SERO-PREVALENCE AND RISK FACTORS OF PESTE DES PETITS RUMINANT (PPR) IN SHEEP IN RIVER NILE AND WHITE NILE STATES, SUDAN

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المستخلص

أجريت هذه الدراسة لإستقصاء وبائية مرض طاعون المجترات الصغيرة (PPR) في الأغنام في ولايتى نهر النيل والنيل الأبيض, تم تحديد الإنتشار المصلي في هاتين الولايتين بإستخدام cELISA كذلك تم إستقصاء في عوامل الخطر المرتبطة بالإنتشار المصلي للمرض في 519 عينة مصل التي تقع ضمن مستوى ثقة 95% (Thrusfield، 2007). تم العثور على نسبة إنتشار 53% (519/275) للولايتين , 56.5%(260/147) فى ولاية نهر النيل و 49.4% (259/128) في ولاية النيل الأبيض. ويعتبر هذا الإختلاف ضئيلاً إحصائياً (P = 0.104) ويعزى هذا إلى حركة الحيوانات الغير محدودة من أجل المرعي والماء فى الولايتين. في التحليل أحادى المتغيرات تم تحديد 15 عامل ذا دلالة إحصائية كان له تأثير كبيرعلى إنتشار مرض طاعون المجترات الصغيرة تمثلت فى الآتى:- المحليات (p- value=0.000) ،الجنس (p- value=0.000)، العمر (p- value=0.000)، تكوين القطيع (p- value=0.002)، طرق الهجرة (p- value=0.000)، النظافة (p- value=0.000)، علامات يعرف بها المرض (p- value=0.011), المواسم (p- value=0.00)، معدلات الإصابة (p- value=0.001)، معدلات الوفيات (p- value=0.00) ، الإجهاض (p-value=0.000)، التأثيرعلى الإنتاج (p- value=0.002)، الفاقد خلال السنة (p- value=0.000)، إستخدام كباش من خارج القطيع (p- value=0.011) والتطعيم ضد المرض (p- value=0.000) , بينما في التحليل متعدد المتغيرات وجد عامل واحد ذا دلالة إحصائية هو الجنس (p- value=0.000) . تعتبر العوامل ذات الدلالة الإحصائية المرتبطة بشكل كبير مع إنتشار المرض فى هذه الدراسة مهمة للتنبؤ بتفشى مرض طاعون المجترات الصغيرة في هاتين الولايتين.

Abstract

"Peste des Petits Ruminants (PPR is a contagious viral disease of small ruminants in Africa and Asia). In this study, the epidemiology of PPR in sheep in River Nile and White Nile States was investigated. For this purpose, the sero-prevalence of PPR in these two states was determined by using cELISA and the risk factors associated with the sero-prevalence were investigated. In a total of 519 serum samples that fall within the recommended 95% level of confidence (Thrusfield, 2007), a 53% prevalence (275/519) was found; 56.5% (147/260) in River Nile state and 49.4% (128/259) in White Nile state. This statistically insignificant difference (P=0.104) in PPR prevalence in the two states could be attributed to similar animal movements and communal grazing and watering among animals of the two states. In the unvariate analysis, 15 statistically significant factors that had an impact on PPR spread were determined. These were: Locality (p-value=0.000), sex (p- value=0.000), age (p- value=0.000), herd composition (p- value=0.002), migratory routes (p- value=0.000), cleaning (p-value=0.000), known signs of PPR (p- value=0.011), season (p-value=0.000), morbidity rate (p- value=0.001), mortality rates (p-value=0.000), abortion (p- value=0.000), affecting production (p-value=0.002), loss during year (p- value=0.000), using outside rams (p-value=0.011) and vaccination of PPR (p- value=0.000) while in multivariate analysis only one risk factor was found statistically significant which is sex (p- value=0.000). Risk factors significantly associated with a cELISA positive status for PPR in this study could be considered as important predictors for the occurrence of PPR outbreaks in these states.

