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Assessment of *Peste Des Petits* Ruminants Antibodies in Vaccinated Yankasa Pregnant Ewes from Nigeria and the Duration of Maternal Immunity in Their Lambs

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ABSTRACT

Introduction: *Peste des petits* ruminants (PPR) or goat plague is a highly contagious viral disease of small ruminants such as sheep and goats with 90% and 100% morbidity and mortality, respectively. This study was aimed at assessing *Peste des petits* Ruminants Virus (PPRV) specific antibodies in vaccinated pregnant ewes and subsequently the passive immunity in their lambs. **Methods:** Seventeen apparently healthy sheep (8 pregnant and 9 non pregnant), 2-3 years old and kept under semi-intensive system of management were used. Ewes were vaccinated with the National Veterinary Research Institute PPR vaccine Nigeria strain 75/1 with a virus titre of 10^3 Tissue Culture Infectious Dose (TCID). Serum Samples were collected from all the sheep before and after vaccination at interval of two weeks for a period of seven months. The resultant (8) lambs were given birth to, blood sample were collected for four month and sera samples were examined using Competitive ELISA (c-ELISA) for the presence of specific PPR-N antibodies. **Results:** The analysed result showed that there was significant difference ($P < 0.05$) in the mean PPRV-N specific antibody c-ELISA values (0-13) before vaccination and the percentage competition protective values (> 50%). However, no significant difference ($p > 0.05$) post-vaccination in both pregnant and non-pregnant ewes was observed throughout the period of the study with mean PPRV-N specific c-ELISA antibodies of 72-86 and 52-86, respectively. The mean PPRV-N specific antibodies values were maintained within the protective value (> 50 %). The result of this study also showed that there was significant difference ($P < 0.05$) with mean PPRV-N specific c-ELISA antibodies (17.3-29.4; 87.5%) of lambs born to vaccinated pregnant Yankassa ewes from 8 weeks. **Conclusion:** This study showed that vaccination does not affect pregnancy with Nigeria 75/1 strain of PPR vaccine in ewes as there was no record of abortion. There was a rapid PPR maternal antibody decay in lambs from the 8th week of age as it was observed that at age 10 weeks, only 37.5 % of the lambs had protective titre. It is therefore recommended that lambs can be vaccinated at 9th week to avoid the window of susceptibility to PPR virus infection.

Citation:

INTRODUCTION

Small ruminant animal production contributes to the food security and sustainable living of most farmers in developing countries like Nigeria. However, the sustainable production of small ruminant animals in Nigeria has been faced with several challenges including the continuous Peste des petits Ruminants Virus (PPRV) attacks on these animals [1]. *Peste des petits* ruminants (PPR) virus is a negative strand RNA virus belonging to the family paramyxovirus of the genus *Morbillivirus* [2]. It has been reported that the morbidity and mortality rate of this virus is in the range of 90%-100% depending on the breed of the animal, general husbandry and susceptibility rate of the animal [3]. Moreover, Victor et al. (2017) [1] found that a high prevalence rate of 71.2% and 63.6% was associated with open grazing and pastoralist systems respectively. The authors further reported higher prevalence (30.8% vs. 29.2%) for Yankassa sheep and West African Dwarf Goats (WAD), respectively.

Despite the continuous prevalence of this virus, the homologous live attenuated strains of PPR virus vaccine (PPRV/Nigeria/75/1) was developed by Diallo et al. (1989) [4]

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and have been reported to be used extensively by both household and field farmers [5] to reduce the incidence of PPR virus disease. The diagnosis of PPR has been achieved through serological and molecular techniques including competitive ELISA (c-ELISA), Immunocapture ELISA (Ic-ELISA), agar gel immunodiffusion (AGID), PCR, isolation on cell culture and haemagglutination inhibition [6] . Furthermore, it is noteworthy that there is no tool in literature to differentiate between the vaccinated and the infected animals.

