the two States were statistically not significant ( $P \leq 0.05$ ) North Kordofan State showing a seroprevalence of 74.5% (298/400), with 95% CI between 70.2 and 78.8 and Kassala State of 66.2% (278/420), with 95% CI between 61.7 and 70.7. There were no significant differences in the seroprevalences estimated for the different localities with Jebelat Al-Shiek and Shiekan localities showing higher prevalences than the other 5 localities (Fig. 3 and Fig. 4). There were no significant differences in the seroprevalences estimated among the different breeds (Fig. 5) and age groups (Fig. 6). Between sexes, seroprevalences were significantly different ( $P \leq 0.05$ ), with females showing a higher seroprevalence than males (Fig. 7).

The numbers of PPR-seropositives were investigated between herd sizes, numbers of males in the herd, numbers of females in the herd and numbers of young animals in the herd. In the univariate analysis, using chi square, only the number of males in the herd ( $p=0.003$ ) was significantly associated with a cELISA positive status for PPR. In contrast, herd size ( $p=0.992$ ), number of females in the herd ( $p=0.852$ ) and number of young animals in the herd ( $p=0.192$ ) were not significantly associated with cELISA sero-positivity (Table 2). However, the numbers of PPR-seropositives apparently varied in herds, depending on whether the owner or the herder did buy animals from outside to increase his herd, by measures taken when introducing new animals into the herd after buying, by farming systems, and by mixing of herds at communal points. However, statistical analysis revealed that none of these factors was significantly associated ( $P \leq 0.05$ ) with the cELISA positivity for PPR (Table 3).

Results of the logistic regression analysis assessing the combined relationship between States, localities, breeds, age groups, and sex with the cELISA positive status for PPR are presented in Table 4. The factors that

were significantly associated with increased odds (Exp (B)) of being cELISA positive included: localities (Jebrat Al-Shiekh, Barra and Al-Girba) and sex (females). Logistic regression analysis was also carried out for assessing the combined relationship between herd management risk factors with the positive reaction for PPR in the cELISA (Table 5). The factors that were significantly associated with increased odds of being cELISA positive included: the number of males ( $\leq 10$ ) and number of young animals ( $> 40$ ) in the herd. However, the variables age groups and mixing of herds were forced into this final regression model.

The summary of responses of sheep owners and herders on vaccination against PPRV infection and the number of vaccinated animals in North Kordofan and Kassala States is contained in Table 6. 48.7% (n=19) of the owners and herders stated that they had vaccinated their animals against PPRV in the past while 51.3% (n=20) of owners and herders did not ever vaccinate their animals before. 68.4% (n=13) of the owners and herders reported that they had vaccinated in the year 2011, 31.6% (n=6) had vaccinated in the period between 2005 and 2010 and none (n=0) had vaccinated before 2000 or between 2000 and 2005. 31.6% (n=6) of the owners and herders vaccinated  $\leq 1000$  animals, 15.8% (n=3) vaccinated  $> 1000-2000$  animals, 21.0% (n=4) vaccinated  $> 2000-3000$  animals, 31.6% (n = 6) vaccinated  $> 3000-4000$  animals, and nobody (n=0) vaccinated more than 4000 animals. 20.0% (n=4) of the owners and herders who did not vaccinate their animals indicated that they did so because vaccine was unavailable, 40.0% (n=8) because vaccine was expensive, 25.0% (n=5) because they saw no need to vaccinate their animals, and 15.0% (n=3) did not give an explanation.

