Molecular Detection of Peste des Petits Ruminants Virus (PPRV) in Goats and Sheep in Ibadan, Oyo State, Nigeria

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sheep,
Vaccine.

Summary
Peste des petits ruminants (PPR) is a vaccine-preventable transboundary animal disease of goats and sheep majorly, and is regarded as a major constraint to small ruminant production especially in developing countries like Nigeria. Despite different strategies that have been employed to control PPR in Nigeria, cases of the disease are still reported in PPR-vaccinated and unvaccinated small ruminant farms. In this study, molecular detection of field PPR virus (PPRV) strains was carried out to determine the presence of PPRV. A total of 135 samples (45 oculo-nasal swabs and 90 tissue samples) were purposively collected between August and October 2020 from goats and sheep at the Akinyele live small ruminant market and at Akinyele and Amosun abattoirs in Ibadan, Oyo State, Nigeria. Using reverse transcriptase-polymerase chain reaction with primers targeting the partial N-gene of PPRV, 10 out of the 135 (7.4%) field samples yielded positive results.. The results of this study reveal that PPRV currently circulates in Ibadan. These findings underscore the need for continuous PPR surveillance, more extensive characterization of circulating PPRV strains and the importance of consistent use of quality vaccines in the country to achieve more effective preventive and control strategies against the disease.

Introduction

Peste des petits ruminants (PPR), similarly referred to as 'syndrome of stomatitis-pneumoenteritis', 'goat plague', 'ovine rinderpest' or 'kata', is a highly infectious disease of sheep and goats. The disease is caused by PPR virus (PPRV), which belongs to the family *Paramyxoviridae* and genus *Morbillivirus*. The virus is tightly linked to the rinderpest virus of buffaloes and cattle, morbilliviruses that affect aquatic mammals, distemper virus that affects dogs and some wild carnivores, and the measles virus of humans (Radostits *et al*., 2000). Peste des petits ruminants is a key transboundary animal disease and has been reported across West, Central, East and some parts of North Africa, the Arabian Peninsula, the Middle East and Asia, although it has been found to be emerging in new areas of the world (Banyard *et al*., 2010, 2014). Regions of the world that are endemic for PPR are known to be important sheep-

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and goat-rearing areas. In Nigeria, the population of sheep and goats was projected to be about 50 million, with goats outnumbering sheep (FAO, 2006). From 1995 to 2000, a study was carried out which documented PPR seroprevalence in some Nigerian States to be 38.34% in goats and 49.26% in sheep. In the same study, PPR was also observed in warthogs and camels (Shamaki *et al*., 2004). Furthermore, across various Nigerian ecological zones, PPR field outbreaks were reported in 2008 (Kazeem *et al*., 2009). Small ruminants are important in providing food and commodities for trade. They provide peasant farmers with basic livelihood, with the meat serving as a chief source of animal protein with an estimation of over 35% (Shamaki *et al*., 2004; Adamu *et al*., 2005). These livestocks sustain the income and employment of many people in the rural communities, contribute draught energy, manure for production of crops and skin for leather industries which also earns foreign exchange. In addition, these animals are used for traditional and religious festivities. However, production of these livestocks which is highly economically important, is extremely hindered by the great morbidity and mortality reportedly associated with PPRV. Depending on its endemicity in an area, great economic losses have been recorded due to 100% morbidity and 80-90% mortality among affected animals despite the availability of vaccines (Adamu *et al*., 2005). PPR is characterized by diarrhoea, fever, oculo-nasal mucopurulent discharges, enteritis, conjunctivitis, erosive-ulcerative stomatitis, broncho-interstitial pneumonia and fibrino-necrotic tracheitis (Kul *et al*., 2007). In severe cases, death results from severe dehydration caused by acute diarrhea or secondary bacterial pneumonia. Abortion has been reported from co-existence of PPRV with pestiviruses (Abubakar *et al*., 2008).