Studies [5, 2] have reported various responses of small ruminants to PPRV vaccine. Djallonke Sheep breed vaccinated with PPRV/Nigeria/75/1 have been reported to produce low rates of maternal antibodies decay [5] and lambs were more susceptible to PPRV disease if vaccinated at a later stage of growth (above 4 months). Bodjo and colleagues have reported that over 70% of lambs tested showed negative response. Thus there is need to vaccinate lambs from 75 to 90 days [5]. In light of this, there is paucity of information in literature on the maternal response and antibody decay of Yankassa sheep predominantly found in Nigeria. Based on this premise, the objectives of this study was to assess the effectiveness of PPRV/Nigeria/75/1 in pregnant Yankassa ewes.

MATERIALS AND METHODS

Ethics Statement

Ethical clearance was obtained for the use of animals in this study in line with the ethics of the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), Kaduna State, in Nigeria.

Animals

The Study was carried out in Zaria, Kaduna, a Northern Guinea savannah zone of Nigeria (latitude11o12 N, longitude 7^{o} 33 E and altitude of 610m). Seventeen Yankassa breed of ewes under natural breeding, semi-intensive system of management was maintained, with history of no PPR vaccination. Ewes of age's 2-3years were screened for helminths and haemoparasites by examination of faecal and blood, respectively. Each animal was duly and properly tagged. Eight pregnant ewes delivered 8 lambs which were used for this study and they were sampled every two weeks for a period of 4 months alongside with 9 non-pregnant ewes. The ewes were not synchronized.

Vaccination

The National Veterinary Research Institute live-attenuated PPR vaccine (batch No. 1/2017) was reconstituted with 50 ml of distilled water Tissue Culture Infectious Dose (TCID = 10^{3}) and administered at 1ml/dose subcutaneously to the dam.

Collection of Blood Sample

Four ml of blood was collected from the dam every 2 weeks. Upon parturition, 2 ml of blood sample was collected from the lambs at 2 weeks intervals for 3 months. The collected blood samples were allowed to clot to obtain sera. The collected sera was then transfered to sterile bottles and stored at -20˚C until examined.

Serology

A kit of c-ELISA (IDvet, Gabrel- France) designed to detect antibodies against the nucleoprotein of PPRV was used, based on the manufacturer's instructions.

Test Procedure

All reagents and samples were allowed to come to room temperature (21˚C ±5˚C) before use. They were also homogenized by inversion or vortex. Twenty-five µl of dilution buffer 13 was added to each well. Twenty-five µl of the positive control was added to wells A1 and B1; 25 µl of the Negative control was added as well to the wells C1 and D1. Twenty-five µl of each samples to be tested was also added to the remaining wells. It was incubated for 45min ±4minutes at 37˚C (± 3˚C). Each well was then washed 3 times with approximately 300 µl of the washing solution and drying of the wells between washings was avoided. The preparation of 1X conjugate was performed by diluting the 10X conjugate to 1/10 in a dilution buffer 4. One hundred µl of the 1X conjugate was added to each well. It was incubated for 30 min ± 3min at 21˚C (± 5˚C). Each well was washed 3 times with approximately 300 µl of the wash solution and also drying of wells between washings was avoided. One hundred µl of the substrate solution was added to each well and was incubated for 15min ± 2min at 21˚C (± 5˚C) in the dark. 100 µl of the stop solution was added to each well in order to stop the reaction and it the optical density (O.D) was read and recorded at 450 nm. The sera were analysed for the PPRV antibodies in the PPR LAB of the Viral Research Division of National Veterinary Research Institute (NVRI), Vom, Nigeria.

Interpretation of the Test

For each sample, the competition percentage (S/N %) was calculated using the Optical Density (O.D):

S/N% = [O.D of the test serum x 100]/[O.D of the Negative control]

S/N % ≤ 50% were considered positive; greater than 50% and less than or equal to 60% were considered doubtful while S/N% > 60% were considered negative.