## DISCUSSION

In this study, the overall seroprevalence of antibodies against PPRV in sheep serum samples collected from the two investigated States of the Sudan was found to be higher than the sero-prevalences reported in Darfur State by El-Rasih (1992) who reported seroprevalences of 12.50% by using Agar Gel Immunodiffusion (AGID) and of 20.0% by using Virus Neutralization Test (VNT). It was also higher than another report by Haroun *et al.* (2002) who reported an overall sero-prevalence of 50.0% by using cELISA and tested 52 serum samples from sheep among samples of camels, goats and yearling calves from different parts of the Sudan. Moreover, it was higher than the reports of Intisar *et al.* (2007), Intisar *et al.* (2009) and Intisar *et al.* (2011). They used Competitive Enzyme Linked Immuno-Sorbent Assay (cELISA) and Immunocaptured-ELISA (IcELISA) and tested many serum samples collected from different animal species including: sheep, camels, and goats from central, northern, eastern and western Sudan. They reported seroprevalences of 67.7, 62.8 and 59.7%, respectively. On the other hand, the overall seroprevalence estimated in this study was found to be lower than the seroprevalence of Banyard *et al.* (2010) who reported a seroprevalence of 93.8% from different States of the country. These dissimilarities between the reported prevalences could probably be attributed to variations in the animal production and husbandry systems practiced in each area. Diagnostic tools used in each study could also have led to the noticed variation, as some diagnostic tools used previously are known for their low sensitivity and specificity. Differences in the sizes of samples tested in each study could also result in the noticed dissimilarities.

**Table 1:** Estimated seroprevalences of PPR by State, Locality, Breed, Age and Sex in North Kordofan and Kassala States and Univariate Analysis for the association between PPR and individual Animal Risk Factor using the Chi square test (April to July 2011)

Risk Factors	No. of tested samples	No. of positive samples	Sero-prevalence (%)	95% CI Lower - Upper	$\chi^2$	p-value
<b>State</b>						
North Kordofan	400	298	74.5	70.23 - 78.77	6.768	0.009
Kassala	420	278	66.2	61.68 - 70.72		
<b>Localities</b>						
Jebrat Al-Shiekh	100	88	88.0	81.63 - 94.37	42.64	0.000
Barra	100	65	65.0	55.65 - 74.35		
Shiekan	100	82	82.0	74.47 - 89.53		
Al-Khaoway	100	63	63.0	53.54 - 72.46		
Kassala	130	84	64.6	56.38 - 72.82		
Wad Al-Hilaiwo	150	86	57.3	49.38 - 65.22		
Al-Girba	140	108	77.1	70.14 - 84.06		
<b>Breeds</b>						
Kabashi	170	132	77.6	71.33 - 83.87	13.53	0.019
Hamari	211	150	71.1	64.98 - 77.22		
Zaghawa	19	16	84.2	67.80 - 100.6		
Garrage	174	108	62.1	54.89 - 69.31		
Dubassy	210	142	67.6	61.27 - 73.93		
Gaash	36	28	77.8	64.22 - 91.38		
<b>Age groups</b>						
$\leq 1$ year	174	114	65.5	58.44 - 72.56	3.546	0.315
$> 1 - 2$ years	123	83	67.5	59.22 - 75.78		
$> 2 - 3$ years	116	84	72.4	64.27 - 80.53		
$> 3$ years	407	295	72.2	67.85 - 76.55		
<b>Sex</b>						
Male	162	95	58.6	51.02 - 66.18	13.00	0.000
Female	658	481	80.2	77.16 - 83.24		
<b>Total</b>	<b>820</b>	<b>576</b>	<b>70.2</b>	<b>67.07 - 73.33</b>		

**Table 2:** Results of univariate associations of herd size, number of males, females, and young animals in herds with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States using the Chi square test (April to July 2011).

Risk factors	Number of samples	Number of positives	% positives	$\chi^2$	p-value
<b>Herd Size</b>					
≤ 100	92	67	72.8	0.265	0.992
> 100 - 200	203	145	71.4		
> 200 - 300	136	100	73.5		
> 300 - 400	44	31	70.5		
> 400	129	93	72.1		
Not Recorded	0	0	0		
<b>Number of Males</b>					
≤ 10	305	237	77.7	11.647	0.003
> 10 - 20	260	177	68.1		
> 20 - 30	0	0	0		
> 30 - 40	0	0	0		
> 40	39	22	56.4		
Not Recorded	0	0	0		
<b>Number of Females</b>					
≤ 100	81	60	74.1	1.352	0.852
> 100 - 200	203	145	71.4		
> 200 - 300	147	108	73.5		
> 300 - 400	27	22	81.5		
> 400	129	93	72.1		
Not Recorded	17	0	0		
<b>No. of Young animals</b>					
≤ 10	7	6	85.7	4.741	0.192
> 10 - 20	15	8	53.3		
> 20 - 30	28	23	82.1		
> 30 - 40	0	0	0		
> 40	112	79	70.5		
Not Recorded	442	0	0		

