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Sero-Prevalence of Peste des Petits Ruminants Virus Antibodies in Sudanese Sheep and Goats before and after Vaccination

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Abstract

Peste des petits ruminants virus (PPRV) antibodies were studied in Sudanese sheep and goats (n = 855) before and after vaccination with a locally produced Nigeria 75/1 vaccine using a commercial competitive ELISA (cELISA) kit. Animals were kept healthy under field conditions, in four states: Blue Nile (n = 250), North Kordofan (n = 189), South Darfur (n = 225) and the Northern State (n = 191). Before vaccination, the overall sero-prevalence of PPRV antibodies was 54.6% (53.2% - 56%, 95% CI); high (64.8% - 76.4%, 95% CI) in Blue Nile State, medium (50.5% - 61.9%, 95% CI) in North Kordofan State and South Darfur State and low (28.6% - 35.2% 95%, CI) in Northern State. In high-risk areas (high sero-prevalence), Blue Nile (70.4%) and North Kordofan (57.7%), middle age groups (7 - 12 and 13 - 18 months) were identified as high-risk age. Middle age groups showed lower sero-prevalence than preceding (3 - 6 months) and subsequent (>18 months) age groups while the risk of exposure increased with age. Current and previous findings suggested a transmission pathway of PPRV involving the South Eastern border (Blue Nile) and neighbouring Central Sudan to North Kordofan. One month after vaccination 88.4% (343/388) of sero-negative animals were sero-converted suggesting the efficacy of the locally produced Nigeria 75/1 vaccine. Even if only individuals in the high-risk age group (7 - 18 months) were vaccinated, the overall

population immunity (OPI) in high-risk areas (the Blue Nile and North Kordofan) would have surpassed the threshold of 70%, which is indicated for blocking PPRV transmission. However, lower vaccination coverage is expected in wider vaccination programmes. These findings primarily justified the targeting of PPR control in Sudan through the vaccination of high-risk age groups in high-risk areas.

Keywords

Peste des Petits Ruminants (PPR), Vaccination Efficacy, Seroprevalence, Herd Immunity, High-Risk Area

1. Introduction

Peste des petits ruminants (PPR) are an important transboundary viral disease of small ruminants [1]. It primarily affects sheep and goats, the main target species, and occasionally some other artiodactyls including camels [2] [3] and small ruminant wildlife [4]-[11]. Cattle and buffalo are considered susceptible, particularly to subclinical infection [1]. PPR in sheep and goats is typically acute, more severe in goats, and marked by fever, oculo-nasal discharge, stomatitis, diarrhea, pneumonia, with high morbidity (100%) and mortality (50% - 80%) in naïve populations [1] [8] [12]-[14]. It is caused by a member of the genus Morbillivirus in the family *Paramyxoviridae* [15], presently known as Small Ruminant Morbillivirus (SRMV) [1]. Small ruminant morbillivirus is a single-stranded negative sense RNA. The viral genome encodes six structural proteins: the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin (H) and polymerase (L) proteins and two non-structural proteins, C and V [16]. Based on the partial nucleotide sequence data of the N [17] and F [18] genes, PPR virus (PPRV) isolates can be classified into four genetically distinct lineages: I, II, III and IV. The geographic specificity of these four lineages has been described, which has led to the emergence of molecular epidemiology of PPRV [19]-[23].

PPRV has a global cost of US$2.1 billion and troubles the livelihoods of 900 million poor and low-income people [24] [25] in developing countries in Africa (apart from most of South Africa), the Middle East and West and South Asia [19]. Moreover, PPRV poses threats to wildlife conservation [10] and can spread well beyond its historical range into East Asia and the European region of Turkey [19] to threaten the European Union (EU). To alleviate poverty in many developing countries and to eliminate risks associated with PPRV circulation, the FAO and WOAH, based on the successful eradication of the related RPV, targeted PPRV eradication by 2030 [24]. Like RPV, PPRV is monotypic; available vaccines can induce immunity against all known genotypes *i.e.*, it is of one serotype, and ensuing immunity, following vaccination or infection, is lifelong. Additionally, like RP, the virus does not persist in the environment, and infection primarily requires contact and results in no carrier state [25]. However, since sheep and goats are

more common than cattle, reproducing more rapidly and having less value per head, the vaccination strategy for PPR is likely to pose more challenges and be more expensive than that for RP [25]. Therefore, to develop an appropriate vaccination strategy, the PPR Global Eradication Programs (PPR GER) requires countries to gain clear insight into the epidemiology of PPR and to update the field situation annually to identify hot spots, transmission pathways and populations critical for virus maintenance [24].

Sudan is a vast country in the upper western corner of East Africa that harbors more than seventy million sheep and goats. Peste des petits ruminants were identified in Sudan in 1972 when they were misdiagnosed as RP [26]. Subsequently, it was reported in the country in many instances in camel, gazelles and cattle [3] [11] [27]-[30]. Early Sudanese isolates of PPRV were of lineage III, and later isolates reflected a predominance of lineage IV [11] [23] [28] [30], while the main target species for infection in Sudan have remained sheep and goats. The seroprevalence of PPR antibodies was consistently higher (mostly within the approximate range of 50% - 70%) in sheep and goats than in other ruminant species [31] [27] [32]. Sheep and goats from, almost, all investigated Sudanese states have shown serological evidence (approximately 30% - 75%) of infection [27] [33]-[35]. The heterologous RP vaccine and, subsequently, the homologous PPR vaccine have been used to control diseases in sheep and goats in Sudan. Saeed *et al.* 2010 [27] and Shuaib *et al.* 2014 [34] observed that despite the lack of so-called Differentiation of Infected and Vaccinated Animals (DIVA) capability, the numbers of vaccinated sheep and goats were too small to affect interpretations of serological surveillance.