Data Analysis

Data collected for antibody values were subjected to one way ANOVA to determine differences between groups using GraphPad Prism version 5 at a 95% confidence level (P< 0.05).

RESULTS

Mean Values of Ewes Pre-vaccination, Pregnant and Non-pregnant Post-vaccination

Pre-vaccination, both pregnant and non-pregnant ewes had no protective antibody levels. Post-vaccination, pregnant and non-pregnant ewes had antibody levels that were protective at 2 weeks post-vaccination, with 13 % to 47 % competition percentage (S/N) of PPRV N antibodies (Table 1). The antibody values pre-vaccination was different significantly (P < 0.05) from those of post-vaccination for both pregnant and non-pregnant ewe. There was no significant difference (P > 0.05) post-vaccination, between the antibody values throughout the period for both pregnant and non-pregnant ewes. However, at the second week post-vaccination, all the ewes showed had protective antibody levels (seroconversion).

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Table 1. Mean and percentage PPR antibody values of pregnant, non-pregnant ewe, pre- and post-vaccination over time.

Period Pre- and Post-vaccination with PPR Vaccine (Weeks)	Mean (SEM)		Percentage (%)		F-Value	*P*-Value
	Non-pregnant (N=9)	Pregnant (N=8)	Nonpregnant (N=9)	Pregnant (N=8)		
Pre-vaccination	97.59[b]±3.2	86.59[b]±10	0	12.5	20.043	0.000*
2	47.59 [a]±8	15.75 [b]±3.8	100	100		
4	45.59 [a]±3.8	13.75 [a]±3.1	100	100		
6	30.4 [a]±5.6	27.28 [a]±5.9	100	100		
8	30.94 [a]±2.4	24.00 [a]±3.4	100	100		
10	29.27 [a]±2.8	21.13 [a]±5.2	100	100		
12	19.44 [a]±3.6	26.63 [a]±8.2	100	100		
14	20.74 [a]±2.7	16.63 [a]±3.9	100	100		
16	13.32 [a]±3.4	24.63 [a]±4.1	100	100		
18	18.72 [a]±3.5	25.13 [a]±3.9	100	100		
20	23.38 [a]±3.4	27.33 [a]±13	100	100		

*significant at $P<0.05$ Mean values with the same alphabet superscript do not differ significantly from each other using a Post HOC Ducan - multiple range test.

Mean and Percentage Values of Maternal Antibodies in Lambs

Lambs had protective maternal antibody levels (100%; 8.88) postpartum up till week 8 (87.5%; 24.25), week 10 (37.5%; 45.60) and week 14 (25%; 63.50). Significant difference ($P < 0.05$) exists in the mean maternal antibody levels of the lambs from week 12 postpartum (Fig. 1).