**Table 3:** Univariate associations of herd management risk factors with cELISA seropositivity

Risk factors	Number of samples	Number of positive	% Positives	Chi square	p-value
<b>Animals from outside</b>					
Yes	310	218	70.3	1.101	0.294
No	294	218	74.1		
<b>Introducing measures</b>					
Isolation	150	108	72.0	1.167	0.761
Giving Drugs	37	24	64.9		
Introduce Immediately	123	86	69.9		
Do not Introduce	294	214	72.8		
<b>Farming systems</b>					
Sedentary	141	108	76.6	2.316	0.509
Semi-Sedentary	224	162	72.3		
Semi-Nomadic	11	8	72.7		
Nomadic	228	158	69.3		
<b>Mixing with herds</b>					
Yes	377	279	74.0	1.655	0.198
No	227	157	69.2		
<b>Where herds mix</b>					
Watering Points	65	46	70.7	0.268	0.605
Pasture	0	0	0		
Watering and Pasture	312	231	74.0		

The overall antibody-prevalence against PPRV in sheep in this study was higher than the prevalences reported by Mulindwa *et al.* (2011) in Uganda (57.6%); Waret- Szkuta *et al.* (2008) in Ethiopia (52.5%); Senyael *et al.* (2009) in Tanzania (45.8%), Abd El-Rahim *et al.* (2010) in Egypt (63.40%); Bidjeh *et al.* (1995) in Chad (34.0%); Olivier *et al.* (2011) in Tunisia (7.5%); Rashid *et al.* (2008a) in Pakistan (28.8%); Wang *et al.* (2008) in China (17.6%); Waret- Szkuta *et al.* (2008) in Turkey (22.4%), Waret- Szkuta *et al.* (2008) in India (33.0%); Al-Majali *et al.* (2008) in Jordan (29.0% in sheep, 49.0% in goats; 60.0 and 74.0% of sheep and goats flocks) and Al-

Afaleq *et al.* (2004) in Saudi Arabia (3.1% in sheep and 0.6% in goats), the latter using a microtiter neutralization assay which is known for its low sensitivity. In Yemen, the seroprevalence of PPRV was found to be 15.0% in sheep and 18.0% in goats (Al-Majali *et al.*, 2008). A plausible explanation for the shooting seroprevalence found in this study could be related to the fact that vaccination against PPRV using a homologous locally produced vaccine that was established in 2002 and planned to control the disease; however, any organized vaccination campaigns are not practiced (Intisar *et al.*, 2009). On top of that, some owners and herders do not

**Table 4:** Results of multivariate analyses of associations of risk factors with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).

Risk factors	Number tested	Number of positive and (%)	Exp(B)	p-value	95% CI for Exp(B)	
					Lower	Upper
<b>Localities</b>						
Wad Al-Hilaiwo	150	86 (57.3)	Ref			
Jebrat Al-Shiekh	100	88 (88.0)	11.41	0.003	2.305	56.515
Barra	100	65 (65.0)	4.400	0.025	1.204	16.073
Shiekan	100	82 (82.0)	1.966	0.370	0.449	8.6140
Al-Khaoway	100	63 (63.0)	1.910	0.402	0.421	8.6750
Kassala	130	84 (64.6)	1.104	0.738	0.619	1.9680
Al-Girba	140	108 (77.1)	2.657	0.000	1.546	4.5670
<b>Breeds</b>						
Garrage	174	108 (62.1)	Ref			
Kabashi	170	132 (77.6)	1.015	0.983	0.256	4.030
Hamari	211	150 (71.1)	1.118	0.640	0.701	1.782
Dubassy	210	142 (67.6)	2.586	0.051	0.994	6.725
<b>Age groups (years)</b>						
≤ 1	174	114 (65.5)	Ref			
> 1 - 2	123	83 (67.5)	1.097	0.723	0.656	1.834
> 2 - 3	116	84 (72.4)	1.424	0.197	0.832	2.439
> 3	407	295 (72.2)	1.302	0.206	0.865	1.960
<b>Sex</b>						
Male	162	95 (58.6)	Ref			
Female	658	481 (80.2)	1.955	0.001	1.294	2.954

**Table 5:** Results of multivariate analyses of associations of risk factors with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).