Local production of a homologous live attenuated vaccine (Nigeria 75/1) against PPR [36] was established in Sudan in 2004 [37]. However, a well-organized vaccination program against PPR has not yet been developed. The objective of this study is to enhance PPR control in Sudan by identifying geographical areas and age groups in sheep and goat populations crucial for PPRV circulation and maintenance. This information aims to prioritize vaccination programs effectively. Additionally, the study evaluates seroconversion following the use of the locally produced PPR vaccine (Nigeria 75/1) under field conditions.

2. Materials and Methods

2.1. Study Area

The study area included four states: the Blue Nile, North Kordofan, South Darfur and the Northern State (**Figure 1**). The first three states are breeding areas, while the Northern State is an important part of a projected disease-free zone. The latter state represents the geographical cluster of northern Sudan, which falls exclusively in the desert ecological zone. The remaining 3 states represent two important geographical clusters [38]: The Western cluster (North Kordofan and South Darfur) and the South Eastern cluster (the Blue Nile state). The Western cluster is the main pastoral area in the country and falls for the most part in the low-rainfall

savannah, in addition to small strips of desert and semi-desert in the North. The south-eastern cluster includes the Nile valley from the South and South-east up to Khartoum state and falls exclusively, apart from Khartoum, in the low-rainfall savannah. The South Eastern cluster is distinguished by large urban centres along the Blue and White Nile and consequent excessive animal movement related to national trade. Three of the surveyed states (Blue Nile, the Northern State and South Darfur) are border areas, while North Kordofan is a central state (Figure 1).

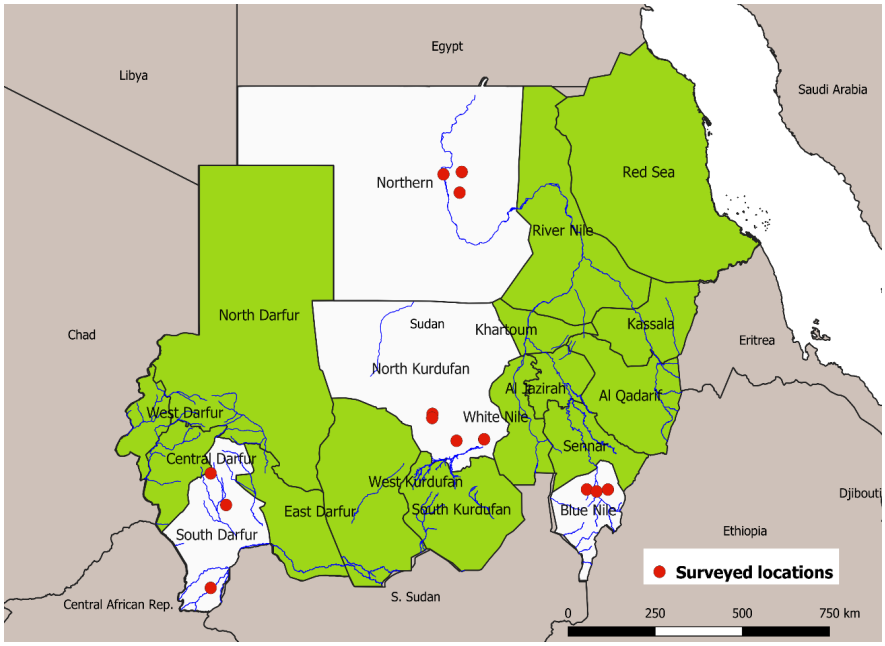

Figure 1. Map of Sudan showing the study area.

North Kordofan State constitutes the western flank of the southeastern cluster, with an area of 185,302 km^2 at the centre of Sudan and populations of 2.7 million goats and 4.2 million sheep [39]. The Blue Nile State has the highest animal density in the country, with populations of 480,718 goats and 4.1 million sheep and an area of 45,844 km^2 bordering Ethiopia and South Sudan [39]. South Darfur is situated far from the Nile valley, bordering the Central African Republic (CAR) and South Sudan, with an area of 81,000 km^2 and 1.7 million goats and 2.2 million sheep [39]. The Northern State has a large area of 348,765 km^2 and relatively low estimates of animal population of 1.2 million goats and one million sheep [39]. It contains border areas with Egypt and Libya.

2.2. Serum Samples

In February 2022, sera were collected from unvaccinated sheep and goats across four states. The sampled animals, all over three months of age and apparently healthy, included 653 sheep and 172 goats. Each sampled animal was ear-tagged and vaccinated with a locally produced PPR vaccine (Nigeria 75/1). The vaccine

batch was transported to the states under the recommended cold chain conditions to ensure its integrity. The same animals were sampled again one month post-vaccination. Throughout the study, animals were closely monitored for clinical signs of PPR infection.

In the first round of sample collection, simple random sampling (SRS) was used to select animals from an available sampling frame of 12 geographical districts or localities (sampling units), 3 in each state (Table 1). The approximate sample size required to estimate the prevalence in an infinite population (large) in each sampling unit was calculated using the following formula [40]:

$$n = \frac{1.645^2 P_{\text{exp}} \left(1 - P_{\text{exp}}\right)}{d^2}$$

where n = the required sample size;

P_{exp} = expected prevalence;

d = desired absolute precision;

1.645 = appropriate multiplier for the required level of confidence.