Introduction

Livestock are very important for both subsistence and economic development of the African continent. They provide a flow of essential food products throughout the year. In some countries such as Sudan, they are major source of government revenue and export earnings.

Sudan had an estimated livestock population of 143 million head of which 51.8 million are sheep, 43.2 million goats, 41.5 million cattle, and 4.4 million camels, in addition to more than two million equines.

A Peste des petits ruminant (PPR) is an acute, highly contagious, infectious, and notifiable transboundary viral disease of domestic and wild small ruminants (Khalafalla *et al.,* 2010). The causative virus belongs to the genus *Morbillivirus* of the family *Paramyxoviridae.* This genus includes measles, rinderpest (cattle plague), canine distemper, phorcine distemper and the morbilliviruses found in whales, porpoises and dolphins. Morbilliviruses are known for their contagious nature and ability to cause some of the most devastating diseases worldwide (Olivier *et al.,* 2011). For many years it was thought that PPR was restricted to the Western part of the African continent until a disease of goats in the Sudan. That was originally diagnosed as rinderpest in 1972, was later confirmed to be PPR (Abubakar *et al.,* 2011). The overall recently detected sero-prevalence (62.8%) is higher than the previously reported by (Intisar et al., 2009) as 50%. Furthermore, in 2004 the virus did emerge in camels in the Eastern region of the Sudan, with a case-fatality rate reaching up to 50% (Khalafalla et al., 2010). Due to an ongoing decrease in available pastureland and forest area, sheep and goats often travel long distances during the dry season in search of fodder and water (Nanda *et al.,* 1996). Hence, movement of animals determines the pattern of PPRV outbreaks and infection (Abd El-Rahim *et al.,* 2010; Abubakar *et al.,* 2011).

Encouraging climatic factors for the survival and spread of the virus contribute to the seasonal occurrence of PPR outbreaks. During the rainy season in Pakistan, the migratory activity of animals is reduced due to the

increased availability of local fodder (Abubakar *et al.,* 2011). The nutritional status of the animals also improves, resulting in an increased resistance to infection. These factors may play a key role in limiting the transmission of the disease (Abubakar *et al.,* 2011; Sarker and Hemayeatul, 2011). Although the outbreaks that occur in West Africa coincide with the wet rainy season, the incidence seems to rise rapidly and reach a peak in winter. This could be related to the dry, cold and dusty weather accompanied with poor nutrition prevails at this time in Pakistan and West Africa (Abubakar et al., 2011; Sarker and Hemayeatul, 2011). PPRV infection was also significantly associated with sex where billy-goats were apparently more prone to PPR infection than nanny-goats (Abubakar *et al.,* 2011; Sarker and Hemayeatul, 2011). Clinically, the disease was characterized by sudden onset of depression, fever, ocular and nasal discharges, sores in the mouth, disturbed breathing and cough, foul –smelling diarrhea and death. The incubation period is 4-5 days. It is an immunosuppressive disease; hence, secondary latent infection may be activated and complicate the clinical picture. It is transmitted by close contact.

Materials and Methods

Study area:

White Nile State:

White Nile State is located in the central region of the Sudan between the longitudes 31.30^{o} and 33.15^{o} and the latitude 12.15^{o} – 15.15^{o}.The state covers an estimated area of 39701 Km2. The state is divided into eight localities, kosti, Tandalti, Elsalam, Rabak, Eljabaleen, Eldoium, Umrimta and Elgeteina. White Nile State borders, Khartoum State to the north, Gezira State, Blue Nile and Sinnar States to the east, North and South of kordofan States in the west and South Sudan country to the south. The dominant climate is Savannah. The annual rainfall ranges from 150–700mm (Annual report of General directorate of animal resources White Nile State, 2011). The human population in the state is estimated as 1.8 million (Annual Report of General Directorate of Animal Resources, White Nile State, 2011).