Risk factors	Number tested	Number of positive and (%)	Exp (B)	p-value	95% CI for Exp(B)	
					Lower	Upper
<b>Number of males</b>						
> 40	39	22 (56.4)	Ref			
≤ 10	305	237 (77.7)	2.80	0.006	1.35	5.83
> 10 - 20	260	177 (68.1)	1.37	0.414	0.64	2.95
<b>No. of young animals</b>						
> 10 - 20	15	8 (53.3)	Ref			
≤ 10	7	6 (85.7)	3.989	0.262	0.356	44.669
> 20 - 30	28	23 (82.1)	3.058	0.148	0.673	13.887
> 40	112	79 (70.5)	4.415	0.014	1.356	14.378
<b>Where herds mix</b>						
Watering Points	51	36 (70.6)	Ref			
Watering and Pasture	312	231 (74.0)	1.316	0.345	0.744	2.329

have the desire to vaccinate their animals because they think that vaccination causes the disease itself, rather than protecting their animals against it. Furthermore, lack of quarantine for infected animals and free movements of animals mainly cattle, sheep, goats and camels as practiced by nomadic and semi-nomadic pastoralists and practicing rampant communal grazing and sharing of water sources are all factors that can play a significant great role in spreading of PPRV, facilitating its transmission among populations of small ruminants and its incursion into new uninfected areas. In Syria, the sheep flock seroprevalences of PPRV was found to be 96.0% (Al-Majali *et al.*, 2008), which is significantly higher than that reported in the present study.

There is no statistically significant difference between the seroprevalences estimated from the two investigated States in this study. Practicing communal grazing and watering by sheep owners and herders throughout the two regions can be taken as an explanation, along with free movements of animals in the regions and into, and out from the regions. Among breeds, seroprevalences were not significantly different. This might be due to the fact that some breeds have low resistance to PPRV infection

and this finding is consistent with the results of Abubakar *et al.* (2011). PPR was significantly associated with breeds where it has been found to be more prevalent in indigenous breeds of Bengali goats than in exotic breeds of goats. The Guinean breeds (West African dwarf, Iagoon, Kindi and Djallonke) are recognized as highly susceptible (Abu bakar *et al.*, 2011). Age groups seroprevalences were also not significantly different. This result would not confirm findings of most studies carried out on PPRV, like that of El-Rasih (1992), Saliki *et al.* (1993), Srinivas and Gopal (1996) and Abubakar *et al.* (2011), who all did confirm a distinction in the susceptibility and the level of antibodies to PPRV in different age groups. However, most of the prevalences in this study statistically were the same, pointing to a more endemic nature of PPR or endemic stability in the two investigated areas than in the study areas of above authors. Females in this study were showing a significantly higher seroprevalence than males. This is divergent from the findings of Abubakar *et al.* (2011) and Sarker and Hemayeatul (2011) who indicated he-goats were apparently more prone to PPR infection than she-goats. Lambs were reported to be the most susceptible age

**Table 6:** Frequencies of responses of sheep owners and herders on vaccination against PPRV and number of vaccinated animals in North Kordofan and Kassala States (survey April to July 2011).