Table 1. Sample frame and sample size.

State	District (sampling unit)	No. of samples	Total no. of samples
Blue Nile	Damazin	84	250
	Rosayris	67	
	Tadamon	99	
South Darfur	Baleel	58	225
	Kass	99	
	Kateela	68	
North Kordofan	Bara	29	189
	El-Rahd and Um Rwaba	101	
	Shaekan	59	
Northern state	Dongla	92	191
	Elburgeeg	55	
	Marawi	44	

According to previous studies, an expected prevalence (P) of 70% was used [41]. The desired absolute precision of 10% was applied at a confidence level of 90%. Accordingly, a sample size of 58 sera in each sampling unit was targeted. Only two sampling units have not been achieved, while a larger sample size was completed in South Darfur and Blue Nile states, where a larger population size and more dense distribution prevail (Table 1). In each sampling unit, at least eight to ten sampling epi-units (herds or collection sites) were visited to achieve a minimum of 25 epi-units in each surveyed state to adhere to statistical theory regarding

unbiased parameter estimates [42].

2.3. Serological Test

Sera were tested using a commercial competitive ELISA (cELISA) kit (ID screen PPR competition) according to the manufacturer's instructions. Sera from different states of different age and sex groups were tested simultaneously. The optical density (OD) was read using an ELEX808 microplate photometer at a wavelength of 450 nm. The results are expressed as the sample positivity percentage (S/N %). Samples were considered positive if the S/N % were ≤50%, negative if ≥60% or doubtful if it was between 50 and 60%.

2.4. Statistical Analysis

Before and after vaccination, the sero-prevalence of PPR-specific antibodies in each population or sub-population was calculated by dividing the number of positive reactions identified by the cELISA by the number of sera tested in that population or sub-population and then multiplying the result by 100.

Negative and doubtful reactors in pre-vaccination sera (before vaccination) in each population or sub-population were identified and used to calculate the sero-conversion rate by the vaccine. Prevalence and conversion rates were compared using 95% confidence intervals (CIs) calculated with the formula $P \pm 1.96\sqrt{p(1-p)/n}$, where P is prevalence, n is the sample size, and 1.96 represents the confidence level [40]. Non-overlapping CIs indicated statistical significance [43]. For overlapping CIs, p-values were calculated using the chi-square test in SPSS, with significance set at $p < 0.05$ [44].

3. Results

3.1. Sero-Prevalence of PPRV Antibodies in Sheep and Goats Before Vaccination

The overall prevalence of PPRV antibodies was 54.6% (**Table 2**), with specific prevalences of 53.9% in sheep and 57.6% in goats, showing no significant statistical difference between species (P = 0.3864; 95% CI: 50.2% - 57.6% for sheep and 50.2% - 65% for goats). However, significant variation was observed across states, with three distinct sero-prevalence levels: the highest in the Blue Nile, moderate in North Kordofan and South Darfur, and the lowest in the Northern State (**Table 2**). Within states, districts in the Blue Nile and North Kordofan exhibited consistently higher sero-prevalence compared to those in South Darfur and the Northern State, except for one district (Kateela) in South Darfur, which showed an exceptionally high sero-prevalence (85.3%) before vaccination. In three states (Blue Nile, North Kordofan, and the Northern State), district-level sero-prevalence aligned proportionally with state-level estimates. However, in South Darfur, two districts reflected low sero-prevalence levels similar to the Northern State, while the third district (Kateela) exhibited the highest rate observed in this study (**Figure 2**).

Table 2. Sero-prevalence and sero-conversion of PPRV antibodies in Sudanese sheep and goats before and after vaccination.

State	Before vaccination				Post-vaccination			
	No. tested	No. positive	Estimated sero-prevalence	95% CI*	No. –ve	No. +ve	Detected sero-conversion	95% CI*
Blue Nile*	250	176	70.4%	64.76% - 76.4%	74	73	98.7%	92.7% - 100%
North Kordofan	189	109	57.7%	54.11% - 61.92%	80	71	88.8%	79.7% - 94.7%
South Darfur**	225	121	53.8%	50.48% - 57.12%	104	99	95.2%	89.1% - 98.4%
Northern State	191	61	31.9%	28.58% - 35.22%	130	100	76.9%	68.7% - 83.9%
Total	855	467	54.6%	53.19% - 56.01%	388	343	88.4%	84.8% - 91.4%

*p value = 0.427.