River Nile State:

River Nile State is located between latitudes 16^{o}- 22^{o} North, and longitudes 32 ^{o} -35 ^{o} South. From the North, it is bordered by the Arab Republic of

Egypt; from the east Kassala and Red Sea States and to the South Khartoum State and from West the Northern State. The State's area is 122.1 thousand square Kilometers and is divided into six localities; Aldamer, Atbara, Shendi, Almatama, Barber and Abuhamed.

Sample Size:

The actual sample size for determining the prevalence rate of PPR in sheep in the River Nile and White Nile States was calculated as based on the following parameters: 95% level of confidence, ±5% desired level of precision and the expected prevalence rate of PPR in sheep (Thrusfield, 2007). The prevalence rate of PPR in sheep in different regions of the Sudan was determined in previous studies as 80% (Wifag, 2009). Therefore, the sample size in this study was determined by using the formula:
Therefore, the sample size in this study was determined by using the formula:
$N= 4P^ Q^/ L^2$
N= Sample size
P^= Expected prevalence
Q^= 1-P^
L= Allowable error (0.05)
The required sample size (n) was determined to be 256 animals from each study state. (Thrusfield, 2007). Thus a total of 512 serum samples from the White Nile State and River Nile State together who included in the study.

Samples collection:

Two serum samples were taken from animals in the selected herds as recommended by OIE (2008). About 5 ml of blood sample was collected from the jugular veins using plain vacutainer tubes. The tubes were kept in a slant position and protected from direct sunlight until the blood clotted and the serum was later separated. The separated serum was transferred into sterile cryovials and kept at -20°C until processed.

Sampling Strategy and Study Design:

A cross-sectional epidemiological study was employed with a multistage sampling strategy with three hierarchical levels of selection. The first level of

selection was the state, the second level was locality (this is non-probability multistage sampling because the selection was based on the non vaccinated localities during the last two year). The third level of the selection was which was selected randomly the location.

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 local distributing agent. The kit contained a user manual with fact sheets, distilled water (30 mL), PBS powder (Sigma, IL), Tween- 20 (100 mL), ELISA plates (Nunc, Maxisorp) anti mouse HRPO conjugate (2 mL), substrate, H_2O_2, OPD tablet (30 mg), antigen (1 mL), strong positive serum (1 mL), weak positive serum (1 mL), negative serum (1 mL) and monoclonal antibody. The c-ELISA test was carried out according to the kit protocol and the manual provided with it the kit.

Test Procedure:

For coating of microplates, PPR antigen was diluted 1:100 in Phosphate Buffer Saline (BPS) and 50 µl of diluted PPR antigen was added to each well of an ELISA plate. Then the plates were covered and incubated at + 4°C over night or placed on a shaker for one hour. The plates were then washed three times with washing buffer, 40 µl Blocking Buffer (BB), PBS 0.1% Tween 20 + 0.3% negative serums, were added to all wells and further 10 µl was added to the monoclonal control wells (F1, F2, G1, G2) and 60 µl to the conjugate control wells (A1, A2). Columns 1 and 2 were used as control and 10 µl of test serum was added to test wells (vertical duplicates), 10 µl of strong positive control serum to controls (B1, B2, C1, C2), 10 µl of weak positive control serum to controls (D1, D2, E1, E2), 10 µl of negative control serum to controls (H1, H2) were added. 50 µl of MAb (1:100 in BB) was added to each well except A1 and A2 (conjugate control 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 then washed three times with the washing buffer and were blotted to dry. Fifty µl of chromogen/substrate (4 µl of H_2O_2 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_2O_2 + H_2SO_4 in one column was 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.