Risk Factors with Levels	Number	%
Vaccination against PPR		
Yes	19	48.7
No	20	51.3
Last Vaccination		
Before 2000	0	0
From 2001 to 2005	0	0
From 2006 to 2010	6	31.6
2011	13	68.4
Number Animals Vaccinated		
≤1000	6	31.6
>1000 - 2000	3	15.8
>2000 - 3000	4	21.0
>3000 - 4000	6	31.6
>4000	0	0
Why Do not You Vaccinate		
Vaccine Unavailable	4	20.0
Vaccine Expensive	8	40.0
Do not Need Vaccine	5	25.0
Reason not Given	3	15.0

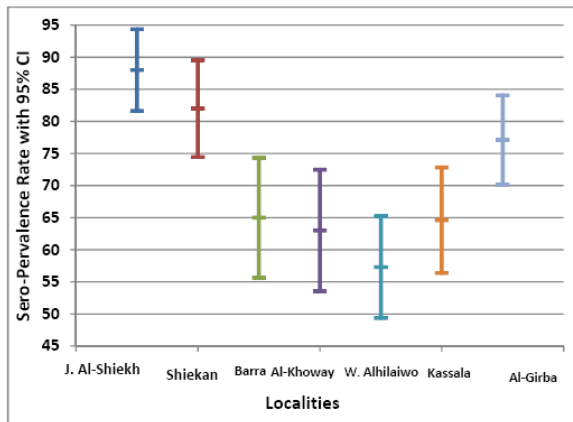
group to PPR infection in some flocks in different areas in the world (El-Rasih, 1992; Saliki *et al.*, 1993; Srinivas and Gopal, 1996; Abubakar *et al.*, 2011). Therefore, a continuously maintained transmission of PPRV from lambs to their dams could be imagined.

Generally few studies, particularly in the Sudan, have addressed risk factors associated with seropositivity to PPRV (Al-Majali *et al.*, 2008). Significant risk factors associated with being cELISA-positive in the univariate analysis in this study were found to be State, locality, breed, sex, and number of males. This is in agreement with reports by Radostits *et al.* (2007), Waret-Szkuta *et al.* (2008), Abd El-Rahim *et al.* (2010), Abubakar *et al.* (2011) and Sarker and Hemayeatul (2011) who stated that the epidemiological patterns of PPRV outbreaks and infections have been observed to be diverse in different ecological systems in various geographical regions. Moreover, PPRV infections in humid areas always occurred in an epizootic form that may have remarkable consequences with morbidity varying from 80 to 90% and mortality from 50 to 80%, while in arid and semi-arid regions, PPR is often fatal and usually occurs as a subclinical or in-apparent infection opening the door for other infections such as Pasteurellosis (Abd El-Rahim *et al.*, 2010; Abubakar *et al.*, 2011). The positive association of State and locality with cELISA PPR-positivity is in disagreement with the findings of Ozkul *et al.* (2002) who indicated that the occurrence of PPRV outbreaks did not vary substantially by geographic locations of livestock tested in Turkey. At the individual animal level, age, and herd size were not significant in the univariate analysis. This is in disagreement with findings of Waret-Szkuta *et al.* (2008), Al-Majali *et al.* (2008), Banyard *et al.* (2010), and Abubakar *et al.* (2011) who pointed out that age appeared to be a risk factor for sero-positive status, and its linear effect suggested that PPRV is highly immunogenic and naturally infected animals remaining positive for a long time. The insignificant association of age with PPRV cELISA positivity in this study indicates that antibodies occur in all age groups and that the virus also is in

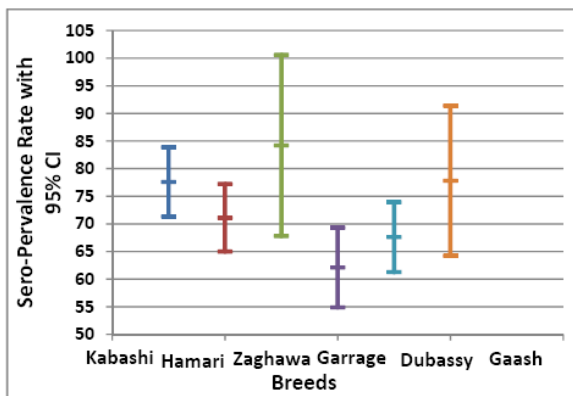
constant circulation in sheep of all ages as has formerly been confirmed by Abu Elzein *et al.* (1990) and Gopilo (2005). This can be elucidated by the fact that animals of the most vulnerable age group (lambs) do die as soon as they contract the virus and only those animals with some resistance do survive; consequently, detection of antibodies in the serum of age groups other than lambs is logical. The insignificant association of herd size to being PPRV cELISA positive could be due to the fact that all owners and herders, with small or large numbers of animals, do practice communal grazing and/or watering; therefore, all animals at these times are at similar risk to be infected with PPRV by coming in contact with infected animals. The same applies to other insignificant potential risk factors addressed in the univariate analysis, which were number of females in the herd, number of young animals in the herd, buying animals from outside, measures taken before introducing the new animal into the herd, production system practiced, mixing herds at communal points, and where herds get mixed. To the best of the author's knowledge, this is the first report of associations of these potential risk factors with having a PPRV cELISA-positive status in the Sudan.