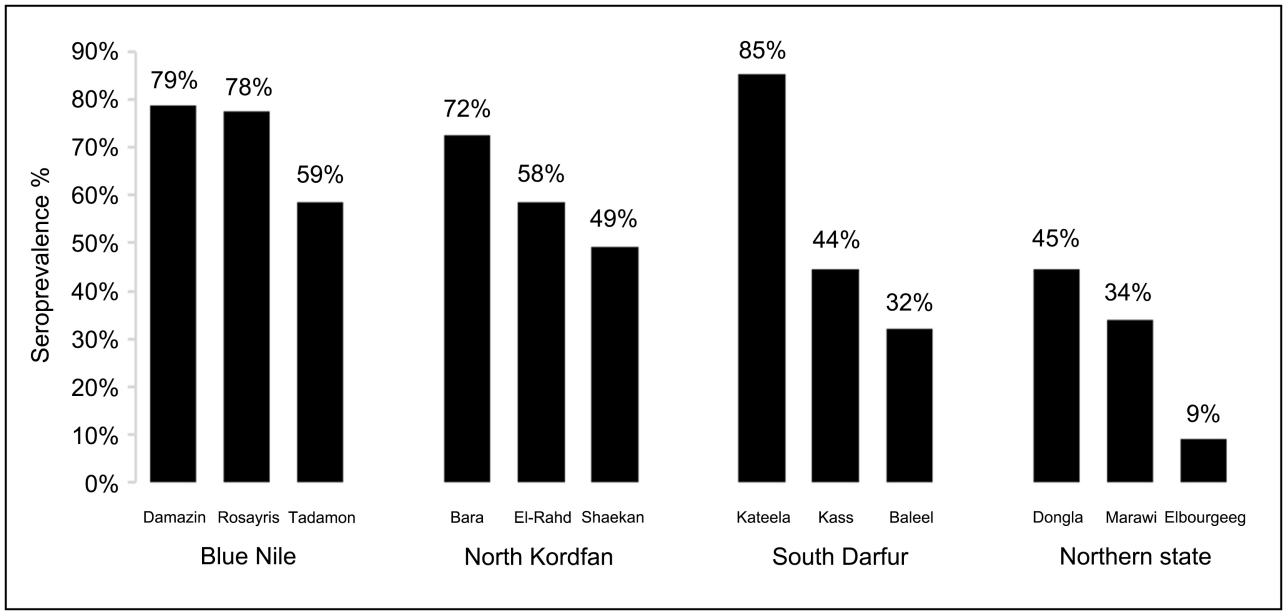

Figure 2. Sero-prevalence of PPRV antibodies in sheep and goats in different districts before vaccination.

Before vaccination, PPRV antibody sero-prevalence was generally higher in older animals (>12 months) and females compared to younger animals (<12 months) and males (Table 3 & Table 4). Statistically significant differences in sero-prevalence between age groups were observed in states with higher prevalence levels (Blue Nile and North Kordofan) but not in states with lower prevalence levels (South Darfur and Northern State). In most states, the highest sero-prevalence was found in the oldest age group (>18 months), except in South Darfur, where this group showed lower sero-prevalence than the 7 - 12 and 13 - 18 months groups. Notably, in the overall test group, the youngest age group (3 - 6 months) displayed slightly higher sero-prevalence than the 7 - 12 months group and was statistically similar (P = 0.05059) to the oldest age group (>18 months).

Table 3. Sero-prevalence of PPRV antibodies in different age groups of Sudanese sheep and goats pre and post vaccination in.

	Age group	<12 months			>12 months		
		3 - 6 months	7 - 12 months	Whole group	13 - 18 months	>18 months	Whole group
Pre-vaccination	Estimated seroprevalence	(28/61) 45.9%	(79/189) 41.8%	(107/250) 42.8	(44/68) 64.7%	(316/536) 59%	(360/604) 59.6%
	95% CI	33.58% - 58.22%	34.82% - 48.78%	36.7% - 48.9%	51.1% - 75.49%	54.86% - 63.14%	55.74% - 63.46%
Post-vaccination	Estimated seroprevalence	(53/61) 86.9%	(174/189) 92.1%	(227/250) 90.8%	66/68 97.1%	500/536 93.3%	566/604 93.7%
	95% CI	78.4% - 95.4%	88.3% - 95.9%	87.2% - 94.4%	93.1% - 100%	91.1% - 95.5%	91.7% - 95.7%

Sero-prevalence in the old age groups did not overlap with those of the young age groups apart from that of the youngest age group (3 - 6 months) which was statistically similar (P value = 0.05059) to that of the oldest age group (>18 months).

Table 4. Sero-prevalence of PPRV antibodies in male and female Sudanese sheep and goats pre- and post-vaccination.

Sex	Pre-vaccination		Post-vaccination	
	Male	Female	Male	Female
Estimated sero-prevalence	(90/202) 44.6%	(377/653) 57.7%	(173/202) 85.6%	(622/653) 95.3%
95% CI	37.8% - 51.4%	53.9% - 61.5%	80.8% - 90.4%	93.7% - 96.9%

Table 5. Comparison of seroprevalence of PPRV antibodies between young (3 - 12 months) and old (>12 months) unvaccinated sheep and goats in different states (dissimilar levels of infection).

State \ Age groups	3 - 12 months	>12 months
Blue Nile [95% CI]	50.7% (37/73) [39.24% - 62.16%]	78.5% (139/177) [72.47% - 84.53%]
North Kordofan [95% CI]	28.9% (15/52) [16.59% - 41.21%]	68.6% (94/137) [60.84% - 76.36%]
South Darfur [95% CI]	51.8% (44/85) [41.2% - 62.4%]	55% (77/140) [46.75% - 63.25%]
Northern State [95% CI]	27.5% (11/40) [13.68% - 41.32%]	33.3% (50/150) [25.75% - 40.85%]

Table 6. Comparison of sero-prevalence of PPRV antibodies between different age groups of unvaccinated sheep and goats in different states (dissimilar levels of infection).