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

Questionnaire Survey:

Semi-structured questionnaires were administered and discussed, based on willingness, of owners and herders of sheep. General subject introductions and clarifications were made immediately after giving out the questionnaires and while discussing. Questions included in the questionnaire covered herd size, males and females within the herd, the probable number of animals involved when outbreaks happen (morbidity and mortality rates), measures taken when introducing new animals into the herd, breed of the animals reared, mixing different species of livestock, mixing herds with each other at pasture or watering points, moving from place to place looking for water and pasture, source of income, farming system practiced, the frequency of PPR outbreaks, season of the year when outbreaks occur, the source and actions to control outbreaks of PPR at local level, and general knowledge and perceptions on PPR, its clinical signs, impact on their animals, their attitude to vaccination and the effect of animal movements on disease spread.

Data Management and Analysis:

All collected data such as age, sex and breed of individual animals and locations during sampling and the laboratory results 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.

For each variable; (age, sex, breed, and locations etc), frequencies (number of observations within each category of the variable) and prevalence rates descriptive statistic were obtained by cross-tabbulation (number of positive valid samples/number of individuals sampled in the variable).

Association between the prevalence of PPR and potential risk factors was investigated by univariate analysis by means of 2-tailed Chi-square test. The level of significance in the univariate analysis was p-value of 0.20. The Logistic Regression model was used to assess the association between the potential risk factors found associated with PPR of P- value of 0.20 and the outcome variable PPR serological status. A risk factor with P-value of 0.05 was considered significantly associated with PPR.

Results

The Overall Sero-Prevalence Rate of PPR:

Generally, antibodies against PPRV were detected in all selected localities within the study regions with variations observed in the sero-prevalence rates according to potential risk factors. The overall sero-prevalence rate was 53% (275/519).

Sero-Prevalence Rate of PPR in River Nile and White Nile States:

The PPR sero-prevalence rate in the River Nile state was estimated as 56.5% (147/260) and in the White Nile state at 49.4% (128/259). No statistically significant difference was observed between the 2 states (P-value= 0.104) (Table1).

Sero -Prevalence Rate of PPR in the Different Surveyed Localities:

There was statistically significant difference in the sero-prevalence rates between the different surveyed localities: Shendi and Almatama localities

showed a significantly higher sero-prevalence rate than the other 3 localities in River Nile State. In the White Nile state Alsalam locality showed a higher sero-prevalence rate while Rabak, Elgableen and Algetena showed lower rates (Table1).

Sero-Prevalence Rate of PPR among Breeds:

There was no statistically significant difference in the sero-prevalence rates estimate among different breeds: Garrage showed a lower sero-prevalence rate of 49.2% (123/250), with 95% CI between 43% and 55.4% than the other breeds. On the other hand, Baladi breed showed a higher prevalence rate of 56.5% (147/260), with 95% CI between 50.47% and 62.53%, while Hamari breed showed a sero-prevalence rate of 55.6% (108/174), with a 95% CI between 23.14 and 88.06 (Table1).

Sero-Prevalence Rate of PPR in Males and Females:

Between sexes, sero-prevalence rates were significantly different. Females showed a higher prevalence rate of 60.4% (95% CI 43.31%-55.49), while males showed a lower prevalence rate of 27.4% (95% CI 19.32% - 35.48%)(Table1).

Results of the Univariate Associations with Sero-positive status against PPR:

In the unvariate analysis, 15 statistically significant factors that had an impact on PPR spread were determined. These were: age (p- value=0.000), herd composition (p- value=0.002), migratory routes (p- value=0.000), cleaning (p- value=0.000), known signs of PPR (p- value=0.011), season (p- value=0.000), morbidity rate (p- value=0.001), mortality rate (p- value=0.000), abortion (p- value=0.000), affecting production (p- value=0.002), loss during year (p- value=0.000), using outside rams (p- value=0.011) and vaccination of PPR (p- value=0.000) while in 8 factors found statistically not significant were determined. These were: herd size (p- value=0.204), seen signs with your herd (p- value=0.516), abortion (p- value=0.297), clean after abortion (p- value=0.768), udder cleaning (p- value=0.908) and veterinary services (p- value=0.480) Table (1).