The multivariate analysis, using logistic regression, with confidence interval of 95%, was used to assess the association between the identified significant risk factors in the univariate analysis in combination with a positive cELISA status for PPR. However, some potential risk factors thought to be important with  $P \leq 0.20$  in the univariate analysis were also entered into the multivariate analysis. This analysis showed association between a cELISA positive status for PPRV infection and locality with sheep of Jebrat Al-Shiekh locality ( $\text{Exp}(B)=11.41$ ), sheep of the Barra locality ( $\text{Exp}(B)=4.400$ ), sheep of Al-Girba locality ( $\text{Exp}(B)=2.657$ ) having been at increased risk of becoming seropositive. This positive association of locality as risk factor is in disagreement with findings of Ozkul *et al.* (2002). However, it agrees with results of Waret-Szkuta *et al.* (2008), Abd El-Rahim *et al.* (2010), Abubakar *et al.* (2011) and Sarker and Hemayeatul (2011) who also pointed to geographic clusters of PPR disease occurrence. When individual risk factors were combined, associations between breeds and cELISA sero-positivity no longer existed. Gopilo (2005) also found no association of PPR status and breeds. Furthermore, the analysis showed there were no significant associations between having a cELISA positive status for PPRV and age. Ozkul *et al.* (2002), Waret-Szkuta *et al.* (2008) and Abd El-Rahim *et al.* (2010), in contrast, found such age dependencies. One explanation for this difference in investigation results could be that PPRV is highly immunogenic and naturally infected animals do remain antibody-positive for a long time after recovery while those animals which are highly susceptible die when they are infected. In the combination of factors, a significant association between being cELISA positive for PPR and sex was established. Females were at increased risk ( $\text{Exp}(B)=1.955$ ) compared to males ( $p=0.001$ ) confirming the results of Rashid *et al.* (2008b), Abubakar *et al.* (2011) and Sarker and Hemayeatul (2011) who found a significant distinction between sexes. They found that PPRV infection was associated with sex where males were at higher risk than females. It has to be considered,



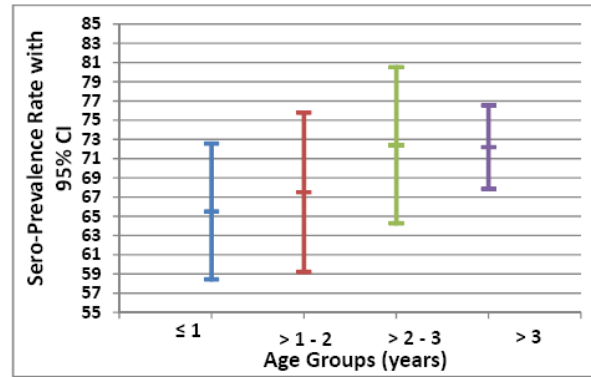


**Fig. 4:** Estimated mean locality seroprevalences for PPR in sheep at study localities in North Kordofan and Kassala States (April to July 2011) with 95% confidence limits