State \ Age groups	3 - 12 months	>12 months		
	3 - 6 months	7 - 12 months	13 - 18 months	>18 months
Blue Nile	(5/9) 55.6%	(32/64) 50%	(29/39) 74.4%	(110/138) 79.7%
North Kordofan	(3/7) 42.9%	(12/45) 26.7%	(8/14) 57.1%	(86/123) 69.9%
South Darfur	(20/44) 45.5%	(24/41) 58.5%	(6/8) 75%	(71/132) 53.8%
Northern State	(0/1) 0.0%	(11/39) 28.2%	(1/7) 14.3%	(49/143) 34.3%

3.2. Performance of the PPR Vaccine

The vaccine effectively converted 88.4% of negative and doubtful sera to positive. Conversion rates exceeded 90% in all states except the Northern State, where it was 76%. District-level conversion rates were 100% in 2/12 districts, ≥90% in 8/12 districts, >85% in 11/12 districts, and lowest (58%) in Elburgeeg, Northern State. The conversion rate at Elburgeeg (58%) was significantly lower (P = 0.00001) than in other districts (92.9%). Post-vaccination sero-prevalence followed conversion trends, exceeding 90% in the Blue Nile, North Kordofan, and South Darfur states, and reaching 84.3% in the Northern State. Overall, sero-prevalence reached 93% (95% CI: 91.3% - 94.7%).

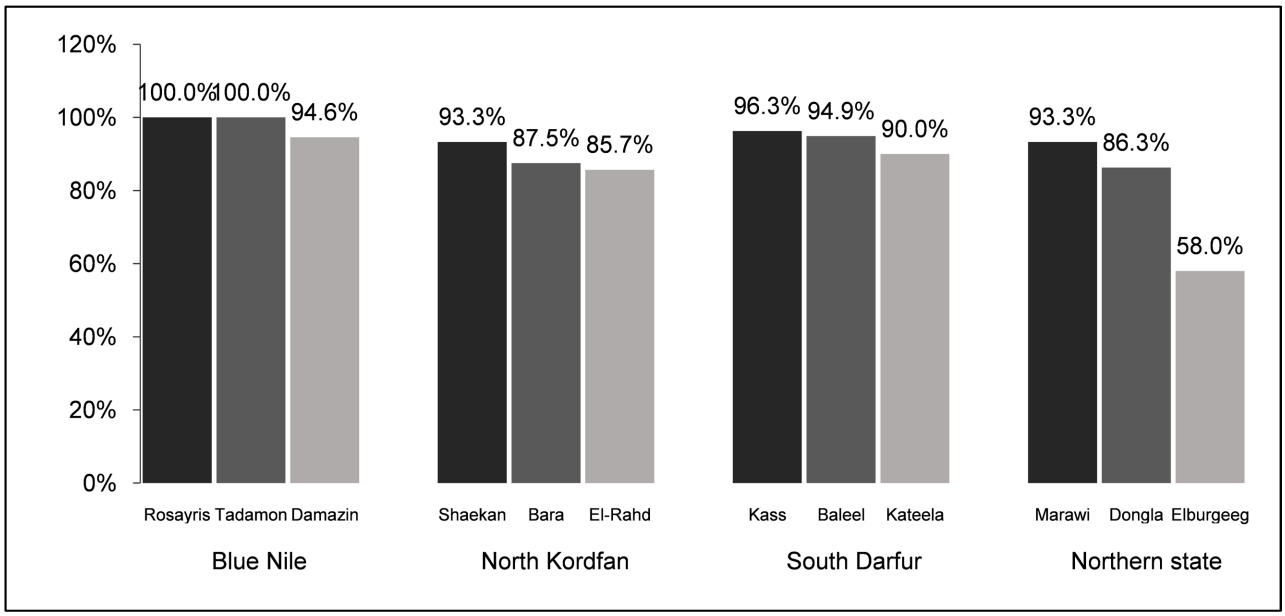

Figure 3. Conversion rate of negative sheep and goat sera (and doubtful) in different districts to positive following vaccination against PPRV.

In a second ELISA round, 3.2% of serum samples reverted to doubtful or negative, narrowing inter-state differences from 31.9% - 70.4% pre-vaccination to 84.3% - 98.4% post-vaccination. Differences among age groups disappeared, with overlapping 95% CIs and P > 0.05, although the youngest group (3 - 6 months) showed the lowest sero-prevalence. Sero-prevalence remained significantly different between males and females post-vaccination (Table 4). Limiting vaccination to 7 - 18-month-old animals would have achieved post-vaccination sero-prevalence rates of 87.2%, 75.5%, 62.2%, and 44.5% in the Blue Nile, North Kordofan, South Darfur, and Northern State, respectively.

No PPR-like clinical signs were observed or reported during the study, and no discrepancies arose from serological testing.

N.B. Discrepancy between sero-prevalence values after vaccination and detected conversion rates (Table 2, Figure 3) was due to that some +Ve sera before vaccination failed to reproduce +Ve values (scored −Ve or doubtful values).