Table1: Univariate associations of risk factors with cELISA PPR-sero-positivity in sheep in River Nile and White Nile States (April 2012):

Risk factor		No. Tested (%)	NO. Positive (%)	df	x²	P value
State	White Nile	259 (49.9)	128 (49.4)	1	2.639	0.104
	River Nile	260 (50.1)	147 (56.5)			
Locality White Nile	Algetena	64 (12.3)	19 (29.7)	8	43.389	0.000
	Alsalaam	65 (12.5)	46 (70.8)			
	Elgableen	65 (12.5)	27 (41.5)			
River Nile	Rabak	65 (12.5)	36 (55.4)			
	Aldamer	52 (10)	24 (46.2)			
	Almatama	52 (10)	34 (65.4)			
	Atbara	52 (10)	25 (48.1)			
	Barber	52 (10)	24 (46.2)			
	Shendi	52 (10)	40 (76.9)			
Sex	Female	402 (77.5)	243 (60.4)	1	39.851	0.000
	Male	117 (22.5)	32 (27.4)			
Age (Month)	2	15 (2.9)	3 (20)	18	127.894	0.000
	3	34 (6.6)	5 (14.7)			
	4	11 (2.1)	2 (18.2)			
	5	25 (4.8)	5 (20)			

	6	22 (4.2)	2 (9.1)			
	7	14 (2.7)	5 (35.7)			
	8	11 (2.1)	2 (18.2)			
	9	2 (0.4)	0 (0)			
	10	2 (0.4)	0 (0)			
	12	48 (9.2)	18 (37.5)			
	18	13 (2.5)	5 (38.5)			
	24	78 (15)	45 (57.7)			
	30	5 (1)	4 (80)			
	36	104 (20)	73 (70.2)			
	42	3 (0.6)	2 (66.7)			
	48	76 (14.6)	61 (80.3)			
	60	32 (6.2)	25 (78.1)			
	72	19 (3.7)	15 (78.9)			
	84	5 (1)	3 (60)			
Breed	Baladi	260 (50.1)	147 (56.5)	2	2.780	0.249
	Garag	250 (48.2)	123 (49.2)			
	Hamari	9 (1.7)	5 (55.6)			

Herd composition	Sheep only	42	(8.1)	32	(76.2)	1	9.877	0.002
	Mixed	477	(91.9)	243	(50.9)			
Herd size	<100	374	(72.1)	204	(54.5)	3	4.591	0.204
	>50	40	(7.7)	15	(37.5)			
	51-100	83	(16.0)	13	(59.1)			
	50	22	(4.2)	43	(51.8)			
Migratory routes	East	307	(59.2)	183	(59.6)	4	33.076	0.000
	Middle	25	(4.8)	9	(36.0)			
	NO	45	(8.7)	8	(17.8)			
	South	40	(7.7)	17	(42.5)			
	West	102	(19.7)	58	(56.9)			
Using outside rams	No	499	(96.1)	270	(54.1)	1	6.540	0.011
	Yes	20	(3.9)	5	(25.0)			

Table (1) continued:

