RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v21i2.5

Tenuche *et al.*/*Sokoto Journal of Veterinary Sciences*, **21**(2): 83 - 88.

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

OZ Tenuche[1]*, BO Emikpe[2], E Godwin[2] & GO Egwu[4]

1. *Department of Veterinary Pathology, University of Abuja, PMB 117, Abuja, Nigeria*
2. *Department of Veterinary Pathology, Kwame Nkrumah University of Science and Technology, Khumasi, Ghana*
3. *Department of Public Health and Preventive Medicine, University of Abuja, PMB 117, Abuja, Nigeria*
4. *Department of Veterinary Medicine, University of Abuja, PMB 117, Abuja, Nigeria*

*Correspondence: Tel.: +2347060692699; E-mail: oremeyiztenuche@gmail.com

Publication History:
Received: 15-12-2022
Revised: 23-04-2023
Accepted: 24-04-2023

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 ($p \leq 0.05$) 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 -20^{o}C 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:

$$PI = \frac{\text{absorbance of the test wells x 100}}{\text{absorbance of the mAb control wells}}$$

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^{o}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 *et 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^{o}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 (Cheesebrough, 1998).

Representative colonies were further subjected to enzymatic (Indole, Ornithine decarboxylase, Citrate utilization test, Hydrogen Sulphide, Methyl red, Vogues-Proskauer test) and biochemical tests for characterisation in peptone water base using various sugars (Fructose, Glucose, Mannitol, Sucrose, Lactose, Galactose, Maltose) as described by Quinn *et al. (*2002).

Isolation and identification of selected bacterial organisms of the nasal flora: Gram-negative bacteria and mixed colonies were sub-cultured on blood and MacConkey agar for 24 hours at 37^{o}C. Isolates obtained were then sub-cultured into other prepared culture media to get pure colonies from the mixed colonies on the plates. Characterization and identification of the isolates were carried out using standard methods (Cheesbrough, 1998).

Statistical analysis

Mean and standard deviation were used to describe the data while Student t test was used to compare the mean of various parameters considered in the study.

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 ($p \geq 0.005$) 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 ^{o}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 ($p\geq0.05$). 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; Zahur *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

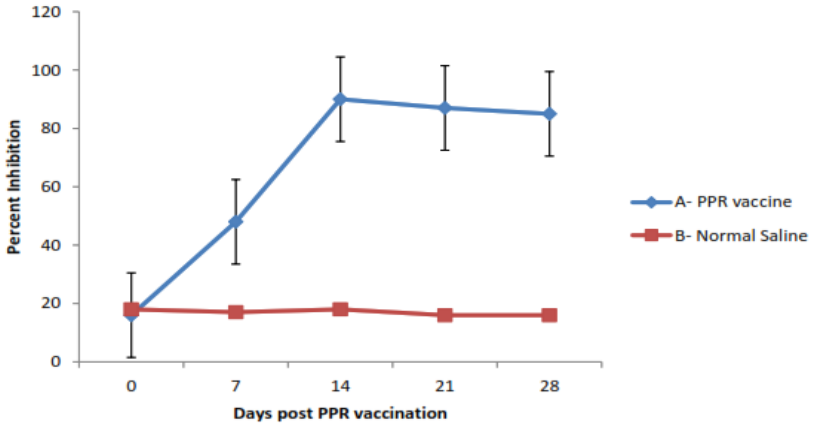

Figure 1: Detection of PPR specific antibodies in PPR-vaccinated Red Sokoto goats

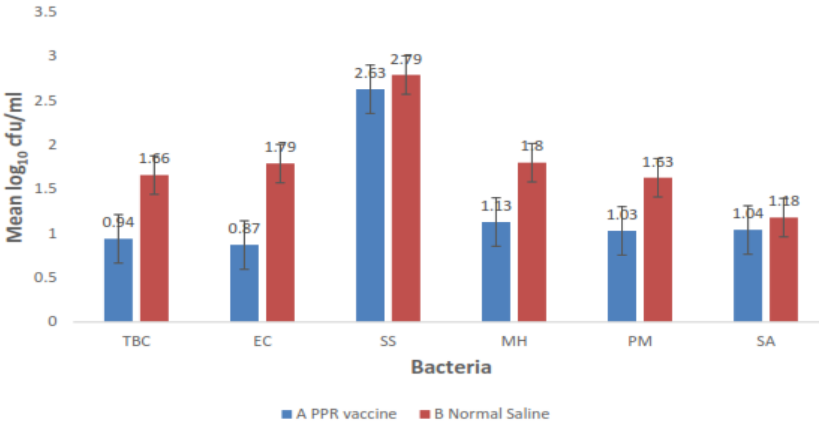

Figure 2: The effect of PPR vaccination on selected upper nasal microflora in Red Sokoto goats