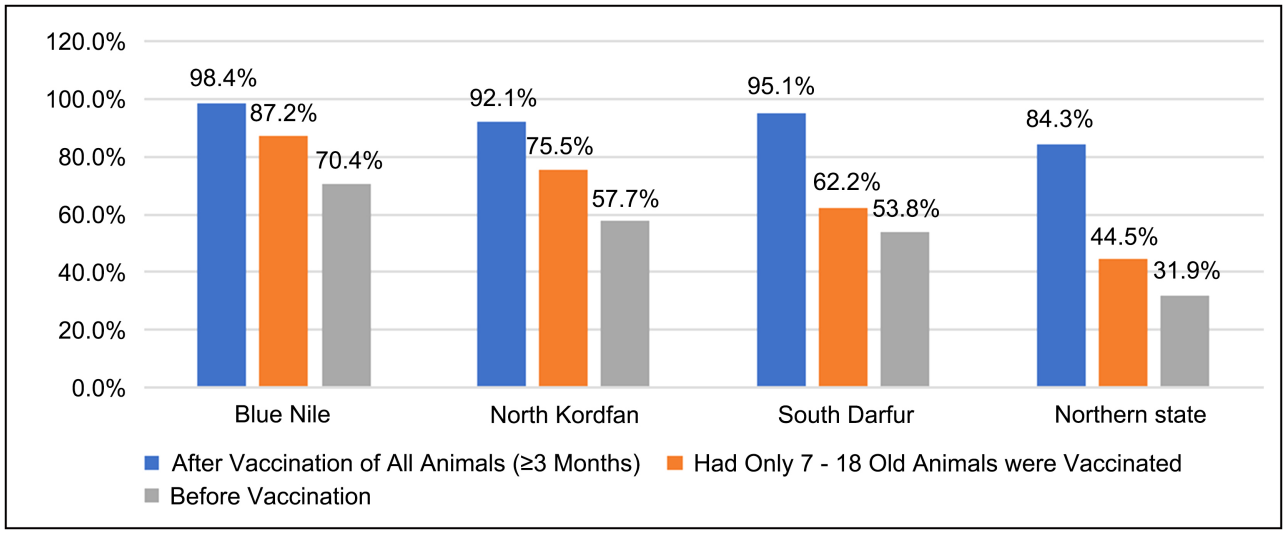

Figure 4. Comparison of Sero-prevalence of PPRV antibodies before and after vaccination of Sudanese sheep and goats in different states.

4. Discussion

The present work aimed to investigate the field performance of the locally produced PPR vaccine; and to update and gain clearer insight into the epidemiology of PPR in Sudan. In the latter contest, it is crucial to survey apparently healthy, unvaccinated flocks. In previous reports, Saeed *et al.* 2010, and Shuaib *et al.*2014 [27] [34] expected that the numbers of sheep and goats vaccinated against PPR in Sudan were too low to affect interpretations of serological surveillance. Serological data obtained in this work largely supported these suggestions. Test animals in the older age groups (>12 months and >18 months) generally showed higher sero-prevalence than young age groups (<12 months) suggesting an increase in the risk of exposure to natural infection with higher age (Table 3). The differences were found to be more significant (statistically) where sero-prevalence was high (Table

5). It was associated with high exposure of young and old animals to PPRV rather than merely the old (**Table 5**). The latter finding challenged the probability of old animals receiving vaccination once during a lifetime in endemic areas, as suggested by some workers [45]. Differences in sero-prevalence between old and young animals have completely disappeared after vaccination (**Table 3**) or had been remarkably low in South Darfur (**Table 4** and **Table 5**). In South Darfur, recent exposure to infection was likely in one district (Kateela) that showed an exceptionally high sero-prevalence (85.3%) similar to what was reported (88.9%, 90.7% and 88.6%) in sheep and goats following PPR outbreak [46] or following PPR vaccination in this work (**Figure 4**). Recent circulation of PPRV in 1/12 of the surveyed districts was not unlikely under the endemic situations probably prevailing in Sudan. These results support the reliability of the outcomes of this work.

However, Serological data on PPR infection in Sudan were not meager; frequently available competitive ELISAs [27] [32]-[34] [46] [47] and rarely other tests such as counter-immuno-electrophoresis (CIEP) [33] have been used. Previous work has determined the extent of PPR infection in different ruminant species with pin-point sheep and goats being the main target species of the disease in the country. the wide geographical distribution of PPR infection in Sudan has been confirmed, however, serological data remain largely tentative and sometimes contradictory regarding high-risk areas. In this work, surveillance was carried out in four Sudanese states, three of which represent the main pastoral and animal breeding areas in the country in South Eastern (Blue Nile) and western Sudan (North Kordofan and South Darfur). The 4th state (the Northern State) represents a distinct ecology, the desert ecosystem, where limited animal breeding and pastoralism are practiced. The detected sero-prevalences in sheep (50.2% - 57.6%, 95% CI) and goats (50.2% - 65% 95% CI), in this study, were statistically similar. Previous reports from Sudan [27] [48] [49] and elsewhere Singh *et al.* 2004 [50] have generally shown slightly higher sero-prevalence in sheep than in goats. In this work, the wide divergence in the numbers of tested sheep (683) and goats (172) and in their origin from different geographical regions (data not shown) with different levels of infection could have resulted in such slight disagreement. In the present work, in different geographical regions, distinct levels of indices of PPR infection were detected: high (64.8% - 76.4%, 95% CI) in South Eastern Sudan (the Blue Nile state), medium (50.5% - 61.9% 95% CI.) in Western Sudan (North Kordofan and South Darfur), and low (28.6% - 35.2%, 95% C.I) in Northern Sudan (Northern state). Similarly, districts (n = 6) in Blue Nile and North Kordofan (higher sero-prevalence) showed higher indices of PPR infection than districts (n = 6) in South Darfur (excluding Kateela) and Northern State (lower sero-prevalence) (**Figure 2**). The lowest sero-prevalence in the Northern State was consistent with the known low density [39] and animal movement in the desert ecosystem in North Sudan. On the other hand, the Blue Nile state shows the highest animal density in the country [39] and uniquely constitutes the South-eastern border of Sudan; the nearest point to East Africa (**Figure 1**). The Blue Nile state