Risk factor		No. Tested (%)		NO. Positive (%)		df	x^2	P value
Cleaning	Weakly	37	(7.1)	16	(43.2)	3	21.383	0.000
	Monthly	104	(20)	74	(71.2)			
	Often	164	(31.6)	71	(43.3)			
	No	214	(41.2)	114	(53.3)			
Known signs of PPR	Yes	469	(90.4)	257	(54.8)	1	6.409	0.011
	No	50	(9.6)	18	(36.0)			
Seen signs with your herd	No	115	(22.2)	64	(55.7)	1	0.421	0.516
	Yes	404	(77.8)	211	(52.2)			
Seasons	All	44	(8.5)	33	(75.0)	3	27.540	0.000
	Rainy	53	(10.2)	14	(26.4)			
	Winter	183	(35.3)	89	(48.6)			
	Summer	239	(46.1)	139	(58.2)			
Morbidity Rates	10.0%	45	(8.7)	23	(51.1)	6	22.434	0.001
	20.0%	65	(12.5)	46	(70.8)			
	40.0%	15	(2.9)	6	(40.0)			
	60.0%	22	(4.2)	13	(59.1)			
	80.0%	242	(46.6)	131	(54.1)			
	90.0%	95	(18.3)	34	(35.8)			
	95.0%	35	(6.7)	22	(62.9)			
Mortality Rates	10.0%	108	(20.8)	79	(73.1)	8	47.523	0.000
	20.0%	30	(5.8)	20	(66.7)			
	25.0%	22	(4.2)	13	(59.1)			
	30.0%	20	(3.9)	12	(60.0)			
	40.0%	10	(1.9)	4	(40.0)			
	50.0%	52	(10.0)	35	(67.3)			
	60.0%	40	(7.7)	15	(37.5)			
	80.0%	82	(15.8)	26	(31.7)			
	90.0%	155	(29.9)	71	(45.8)			
Abortion	No	48	(9.2)	22	(45.8)	1	1.086	0.297
	Yes	471	(90.8)	253	(53.7)			
Abortion Rates	.0%	18	(3.5)	15	(83.3)	10	62.513	0.000
	2.0%	15	(2.9)	1	(6.7)			
	4.0%	72	(13.9)	50	(69.4)			
	5.0%	15	(2.9)	5	(33.3)			

	6.0%	16	(3.1)	3	(18.8)			
	7.0%	15	(2.9)	6	(40.0)			
	15.0%	15	(2.9)	5	(33.3)			
	20.0%	105	(20.2)	46	(43.8)			
	30.0%	46	(8.9)	37	(80.4)			
	50.0%	52	(10.0)	21	(40.4)			
	70.0%	150	(28.9)	86	(57.3)			
Clean after Abortion	Yes	120	(23.1)	65	(54.2)	1	0.087	0.768
	No	399	(76.9)	210	(52.6)			
Affecting Production	No	50	(9.6)	16	(32.0)	1	9.783	0.002
	Yes	469	(90.4)	259	(55.2)			

Table (1) continued:

Risk factor		No. Tested (%)		NO. Positive (%)		df	x^2	P value
Udder cleaning	Yes	180	(34.7)	96	(53.3)	1	0.013	0.908
	No	339	(65.3)	179	(52.8)			
Veterinary services	Yes	110	(21.2)	55	(50.0)	1	0.500	0.480
	No	409	(78.8)	220	(53.8)			
Loss during Year	6	55	(10.6)	34	(61.8)	13	47.931	0.000
	7	20	(3.9)	12	(60.0)			
	10	50	(9.6)	27	(54.0)			
	15	15	(2.9)	5	(33.3)			
	18	15	(2.9)	5	(33.3)			
	20	110	(21.2)	69	(62.7)			
	25	31	(6)	18	(58.1)			
	30	60	(11.6)	19	(31.7)			
	40	15	(2.9)	1	(6.7)			
	45	15	(2.9)	10	(66.7)			
	50	15	(2.9)	10	(66.7)			
	60	54	(10.4)	30	(55.6)			
	80	20	(3.9)	5	(25.0)			
	100	44	(8.5)	30	(68.2)			
Vaccination of PPR	Yes	140	(27)	93	(66.4)	1	13.906	0.000
	No	379	(73)	182	(48.0)			

Results of Multivariate Analysis of Associations with PPR-Sero-positive Status:

Potential risk factors with $p \leq 0.20$ in the univariate analysis were entered into the Logistic Regression model. The only factor found statistically significantly associated with increased odds of being cELISA positive was sex (p-value= 0.000).

Discussion

This study showed that the sero-prevalence rate of PPRV was considerably high in the two studied regions. The sero-prevalence rates estimated in River Nile State was 56.5% (147/260) and in White Nile State was 49.4% (128/259). While many studies have been conducted on PPR in the Sudan, only few has included investigations on potential risk factors contributing to the occurrence and spread of PPR amid small ruminants populations.