Key:

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

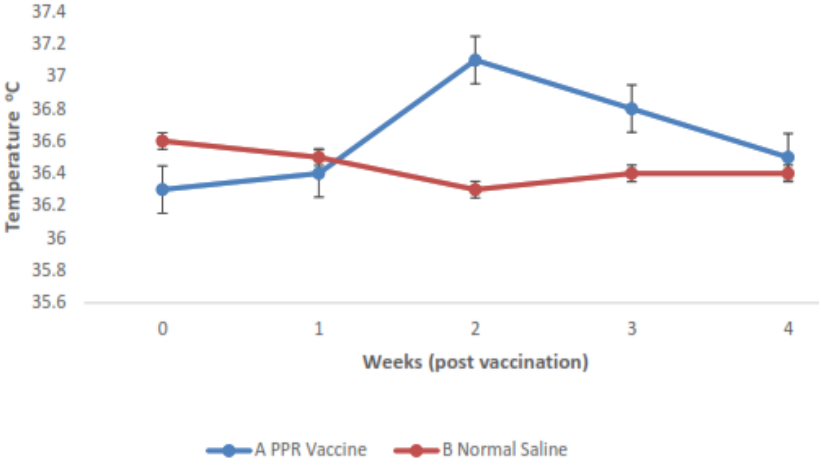

Figure 3: The effect of PPR vaccination on average temperature rates in Red Sokoto goats

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 and14 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.

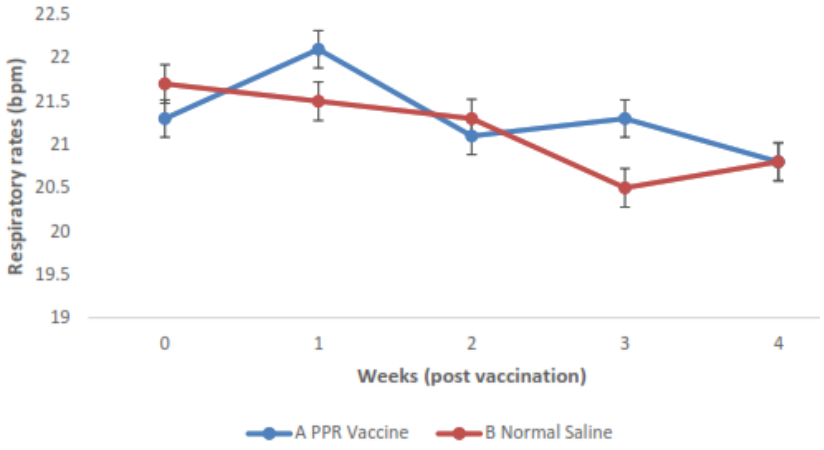

Figure 4: The effect of PPR vaccination on average respiratory rates in Red Sokoto goats

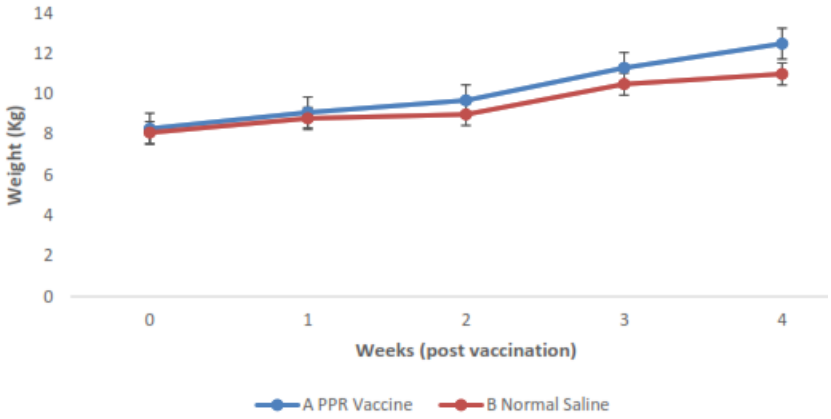

Figure 5: The effect of PPR vaccination on average weight in Red Sokoto goats

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.

Funding

No funding was received

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Bello SA, Akintunde OG, Sonibare AO & Otesile EB (2016). Effect of sex, age and time of the day on vital parameters of apparently healthy West African Dwarf goats in Abeokuta, Nigeria. *Alexandria Journal of Veterinary Sciences*, **49**(2): 18-23.