In some cases, PPRV is considered more dangerous in goats than sheep; nonetheless, outbreaks affecting both sheep and goat populations have been described (Chauhan *et al*., 2009; Wang *et al*., 2009). Furthermore, in some cases, goats seem uninfected, whereas sheep have great rates of morbidity and mortality (Yesilbag *et al*., 2005). The reason for this is yet unknown although the infecting viral strain(s) and host species seem to play crucial roles. Also, although only one PPRV serotype is recognized, diverse strains of the virus which differ in virulence when experimentally inoculated into the same breed of goats have been reported (Couacy-Hymann *et al*., 2007a). In addition, various breeds of goats are known to react in several ways to disease with the same virus (Diop *et al*., 2005). Asymptomatic infection has also been observed in some species (Bidjeh *et al*., 1995, Ezeibe *et al*., 2008).

In Nigeria, PPR remains an endemic disease of sheep and goats and is the most important single cause of high morbidity and mortality in these animals, thereby limitating livestock production (Saliu *et al*., 2008). Hamdy and Dardiri (1976) reported the financial loss associated with PPR annually in Nigeria to be about USD 1.5 million which is about USD 700 million in recent budget. Bearing in mind the economic losses resultant from this infection, the necessity therefore remains for continued monitoring of PPR in Nigeria for early detection, and effective management and control.

Vaccination with homologous PPR vaccines has been the control technique yet sporadic cases on both vaccinated and unvaccinated animals have been reported, thus the disease remains a major problem (Luka *et al*., 2011). Furthermore, Nigeria shares boundaries with Benin Republic to the West, Cameroon and Chad to the East and Niger Republic to the North. These land borders are porous, permitting continuous influx of pastoralists and their livestock into Nigeria. Considering this regular cross-border movement of pastoralists and their livestock, and the incessant cases of PPR in small ruminants in Nigeria, it is important to regularly carry out surveillance to detect the circulating field PPR virus(es). The study was designed to investigate the presence of PPRV in goats and sheep in Ibadan, Oyo State, Nigeria using molecular-based techniques.

Materials and methods

Study site and population

This study was conducted in Ibadan, the capital city of Oyo State, South-Western Nigeria (Figure 1).

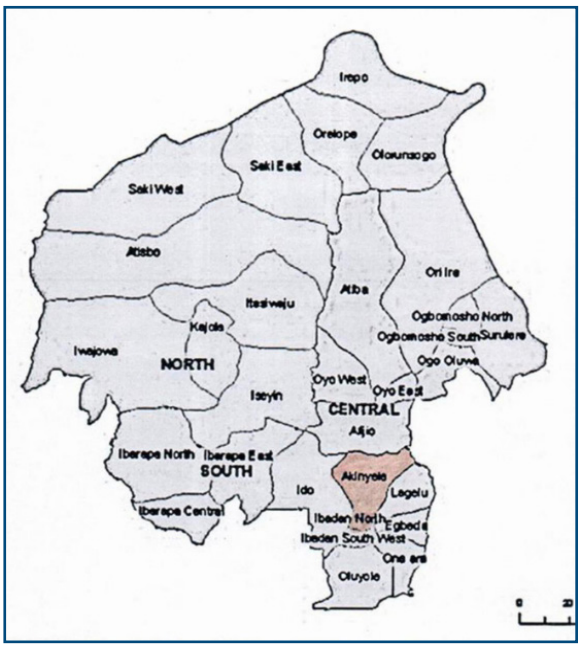

Figure 1. *Map of Oyo State showing the study areas in Ibadan.*

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Ibadan is located on coordinates 723'47"N and 3 55'0"E.

It lies in the tropical rainforest zone and has a population of about 3.7 million people as at 2021 with over 6 million living in the metropolis. The city has both tropical wet and dry climates; the wet period extends from March to October while the dry period runs from November through February (Wells, 2008).

Sample size determination

Using the formula of Thrusfield and Christley (2005), the size for the field samples (from goats and sheep) collected was calculated to be 135.

This was based on an estimated prevalence (Pexp) of 9.7% from a previous study (Mantip *et al*., 2016), reliability coefficient (Z) of 1.96 at 95% confidence interval and absolute precision (d) of 5%, using the formula $n = Z^2 Pexp (1-Pexp) / d^2$

The commercially available PPR vaccine in Ibadan was also included in this study for comparative molecular assay.

Sampling technique

A purposive sampling technique was used for the field sample collection. Only sheep and goats for sale or those having clinical signs suggestive of PPR at slaughter were included in this study.