In this study, the overall sero-prevalence rate of antibodies against PPRV in sheep serum samples collected from nine localities in River Nile and White Nile states of the Sudan was found to be near to the overall sero-prevalence rates reported by Intisar *et al.* (2007), Intisar *et al.* (2009), Osama (2010), Intisar *et al.* (2011) and lower than that found by Yassir *et al.* (2011) and Wifag *et al.* (2009). The variation could probably be attributed to dissimilarities in the size and method of collection of tested samples in each study. The variation of investigated areas could be another point of difference, considering the fact that each area has its specific and unique indigenous components and risk factors. Furthermore, the current discord results could probably be explained by differences in the investigated animal production systems and husbandry in each area. Diagnostic tools used in each study could also have led to the noticed variation.

The overall antibody-prevalence rate against PPRV in sheep serum samples collected from the nine localities in River Nile and White Nile states of the Sudan was near to rates reported by Abd El-Rahim *et al.* (2010) for Egypt (63.40%). A plausible explanation for the highst sero-prevalence rate found in this study could be related to unorganized vaccination against PPRV, using

a homologous locally produced vaccine established in 2002 and was planned to be used in control of the disease. On other hands this high sero-prevalence could be due to the fact that some owners and herders do not 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, that is 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 small ruminants populations and its incursion into new uninfected areas.

The sero-prevalence of PPR in sheep serum samples that were collected from the five surveyed localities of River Nile State of the Sudan was in agreement with the sero-prevalence reported by Intisar *et al.* (2009) and higher than that reported by Intisar *et al.* (2011).

There is no statistically significant difference in the States sero-prevalence rates estimated in this study. Practicing communal grazing and watering by sheep owners and herders in the two states could be taken as an explanation, along with free movements of animals in between the two States.

Samples from Shendi and Almatama localities in River Nile State showed significantly higher sero-prevalence rates than that from Aldamer, Atbara and Barber localities. In contrast, in White Nile State, the sero-prevalence rates are significantly higher in Alsalaam and Rabak samples than that of Elgableen and Algetena localities. The high sero-prevalence rates in these 2 localities in each State may underline a different PPR pattern in comparison to other areas. Abubakar et al. (2008) reported that PPRV spread is sometimes enhanced through migrations. Therefore, these differences could be ascribed to the continuous and intensive movements of animals (cattle, sheep, goats, and camels) in particular areas in White Nile State. Animals are trekked from South Sudan towards the northern part of the Sudan looking for pasture, water, and running away from biting insects, ticks and tick-borne diseases (TBDs). On the other hand, animals are also trekked from different states to the markets in the capital Khartoum for purpose of trading and most of these trades animals go far to River Nile State. Of course these movements

of animals through different States facilitate the spread of infectious diseases, including PPR, by contaminating the shared water sources and pasture as animals move from place to place.

The Baladi and Hamari breed showed the highest PPR sero-prevalence rate, while, in contrast, the Garrage breed showed the lowest. This can be explained by the fact that some breeds have resistance to PPRV infection. This result is consistent with Sudan field results of Abu bakar *et al.* (2011).

Animals between 24 and 84 month old showed the apparently highest age group sero-prevalence rate and animals younger than 1 year showed the apparently lowest rate. This result would confirm findings of Abubakar *et al.* (2011), who did confirm a distinction in the susceptibility and the level of antibodies to PPRV in different age groups. However, all rates in this study were statistically the same, pointing to a more endemic nature of PPR or endemic stability in the two studies areas.

Females showed a significantly higher sero-prevalence rate than males. This is in disagreement with findings of Abubakar *et al.* (2011) and Sarker and Hemayeatul (2011). Lambs were the most susceptible age group to PPR infection in the study flocks of Abubakar *et al.* (2011). Therefore, a continuously maintained transmission of PPRV from lambs to their dams could be imagined.