encompasses border areas with South Sudan, such as South Darfur, and border areas with southern Ethiopia. Molecular data indicated that earlier Sudanese isolates of PPRV were Lineage III which is known to be circulating in East Africa including Ethiopia [19] [23]. In Central and West African countries, which are closer to Western and South Western Sudan, different lineages, lineage I and II, respectively prevailed [19] [23]. The emergence of the Asian lineage of PPRV (lineage IV) in Sudan [28] and other African countries was an illustration of the transboundary nature of PPRV. Network analysis of unique sequences (haplotype) from the 101 N-gene and the 103 F-gene of lineage IV revealed the presence of multiple clusters in isolates from Sudan [22]. Some of these Sudanese clusters showed closer associations with isolates from other countries than with other Sudanese clusters, indicating the likely multiple waves of introduction of PPRV from neighbouring countries [22]. Accordingly, molecular data have highlighted the epidemiological links between PPR outbreaks in Sudan and neighbouring countries, mainly in East Africa, and have added particular significance to the high indices of PPR infection in the Blue Nile state. Other North-eastern border areas in the country are adjacent to northern areas in neighbouring countries where, like in Sudan, less circulation of PPRV is expected.

The medium indices of PPR infection detected in western Sudan were lower (50.5% - 57.12%, 95% CI) in South Darfur at the South Western border than in North Kordofan (54.1% - 61.9%, 95% CI) in Central Sudan. Sero-prevalence's detected in districts in South Darfur was not uniform; it was very high in one district and low in two districts which once more signifies the relatively low sero-prevalence in South Darfur compared to North Kordofan (**Figure 2**). South Darfur borders South Sudan and CAR, while North Kordofan is a central state comprising most of the western flank of the Nile Valley (**Figure 1**). In another instance, Abdalla *et al.*, 2012 [48], North Kordofan also showed high sero-prevalence [68.4% (n = 215)] similar to that of the Blue Nile [69.3% (n = 280)] and significantly higher than that of Al Qadarif [28.6% (n = 105)], a border state in Eastern Sudan. Saeed *et al.* 2017 [32] reported high sero-prevalence's of PPR-specific antibodies in sheep from central (White Nile, Al Gazeera and Blue Nile) states [70.7% (n = 1674)], Darfur (North and South Darfur) states [68.1% (n = 4062)] and Kordofan (North and West Kordofan) states [58.3% (n = 585)]. Constantly significant indices of PPR infection were detected in Central states including North Kordofan; higher in some instances than border areas in Eastern and Western Sudan. A working hypothesis of PPR circulation in Sudan involves the intense circulation of the infection in the breeding and border area of the Blue Nile state and in adjacent Central Sudan, where main animal markets and animal trade exist; this possibility is worthy of further investigation.

Under field conditions and in the absence of any PPR-like clinical signs, vaccination with the locally produced PPR vaccine (Nigeria 75/1) produced an antibody response in 88.4% (343/388) of negative sheep and goats, as detected by cELISA. The commercially available cELISA tool is indicated by WOAH 2022 [1]

for assessing antibody responses following PPR vaccination or infection and is among the most commonly used tests for this purpose [51]. A 100% sero-conversion in experimental animals (mainly goats) vaccinated with the Nigeria 75/1 vaccine was reported in laboratory studies [51]-[53]. After a vaccination campaign in all of Somalia with the Nigeria 75/1 vaccine, individual animal sero-prevalence increased from 62% before the vaccination campaign to 76% [54]. The N-specific antibodies detected by the employed cELISA are not neutralizing antibodies. Nonetheless, they are considered indicators of an ongoing T-cell mediated immune response [53]. Evaluation of antibody responses following PPR vaccination using virus neutralization test (VNT), H-antigen-specific antibodies cELISA which are neutralizing antibodies [55] or N-antigen-specific antibodies cELISA and challenge studies reported no discrepancy between sero-conversion and protection [51]-[53]. On the other hand, Saravanan *et al.* 2010 [56] reported 6/6 protection against challenge in goats vaccinated with a PPR vaccine but 4/6 sero-conversion by VNT and cELISA. The Nigeria 75/1 is one of two commonly used PPR vaccines, and it is universal efficacy has been strongly established against the four known genetic lineages of PPRV [52] [57] [58]. In addition to suggesting the efficacy of the locally produced Nigeria 75/1 vaccine, the findings presented here support its universal efficacy, particularly in sub-Saharan Africa, where poor veterinary infrastructure generally prevails.

Sero-conversion rates were approximately 90% in three states and 76.9% in the fourth state (the Northern State). It was consistently around 90% in 11/12 surveyed districts, apart from one district, Elburgeeg, in Northern State where it was 58% (29/50). Two out of the three districts in the Blue Nile state have shown 100% sero-conversion (**Figure 3**) which was also consistent with the comparatively lower numbers of sero-negative animals in the Blue Nile (74) and the high sero-conversion rate (88.4%) reported in this work. Alternatively, sero-conversion at Elburgeeg was statistically significantly different from that at other districts ($p = 0.00001$). The Nigeria 75/1 vaccine is thermolabile [36] and maintenance of the cold chain in the field is necessary to achieve acceptable vaccine performance and efficacy. After reconstitution of the vaccine material, it is preferred to be administered within 30 minutes [24]. The vaccine delivery mechanism, including the cold chain, seemed to be functioning efficiently in 11/12 of the vaccinated districts. A low post-vaccination sero-conversion rate (61.13%) in Ethiopia, Faris *et al.* 2012 [59], similar to that observed in Elburgeeg, was attributed to the inadequacy of the cold chain. It is to be expected that at areas like Elburgeeg where little acquaintance with the disease has been made (low sero-prevalence), observation of vaccine delivery mechanisms and cold chain recommendations would be less strict.