A total of 135 samples were purposively collected from Akinyele live small ruminant market, and Akinyele and Amosun abattoirs in Ibadan, Oyo State between August and October, 2020. The distribution of the animals sampled based on species, breed, sex, age and site of sample collection is presented in Table I.

The animals are often brought to the market from villages around Ibadan and these markets also serve as transit points to other larger markets where the animals are re-sold for profit.

Table I. *Distribution of the animals sampled in Oyo State, Nigeria based on location, species, breed, number, sex and age.*

Location	Species	Breed	Number	Sex	Age	Total number of animals sampled
Akinyele live small ruminant market	Caprine	Red Sokoto	9	Male	>1 year	45
		Red Sokoto	7	Female		
	Ovine	West African Dwarf	4	Male		
		West African Dwarf	3	Female		
		West African Dwarf	7	Male	>1 year	
		West African Dwarf	14	Female		
		Sahel	1	Female		
Amosun Abattoir	Caprine	Red Sokoto	9	Male	>1 year	45
		Red Sokoto	5	Female		
	Ovine	West African Dwarf	6	Male		
		West African Dwarf	9	Female		
		West African Dwarf	5	Male	>1 year	
		West African Dwarf	11	Female		
Akinyele Abattoir	Caprine	Red Sokoto	5	Male	>1 year	45
		Red Sokoto	9	Female		
		West African Dwarf	8	Male		
		West African Dwarf	7	Female		
		West African Dwarf	10	Male	>1 year	
		West African Dwarf	6	Female		

Sample collection

Forty-five oculonasal swabs were collected from Akinyele live small ruminant market, and 45 tissue samples each from Akinyele and Amosun abattoirs. The tissue samples comprised lung, spleen and mesenteric lymph nodes. The samples were properly labeled with designated sample identity tags, site of swab collection or type of tissues collected and date of sample collection.

Oculo-nasal swabs collected from live small ruminants were transported in viral transport medium containing 2000 units/ml of penicillin,

2 mg/ml of streptomycin, 0.05 mg/ml of gentamycin and 100 units/ml of mycostatin in a cool flask with ice packs. Tissues (lung, spleen and lymph node) were collected from carcasses of slaughtered animals. All samples collected were transported in cool flasks with ice packs to the Virology laboratory, Department of Veterinary Microbiology, University of Ibadan where they were stored at -20°C under constant power supply for approximately one week before being transported in a cold chain to the National Veterinary Research Institute (NVRI), Vom, Plateau State where they were stored at -80°C until processed for PPRV detection.

Preparation of necropsy tissues

Necropsy tissues were processed in the as previously described (Mantip *et al*., 2016). Briefly, 10% suspensions of the tissues were prepared by pooling 1 g of lung, spleen and lymph node from the same animal and macerating them using a mortar and pestle. Thereafter, sterile sand and phosphate-buffered saline were added. The suspension was centrifuged at 2500 g for 5 min and the supernatant pipetted into cryovials, labelled properly and stored at -20°C. The leftover tissues were kept at -80°C for further usage.

Molecular detection of PPRV in goats and sheep

Viral Ribonucleic Acid (RNA) extraction

Viral RNA was extracted directly from the viral transport medium containing swab samples collected from the live small ruminant market and homogenized tissue samples from the abattoirs. The extraction was performed using the QIAamp Viral RNA Mini kit (QIAgen, Hilden, Germany) according to the manufacturer's instruction. The procedure was carried out as follows: 560 µL of the prepared buffer AVL (QIAgen, Hilden, Germany) containing carrier RNA was pipetted into a 1.5 ml microcentrifuge tube. 140 µL of homogenized tissue samples or pulse-vortexed viral transport media containing the swab was pipetted into the buffer AVL containing carrier RNA in a microcentrifuge tube.

This was mixed by pulse-vortexing for 15 seconds and incubated at room temperature for ten minutes after which it was briefly centrifuged to remove drops from the inside of the lid. 560 µL of ethanol (96-100%) was added to the mixture which was pulse-vortexed for 15 seconds and briefly centrifuged. 630 µL of the solution was aliquoted to the QIAamp Mini Spin Column (in a 2 ml collection tube) and centrifuged at 8000 rpm for 1 min.