Knowledge of risk factors associated with PPR is an important pre-requisite for the design and implementation of effective control strategies and for management programs that can lead to the control and eradication of the virus. An understanding of these risk factors and their association and contributions to the occurrence and spreading of PPR among small ruminants populations also is a good aid for clinical diagnosis and for determining PPR's epidemiology and patterns.

In the current study, univariate analysis using the Chi square, with a confidence interval of 95% and at a p-value of ≤ 0.05 was used to identify potential risk factors associated with cELISA-positivity for PPRV infection. Significant risk factors associated with being cELISA positive in the univariate analysis were found to be locality, sex, age, herd composition and vaccination. This is in agreement with what has been reported by Abd El-Rahim *et al.* (2010), Abubakar *et al.* (2011) and Sarker and Hemayeatul

(2011). At the individual animal level herd size was not significant in the univariate analysis. This is in disagreement with findings of Abubakar *et al.* (2011). The significant association of age with PPRV cELISA, positivity indicates that the virus is in constant circulation in sheep of different ages.

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, such as a State. Also another insignificant potential risk factor addressed in the univariate analysis is abortion. Most of animal owners said abortion was caused by another disease. Also most of the owner's knowledge about signs of the disease is the same. The insignificant association for hygiene after abortion, and udder cleaning could be due to the fact that most of animal owners do not practice this type of cleaning. In respect to for veterinary services, most of animal owners went to pharmacy to buy animal drugs without consulting veterinary officer in there locality.

The significant association of mortality rates, morbidity rates, abortion rates and losing during year to being PPRV cELISA positive could be due to the effect of virus in animals, as the virus cause high mortality and morbidity.

From the risk factor, use of vaccination we found that the vaccination use has significant association with PPRV cELISA positive. From that we can conclude that vaccination is good measure to control the disease.

The multivariate analysis, using logistic regression, with a confidence interval of 95% and a p- value of ≤ 0.05 was used to assess the association between identified significant risk factors in the univariate analysis in combination towards 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 significant association between being cELISA positive for PPRV infection and locality. This association of locality as a risk factor is in agreement with the findings of 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, while our result are in disagreement with the results of Ozkul *et al.* (2002).

Furthermore, the analysis showed there were no significant associations between being a cELISA positive for PPRV and age. Abd El-Rahim *et al.* (2010), in contrast, found such age dependencies. One explanation for this difference 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 multivariate analysis, a significant association between being cELISA positive for PPR and sex was established. Females were at increased risk as compared to males (p=0.000). Sarker and Hemayeatul (2011). Females are subjected to more stressing factors like pregnancy and lactation; in addition, the productive life span of females is longer than that of males.

No significant association between being cELISA positive for PPR 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.

Abbreviations:

PPR: Peste des Petits Ruminants; PPRV: Peste des Petits Ruminants virus; c ELISA: competitive Enzyme- Linked Immunosorbent Assay.

Acknowledgments:

I would like to express my deep and sincere gratitude and appreciation to my supervisors Prof. Abd-Alhamid A. M. El-Fadil, College of Veterinary Medicine, Sudan University of Science and Technology for his excellent guidance, support, and constant encouragement throughout this study.I am grateful to Dr. Khedir Alfakki Director General of General Directorate of Animal Health and Epizootic Diseases Control, Ministry of Animal Resources and Fisheries for organizaing the field mission with the states Directors. I would also like to thank Dr. Alwia Mohammed, Head of EELISA Unit, General Directorate of Animal Health and Epizootic Diseases Control, Ministry of Animal Resources and Fisheries and Dr. Alyaa Hassan and Dr. Rehab Mohammed Nour and the staff of the EELISA Unit for their endless and kind help during carrying out the cELISA test.

I would also like to thank the General Directors of Animal Resources of River Nile and White Nile States for their support, help, and facilitating the field missions and sampling.

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