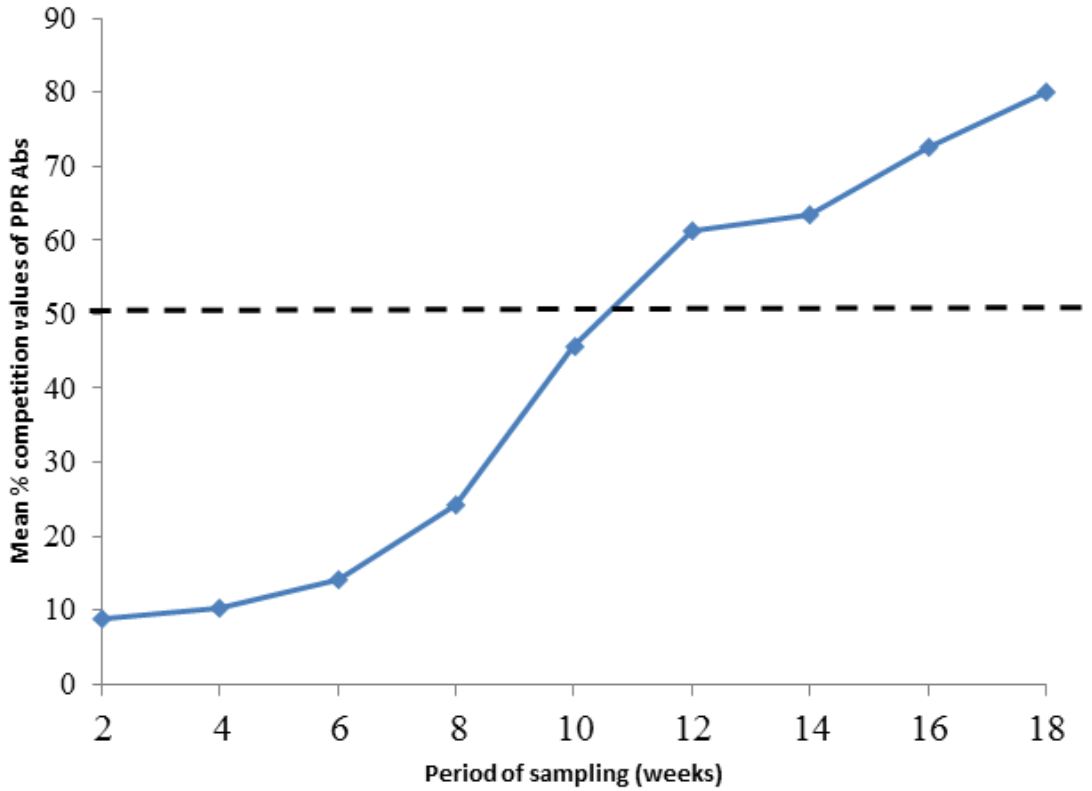

Fig. 1. Mean percentage competition values of maternal antibody in lambs with time.

Mean Percentage Pompetition Values of Maternal Antibodies in Lambs and Pregnant Ewe

The expected decline in lambs' antibody titre confirms the half-life of IgG of about 28 days is contrasted by virtually identical titres in ewes over time (Fig. 2).

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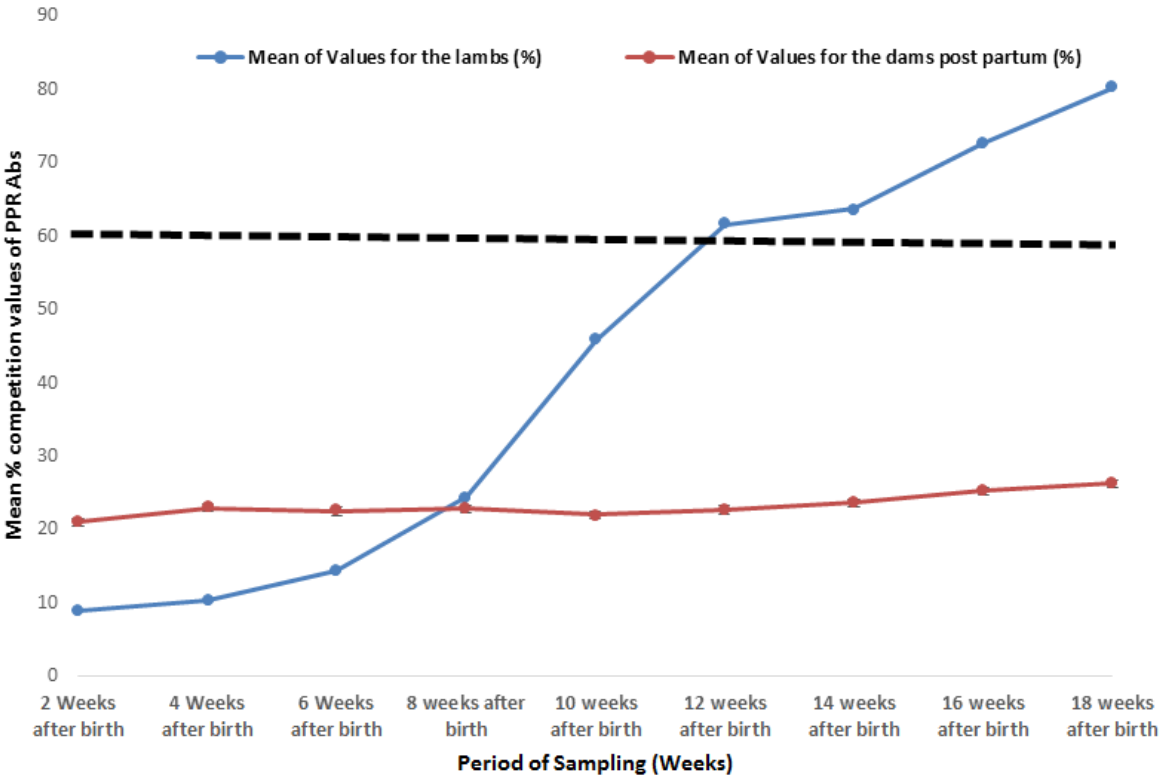

