Effects of *peste des petit ruminants* N/75/1 vaccine on nasal bacterial flora and clinical indices in Red Sokoto goats

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**Abstract**

Peste des petit ruminants virus (PPRv) and *Mannheimia haemolytica* have been commonly implicated in naturally occurring pneumonia in sub-Saharan Africa and account for huge economic losses as they are associated with high morbidity and mortality. The Nigerian 75/1 PPR vaccine has been proven to be efficacious in the control of PPR. However, there is a paucity of information on the ability of this live attenuated vaccine to modulate bacteria of the nasal flora to control or confer protection against secondary bacterial infection induced especially by *M. haemolytica*. Nasal swabs and blood samples were collected for bacterial count and serology respectively. This study describes the effect of the N/75/1 PPR vaccine on the nasal bacteria flora and clinical indices of Red Sokoto goats. Ten (10) Red Sokoto goats were divided into two groups of five animals each. In one group, 1ml of PPR vaccine (2.5 TCID50) was administered subcutaneously while the other group served as control and 1ml of normal saline was administered subcutaneously. The results showed that for the PPR vaccinated group, although there was a significant decrease (p≤0.05) in nasal bacteria counts for *Mannheimia haemolytica*, *Pasteurella multocida* and *Escherichia coli* compared to the control group it was not sufficient to clear out these potentially pathogenic bacteria from the nasal flora. Also, in the PPR-vaccinated group, the only clinical symptom observed was a mild transient and self-limiting hyperthermia at about 2 weeks post-vaccination which may be attributed to the initiation and enhancement of immune responses. Hence, it can be deduced that the PPR vaccine may moderately inhibit the colonization of certain pathogenic bacteria of the nasal microbial flora but however, it is incapable of conferring total immunity against the secondary bacterial infection in naturally occurring pneumonia.

**Keywords:** Bacterial flora, Goats, Nasal cavity, Nigeria, PPR vaccine

**Introduction**

Small ruminants (especially goat) farming contributes largely to the nutritional, economic and social security of the rural farmers especially women and children who keep and rear them (Kaumbata *et al.*,...
2020), as they are a good source of animal protein or income when sold (Lohani & Bhandari, 2021). Limitations to goat productions have been attributed to certain factors which include: poor management practices (Nwachukwu & Berekwu, 2020); increase in the costs of treatment (Yatoo et al., 2019) and inadequate vaccination strategies (Ezeano et al., 2019). All of these factors predispose susceptible animals to endemic disease conditions like Peste des petits ruminants (PPR).

Peste des petits ruminants is a viral ruminant disease of importance in African, Asian and Middle Eastern countries (Idoga et al., 2020). PPR is a disease caused by a paramyxovirus characterised by severe respiratory distress, diarrhoea and ultimately death (Ugochukwu et al., 2017). The huge economic losses attributed to the disease are due to its highly debilitating nature and high morbidity and mortality rates. An important mechanism of pathogenicity of the virus lies in its ability to disrupt the integrity of the respiratory mucosa thereby predisposing the respiratory epithelium to invasion and colonization by potentially pathogenic bacteria which are commensals of the nasal flora. Hence, the pathology often associated with a PPR outbreak is exacerbated by secondary bacterial infections which also result in delayed healing of the pulmonary parenchyma (Jarikre et al., 2017; Jarikre & Emikpe, 2019).

In Sub-Saharan Africa, PPR N/75/1 vaccine administered via the subcutaneous route has been recommended and has proven effective in the control of PPR in small ruminants (Mahapatra et al., 2020). However, there is no information (to the best of our knowledge) on the effect of PPR vaccine on the nasal bacterial flora in goats. This study, therefore, highlights the effect of the subcutaneous administration of PPR vaccine on the nasal bacterial flora as well as on other clinical indices like temperature, respiratory rates and weight.

Materials and Methods

Study area

This study was conducted at the Experimental Animal Unit and Diagnostic Pathology Laboratory of the Veterinary Teaching Hospital, University of Abuja.

Experimental animals

Ten Red Sokoto goats were purchased from recognised breeders in Abuja, Federal Capital Territory. They were aged between 6 months and 1 year with an average weight of 8.9 Kg. The goats were divided into two groups (A and B) consisting of five animals each and housed in well partitioned fly-proof pens with concrete floors. The experimental animals were conditioned for two weeks, during which they were pre-treated with 10% Oxytetracycline (L.A) at 1ml/10Kg intramuscular injection stat; Ivermectin 1ml/50kg subcutaneous injection and Vitamins at 10mg/L of water were administered to alleviate stress for 5 days. They were fed on wheat bran/grass twice a day and clean drinking water was provided ad libitum. Adequate measures were taken to minimize discomfort or pain to the animals during the period of the experiment.