Sero-prevalence of PPRV antibodies in the tested animals increased from 54.6% (467/855) before vaccination to 93% (795/855) one month after vaccination. Accordingly, the range of differences between states (**Table 2**) largely decreased (**Figure 4**) and the differences between age groups disappeared (overlapping 95% CI)

(Table 3). Interestingly, after vaccination, the youngest age group (3-6 months), unlike in unvaccinated animals, exhibited, among the different age groups, the least sero-prevalence (Table 3) suggesting a degree of interference between maternal immunity and vaccination. Ata *et al.* 1989 [60] and Bedjeh *et al.* 1999 [61] indicated that maternal antibodies remain detectable up to 6 months of age and maintain protective effects up to 3.5 and 4.5 months in lambs and kids respectively. Significantly higher sero-prevalence persisted in females than in males before and after vaccination, which is consistent with higher ser-prevalence of old animals than in young animals (3 - 6 months) before and after vaccination. Acharya *et al.* 2018 [62] explained differences in sero-prevalence between females and males by their different proportions of old and young groups in herds. Females are usually kept for longer periods in herds for reproduction, while males are sold for meat production. Several other studies also reported higher sero-prevalence in females than in males [48] [49] [63].

The Sero-prevalence of PPRV antibodies detected during this work after vaccination was a precise measure of the response to vaccination. These indices represented vaccinated population immunity (VPI). The quality of the vaccination programs depends on vaccine coverage in addition to the VPI, while the best indicator of breaking virus transmission is overall population immunity (OPI). The results presented in Figure 4 show herd immunity after vaccination of all animals or when only middle age group (7 - 18 months) animals were vaccinated. The former was strictly VPI while the latter was OPI of the experimental herd had only middle age group was vaccinated. However, in both cases vaccination coverage was 100% which is unachievable in a wide vaccination program. The middle age groups (7 - 12 and 13 - 18 months) were selected for this demonstration for two reasons. First, it showed, particularly in Blue Nile and North Kordofan, lower sero-prevalence than preceding and subsequent age groups (Table 6) while the risk of exposure to PPR infection increased with age (Table 3) *i.e.* high-risk age. Second, before vaccination, it was demonstrated in South Darfur, when recent circulation of PPRV was suspected, there was a higher sero-prevalence than those in the higher and lower age groups (Table 6). Evidently, VPI values were high; above 90% in three states and decreased to 84% in the Northern State. On the other hand, values of OPI (if only middle age groups were vaccinated) have a range as low as 44.5% (Northern State). It reached 75.5% in North Kordofan and 87.2% in Blue Nile; well above the threshold of 70% indicated by some workers [64] [65] for breaking of effective transmission of PPRV. In state-wide vaccination programs, lower vaccination coverage than the one applied in this work (100%) is expected, yet the achieved herd immunity in these two states is unlikely to fall considerably below 70% since pre-vaccination sero-prevalence was already high. These findings suggested targeting control efforts of PPR in Sudan by vaccination of high-risk age groups in high-risk areas such as Blue Nile and North Kordofan.

The study's limitations include restricted geographic coverage, reliance on serological data without molecular diagnostics, and a lack of long-term follow-up to

assess vaccine efficacy and immunity duration. Regional variations in vaccination outcomes highlight potential cold chain and delivery challenges. Broader geographic studies, integration of molecular tools, and extended monitoring are needed to address these gaps and enhance PPR control strategies in Sudan.

5. Conclusion

This study confirms the efficacy of the locally produced PPR vaccine in inducing seroconversion and highlights regional variations in antibody prevalence across Sudan. The findings support its use in national vaccination campaigns and offer valuable insights for PPR control policies. The research contributes to global PPR eradication efforts, strengthening strategies to enhance animal health, food security, and economic stability.

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Ethics Approval and Consent to Participate

This study strictly follows institutional, national, and international ethical guidelines, including the Basel Declaration and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Approval was obtained from the Central Veterinary Research Laboratory/academic committee (No. 021/092-8004365). Sample collection was conducted by trained veterinarians with owner permission, prioritizing animal welfare. Vaccination was performed by state veterinary authorities using a certified vaccine that passed all quality control tests. The study emphasizes ethical standards, animal welfare, scientific integrity, transparency, rigor, and reproducibility. Relevant details are included to ensure critical evaluation and replication, with all authors voluntarily participating in the research.

Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Authors' Contributions

Conceptualization: (Omer Algezoli, Selma Kamal, Tageldin Nour, Mohamed Abdalla); Methodology: (Omer Algezoli, Yazeed Raouf, Mozdalifa Alamin, Hiba Ali); Sampling, vaccination and monitoring of animals: Mohamed Aljameel., Sulieman Ahmed, Ibtesam Fadul Elsied, Sir Elkhatim Salih; Analysis (Omer

Algezoli, Mozdalifa Alamin, Yazeed Raouf, Selma Kamal) Writing and editing: (Omer Algezoli, Yazeed Raouf, Mohamed Abdalla); Supervision: (Tageldin Nour, Mohamed Abdalla). All the authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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