Chaussee MS, Sandbulte HR, Schuneman MJ, Depaula FP, Addengast LA, Schlenker EH & Huber VC (2011). Inactivated and live, attenuated influenza vaccines protect mice against influenza: *Streptococcus pyogenes* super-infections. *Vaccine*, **29**(21): 3773–3781.

Cheesbrough M (1998). Microscopical techniques used in Microbiology, culturing bacterial pathogens, biochemical tests to identify bacteria. *District Laboratory Practice in Tropical Countries,* **2**(2): 35-70.

Enchery F, Hamers C, Kwiatek O, Gaillardet D, Montange C, Brunel H & Bataille A (2019). Development of a PPRV challenge model in goats and its use to assess the efficacy of a PPR vaccine. *Vaccine*, **37**(12): 1667-1673.

Ezeano CI, Obiegbuna JC & Chukwuigwe O (2019). Obstacles to small holder sheep and goat rearing in rural communities of Anaocha Local Government Area of Anambra State, Nigeria. *International Journal of Applied Sciences and Engineering,* **7**(2): 45-53.

Herigstad B, Hamilton M & Heersink J (2001). How to optimize the drop plate method for enumerating bacteria. *Journal of Microbiological Methods*, **44**(2): 121-129.

Hamzaoui S, Salama AAK, Albanell E, Such X & Caja G (2013). Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. *Journal of Dairy Science*, **96**(10): 6355-6365.

Idoga ES, Armson B, Alafiatayo R, Ogwuche A, Mijten E, Ekiri AB, Varga G & Cook AJC (2020). A Review of the current status of peste des petits ruminants epidemiology in small ruminants in Tanzania. *Frontiers in Veterinary Science*, doi.10.3389/fvets.2020.592662.

Jarikre TA, Ohore GO, Oyagbemi AA & Emikpe BO (2017). Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs. *International Journal of Veterinary Science and Medicine*, **5**(2): 143-147.

Jarikre TA & Emikpe BO (2019). Immunohistochemical evidence of multiple viral and bacterial associations in caprine pneumonia in Nigeria: Implications for vaccines. *Acta Veterinaria Eurasia*, **45**(1): 1-8.

Lohani M & Bhandari D (2021). The importance of goats in the World. *Professional Agricultural Workers Journal*, **6**(2): 9-21.

Mahapatra M, Selvaraj M & Parida S (2020). Comparison of immunogenicity and protective efficacy of PPR live attenuated vaccines (Nigeria 75/1 and Sungri 96) administered by intranasal and subcutaneous routes. *Vaccines*, **8**(2): 168-179.

Marques JI, Leite PG, Lopes-Neto JP, Furtado DA & Lopes FFDM (2021). Estimation of rectal temperature of goats based on surface temperature. *Engenharia Agrícola*, **41**(6): 591-598.

Miles AA, Misra SS & Irwin JO (1938). The estimation of the bactericidal power of the blood. *Epidemiology and Infection*, **38**(6): 732-749.

Nwachukwu CU & Berekwu N (2020). Production and management of goat rearing in rural areas of Ezinihitte Mbaise, Imo State, Nigeria. *Agro-Science*, **19**(3): 25-31.

Kaumbata W, Banda L, Mészáros G, Gondwe T, Woodward-Greene MJ, Rosen BD, Van Tassell CP, Solkner J, & Wurzinger M (2020). Tangible and intangible benefits of local goats rearing in smallholder farms in Malawi. *Small Ruminant Research*, doi.10.101016/j.smallrumres.2020.106095.

Quinn PJ, Markey BK., Carter ME, Donnelly WJC & Leonard FC (2002). *Veterinary Microbiology and Microbial Disease*. Blackwell Science. USA. Pp 536-573.

Ugochukwu IC, Aneke CI, Ezeasor CK, Msheila WP, Idoko SI, Kwabugge AY, Shoyinka SVO, Chineme CN, Chah KF & Ugochukwu EI (2017). Pathomorphology and aerobic bacteria associated with pneumonia in small ruminants slaughtered at the Nsukka abattoir. *Animal Research International*, **14**(1): 2644-2651.

Yatoo IM, Raffiq PO, Tauseef BS, Ahmed BR, Gopalakrishnan A, Karthik K, Dhama K & Vir-Sing S (2019). Contagious caprine pleuropneumonia: A comprehensive review. *The Veterinary Quarterly*, **39**(1): 1–25.

Zahur AB, Hamid I, Ullah A, Afzal M, Latif A, Ullah RW, Umer F, Samo MH & Jahangir M (2014). Peste des petits ruminants vaccine (Nigerian strain 75/1) confers protection for at least 3 years in sheep and goats. *Journal of Biosciences and Medicines*, **2**(6): 27-33.