The study was reviewed and approved by the ethical board of the Faculty of Veterinary Medicine, University of Abuja (UAECAU/2020/0028).

Experimental design

Experimental animals were divided into two groups (A and B) of 5 animals each. Goats in group A received 1ml of reconstituted N/75/1 PPR vaccine via subcutaneous route; while goats in group B received 1ml of normal saline subcutaneously.

Clinical indices: Respiratory rates and rectal temperatures were taken and recorded once, daily (morning hours) with the use of a stethoscope (Hamzaoui et al., 2013) and a digital thermometer (Marques et al., 2021) respectively. This was carried out throughout the course of the experiment. The weight was measured and recorded once, weekly using a manual weighing scale.

Serology

Blood sample (3mls) was collected via jugular venipuncture into clean, well-labelled, plain vacutainer tubes from each experimental animal once, weekly. This was done for a duration of five weeks. Blood collected was allowed to clot and then stored overnight at 4ºC. The sera collected were centrifuged at 1 500 rpm for 10 minutes (WOAH, 2008). These samples were stored at -20ºC in well labelled 2ml cryotubes to determine PPR antibody levels in the blood.

Enzyme-linked immunosorbent assay (ELISA): This test was performed using the direct technique (Goat PPR ELISA Kit, BIOTUVA Life Sciences™ United Kingdom) and the procedure as described by the manufacturer was strictly adhered to. Absorbance was measured using the Spectrophotometer and determined at 450nm. The percentage of inhibition (PI) was calculated using the following formula:

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PI = \frac{\text{absorbance of the test wells} \times 100}{\text{absorbance of the mAb control wells}}
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Percentage inhibition values were recorded as positive when greater than 50 (>50).

Bacteriology
Nasal swabs were collected once, weekly throughout the duration of this study which lasted for four weeks.

Sample collection: For bacteriology, nasal swabs were collected aseptically from each tagged goat after thorough cleaning of the external nares with a disinfectant. Collection was done by introducing sterile applicator sticks with cotton tip into each nostril and gently swirling around to get a representative bacterial sample from each nostril. The swab sticks were then dipped into bijou bottles containing 2mLs of BHI broth and the ends of the sticks were carefully cut and bottle caps were closed and tightened. The samples were transported in ice-packs to the Bacteriology Laboratory of the Microbiology Department, University of Abuja, for bacterial isolation.

Culture media: Nutrient agar and sheep blood agar (Oxoid™) were the general all-purpose media while Brain heart infusion broth (Oxoid™) served as the transport medium. The following agar were used as selective and differential media for the species identification and enumeration: MacConkey agar, Eosin Methylene Blue (EMB) agar, Mueller Hinton Agar, Sheep Blood agar and Bair Parker agar for Escherichia coli, Streptococcus spp., Pasteurella multocida, Mannheimia hemolytica and Staphylococcus aureus and prepared according to manufacturer’s specification:

Bacterial culture: Swabs from the original sample were inoculated onto all-purpose media (Nutrient agar and Sheep Blood agar) and incubated at 37°C for 24 hours.

Enumeration of selected bacterial species: The bacterial colony counts were performed following standard methods as described by Miles et al. (1938) as modified by Herigstad el al. (2001). The bacterial cells were suspended and homogenised using BHI broth in tenfold serial dilution. 0.1mL of each dilution was seeded onto each of the five sections drawn on a petri dish in the different selective and differential agar. The inoculated plates were then incubated at 37°C for 24 hours. Following incubation, plates with the highest dilution with colonies of between 30 and 300 were enumerated as colony forming units (CFU) per mL, i.e. (No. of colonies x Dilution x Dilution factor).

Bacterial identification and Characterisation: Colonies were identified based on morphology and Gram staining (Cheesbrough, 1998).

Results

ELISA test revealed that the PPR-vaccinated goats (group A) had significantly higher antibody titres (p<0.05) compared to the unvaccinated goats (group B). This increase in percent inhibition was observed from day 7 post-vaccination (46%) and peaked at day 14 post-vaccination (90%) in group A. Although there was a mild decline in antibody titres by days 21 (87%) and 28 (85%), the values remained significantly higher in the PPR vaccinated group (Figure 1).