Fig. 2. Relationship between mean percentage competition values of maternal antibody level in the Lambs and the mean value of antibody titre in ewe to PPR over time.

DISCUSSION

The PPRV N antibodies obtained from this study were maintained below the threshold S/N value (50%) and ranged between 4 to 50% and so there was no significant difference in the values post-vaccination against PPRV for both pregnant and non-pregnant, indicating adequate protective titre, which may be suggestive of protective humoral response. This is similar to the reports of [7]. Two weeks post-vaccination, mean percentage titre in pregnant and non-pregnant ewes were 15.75% and 47.59% respectively; thus, suggesting rapid seroconversion with peak value observed at week 4 (13.75%) in pregnant ewes. However, the peak titre (14.32%) for the non-pregnant ewes was observed at 4 month post-vaccination and this is similar to the report of [8] who observed peak titres in sheep at 4 months post-vaccination. This suggested that pregnancy enhances the rapid seroconversion of the vaccine as observed in this study [9] reported a different trend in the frequency distribution of S/N values for goats and sheep and this disparity may be due to the differences in breed (foulbe breed of sheep), age, health status of animals, management conditions (Intensive System) and vaccine (Sungri strain) used.

The significant difference ($P < 0.05$) in the antibody values pre- and post-vaccination indicated that all ewes before vaccination were not protected against PPRV. A probable explanation is that the animals used in this study were reared under intensive management system with no contact with other animals of the area and no history of PPR. This observation is in agreement with those reported by [4], [10] and [11]. Lambs had protective antibody level postpartum until week 10 – 12 where less than 50% (37.5%) maintained the protective levels and there was significant difference ($P < 0.05$) from up until week eighteen postpartum. This study showed that lambs should be vaccinated at 9 weeks of age to avoid the window of susceptibility. This is in contrast to the findings of [12] and [7] who reported that vaccination should be carried out after 4 months of age using Sungri strain. This variation may be attributed to the type of vaccine and the breed of sheep used. That would beg the question as to why there is no such decline in the adults. This could be as a result of constant boosting by (residual) live virus or perhaps the only one shot of the vaccine given to the ewes maybe responsible for this behavior.

In conclusion, there was significant difference ($P < 0.05$) in the mean antibody values pre-vaccination as compared to post-vaccination and this indicated that the PPR N antibodies pre-vaccination were below the protective values (> 60%), suggesting that all were not protected against PPR. The NVRI vaccine was safe in both pregnant and non-pregnant Yankassa breed of ewes. There was a rapid PPR maternal antibody decay in lambs after 8 weeks of age. This study has indicated that the lambs should be vaccinated at 9 weeks of age to avoid the window of susceptibility as less than 50% (37.5%) of the lambs had protective titre at 10 weeks of age. Further studies should be carried out to demonstrate the role of cell mediated immunity in the protection against challenge with virulent PPRV. Moreover, studies should be conducted in other breeds of sheep as to determine the pattern of PPR antibody decay in lambs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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