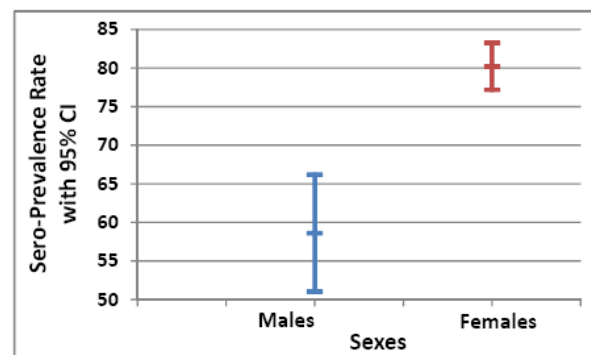


**Fig. 5:** Estimated mean breed seroprevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011) with 95% confidence limits

however, that females are subject to more stressing factors like pregnancy and lactation; in addition, the productive life span of females is longer than that of males. The proportionally higher number of females in herds in comparison to males could be another explanation, why, statistically, females were found to be of increased risk of attaining a seropositive status in this study. The analysis further showed that there was a significant associations between being cELISA positive for PPR and the number of males in the herd, with low number of males ( $\leq 10$  males) in herds being at higher risk ( $\text{Exp}(B) = 2.802$ ;  $p\text{-value} = 0.006$ ) when compared to the reference category (herds having  $>40$  males). Likely reasons range from biological, managerial or genetic factors. However, more studies are warranted to explore a more convincing scientific sound explanation. The number of young animals in the flock was found to be associated with seropositivity in the cELISA. Herds having  $>40$  young animals were about 4.5 times at higher risk when compared to the reference category, herds having between 10 and 20 young animals. Ozkul *et al.* (2002) and Abd El-Rahim *et al.* (2010) indicated that young animals, both of sheep and goats, after losing maternal immunity are at higher risk than adults and have better chance to become sero-positive to PPRV. Therefore, the higher the number



**Fig. 6:** Estimated mean age group seroprevalences for PPR in sheep in North Kordofan and Kassala States (April to July 2011) with 95% confidence limits



**Fig. 7:** Estimated mean sex seroprevalences for PPR in sheep in North Kordofan and Kassala States (April to July 2011) with 95% confidence limits

of young animals is in herds, the higher is the number of PPRV. No significant association between being cELISA positive for PPRV and where herds get mixed could be established. This could be related to the fact that PPR is transmitted from infected animals to susceptible ones by contact, whether the contact happens at watering points, pastures or at both.

Less than half of the owners and herders answering the questionnaire had vaccinated their animals against PPRV. The majority of owners and herders do reject vaccination because they think that vaccination causes the disease rather than protecting their animals against it. It also is possible that a considerable number of owners and herders does not vaccinate because they have to pay vaccination fees sometimes. Wifag (2009) also reported only one-third of owners and herders vaccinating against PPRV. In this study, more than half of the owners and herders who vaccinated their animals did so in the year 2011, rather than in previous years. Whether these 2011 vaccinations are related to the increasing number of outbreaks as well as to the economic impact of these outbreaks, must remain unanswered. The study showed that the number of vaccinated animals is very small. It is obvious that this low number of vaccinated animals against PPRV in the Sudan will not lead to effective containment and control of PPRV due to the fact that the Sudan has millions of susceptible host animals. Vaccination campaigns further on are not well organized

since they have been established in 2002 (Intisar *et al.*, 2009). The educational status of the owners and herders, their unawareness of the benefits of vaccination and the fees of vaccination could all be probable explanations why only very small numbers of animals are vaccinated. Also, vaccine availability plays an essential role. More than half of the owners and herders who had not vaccinated their animals before indicated that vaccine was unavailable.

In conclusion, PPRV according to serological diagnosis is prevailing in sheep in North Kordofan and Kassala States of the Sudan at a very high seroprevalence. Based on the results of the study, risk factors associated with PPRV occurrence in the two States are locality, breed, sex and number of males in the herd. In contrast, age, herd size, number of females in the herd, number of young animals in the herd, buying animals from outside or herd increases by breeding, measures taken when introducing new animals, different farming systems, mixing different herds at communal points and at what places herds mix were found not to be significantly associated with the occurrence of PPRV. A Differentiation of Infected from Vaccinated Animals (DIVA) is essential and ultimately discriminating since current tests do not differentiate between PPRV-induced and vaccine-induced antibodies. Legislation should be improved, updated and enforced to ensure that sheep and other livestock movements are controlled through the implementation of a permit system for livestock movement and road check points. In addition, all sheep owners and herders should compulsorily vaccinate their animals annually.

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