Experimental animals in group A (PPR vaccinated), recorded a significant decline in bacteria counts for total bacteria (p = 0.0001), Mannheimia haemolytica (p = 0.038), Pasteurella multocida (p = 0.021) and Escherichia coli (p = 0.0001) compared to that of animals in the unvaccinated control group B (Figure 2). A slight but insignificant (p ≥ 0.005) reduction was also recorded in group A for total bacteria count, Staphylococcus aureus and Streptococcus sp. Compared to group B (control).

The daily temperature rates recorded in both groups (A and B) fell within the range of 36 - 37.5 °C in both groups and there was a mild increase in temperature at day 14-16 post-vaccination (Figure 3) which subsided subsequently with no statistical significance (p≥0.05). The respiratory rates also fluctuated but values remained within the range of 21-22.5 bpm for both the vaccinated (group A) and unvaccinated (Group B) goats (Figure 4).

All the experimental animals in both groups (A- PPR vaccinated; and B- Normal saline) remained apparently healthy throughout the course of the experiment. However, an average weight gain
(12.5Kg ±0.15) was observed in group A (PPR vaccinated) compared to 11Kg (± 2.33) recorded in animals from group B (unvaccinated group) at 28 days post-vaccination (Figure 5).

Discussion
The ability of the Nigerian PPR 75/1 vaccine to produce specific antibodies against PPR viral antigen was clearly demonstrated in this study evidenced by the high titres recorded from 14 days post-vaccination till the end of this study (28 dpv). This shows the ability of the PPR N/75/1 vaccine to boost a strong immunological response in goats and agrees with previous findings (Jarikre & Emikpe, 2019; Zahir et al., 2014). The interaction observed in this study showed that although PPR vaccine had an inhibitory effect on *M. haemolytica*, *P. multocida* and *E. coli* (p≥0.05) there was no significant effects on the other selected bacteria species (*Streptococcus* sp, *Staphylococcus aureus* and Total bacteria count). The likely explanation for this is that both *E. coli* and *M. haemolytica* are Gram-negative organisms and possess outer membrane lipopolysaccharide which is easily recognised as potent antigens by circulating antibodies induced by the PPR vaccine. In conjunction to this observation, the relatively thin peptidoglycan wall of these gram-negative bacteria (*M. haemolytica* and *E. coli*) could have increased their susceptibility to antibody infiltration and subsequent destruction. Few studies have evaluated the effect of a viral live attenuated vaccine on the nasal bacterial flora, however there are reports that some influenza vaccines limit the severity of

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**Figure 1**: Detection of PPR specific antibodies in PPR-vaccinated Red Sokoto goats

**Figure 2**: The effect of PPR vaccination on selected upper nasal microflora in Red Sokoto goats

**Key**:
- TBC: Total bacteria count
- EC: *Escherichia coli*
- SS: *Streptococcus* sp
- MH: *Mannheimia haemolytica*
- PM: *Pasteurella multocida*
- SA: *Staphylococcus aureus*

**Figure 3**: The effect of PPR vaccination on average temperature rates in Red Sokoto goats
secondary bacterial infections but does not completely protect against the same infections in mice (Chausse et al., 2011). The observable clinical indices such as temperature, respiratory rates and weight gain were also evaluated post-vaccination (pv). All parameters remained within the normal range as reported earlier (Bello et al., 2016); except for transient hyperthermia observed in the PPR vaccinated group between days 9 and 14 post-vaccination which could be described as a self-limiting physiologic homeostasis, or it could be due to the activation and enhancement of humoral immune responses attributable to the PPR vaccine. This finding has been previously reported to occur between days 7 and 21 post-vaccination with the use of the 75/1 PPR vaccine in goats (Mahapatra et al., 2020). Also, the vaccinated goats (group A) may have recognised the live attenuated viral component of the vaccine as a foreign pathogen; thereby triggering the release of pyrogenic cytokines in minimal quantities which simulates the natural infection. Finally, vaccinated goats (group A) showed a slight increase in weight (P>0.05) compared to the unvaccinated group (B), this clearly indicates that PPR vaccine does not have any effect on growth. This finding corroborates that of Enchery et al. (2019) who reported that PPR vaccine had no effect on weight gain in vaccinated animals prior to challenge with the PPR virus.

In conclusion, the PPR vaccine (N/75/1) may have shown some inhibitory effect on M. haemolytica count in the upper respiratory microflora and has no negative effect on clinical homeostasis. It is however insufficient in its capacity to control/prevent mannheimiosis in Red Sokoto goats.

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Conflict of Interest
The authors declare that there is no conflict of interest.

References