The tube containing the filtrate was then discarded. Thereafter, 500 µL Buffer AW1 (QIAgen, Hilden, Germany) was added to the spin column and centrifuged at 8000 rpm for 1 min. The spin column was placed in a clean 2 ml collection tube while the tube containing the filtrate was discarded. 500 µL of Buffer AW2 (QIAgen, Hilden, Germany) was added to the spin column and centrifuged at 14,000 rpm for 3 min.

The QIAamp mini spin column was placed in a clean 1.5 ml microcentrifuge tube while the tube containing the filtrate was discarded. 60 µL of Buffer AVE (QIAgen, Hilden, Germany) was added to the spin column and incubated at room temperature for 1 min. This was then centrifuged at 8000 rpm for 1 min. Finally, the labeled 1.5 ml tube containing the eluted RNA was kept at -80°C before moving to the PCR mix room. For positive control, the viral RNA was extracted from a previously known positive PPR sample while a non-template control was used as negative control.

One-Step Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

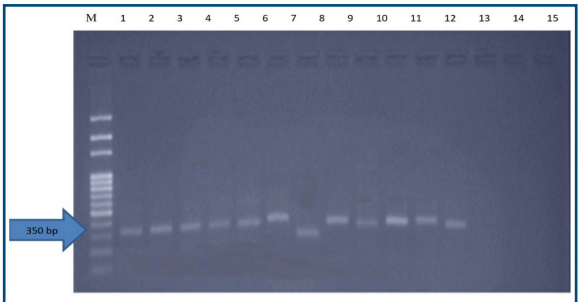
One-Step RT-PCR was performed for the amplification of a 350 base pair fragment of the N-gene using the following primers:

- Forward primer: PPR-NP3 (5'-GTC TCG GAA ATC GCC TCA CAG-3')
- Reverse primer: PPR-NP4 (5'-CCT CCT CCT GGT CCT CCA GAA-3'),

and using a 25 µL final reaction volume, mixtures containing 12.75 µL RNase-free water, 5 µL PCR buffer 5X, 0.5 µL dNTPs, 0.5 µL One-Step RT-PCR Enzyme Mix, 0.25 µL RNase Inhibitor, 5 µL of RNA template and 0.5 µL each of the primers. A 96-well thermocycler (Applied Biosystems) (Biopolis, Singapore) was used for the amplification. It was set to start at 50°C for 30 minutes, followed by initial denaturation at 95°C for 15 minutes. Thereafter, 35 cycles of denaturation, annealing and elongation of the templates were achieved at the cycling conditions of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute, respectively. Final extension of the templates was achieved at 72°C for 10 minutes. The amplified PCR products were analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide (1 µg/mL) and run at 120 V for 30 minutes. The bands were visualized under an ultraviolet transilluminator.

Results

Agarose gel electrophoresis of amplified products of the 135 field samples tested revealed that only 10 (7.4%) yielded the expected 350 bp band as previously described (Couacy-Hymann *et al*., 2002) (Figure 2).



M = 100 bp molecular weight marker; Lanes 1-10 = positive bands for the tissue samples; Lane 11 = positive band for NVRI PPR vaccine; Lane 12 = positive control; Lane 13 = negative control.

Figure 2. *RT-PCR product bands (350 bp) of amplified PPR virus N- gene.*

Out of the tissue samples collected at Amosun abattoir, only one (7.1%) of 14 female West African Dwarf (WAD) goats, 4 (26.7%) of 15 male WAD goats, none (0%) of 11 female WAD sheep and one (20.0%) of 5 male WAD sheep tested positive for PPRV RNA, while for the tissue samples collected at Akinyele abattoir, one (5.6%) of 18 female WAD goats, 3 (23.1%) of 13 male WAD goats, none (0%) of 6 female WAD sheep and none (0%) of 10 male WAD sheep were positive.

In addition, none (0%) of the 45 oculo-nasal swab samples collected from goats and sheep at Akinyele live small ruminant market was positive for PPRV nucleic acid (Table II).

Analysis of the results based on abattoir location showed that of the 45 of the samples each from Amosun and Akinyele abattoirs, only six goats and sheep (13.3%) and four goats (8.9%) yielded the expected 350 bp DNA band, respectively.

Table II. *Description of the samples that tested positive for PPRV by RT-PCR.*

Location	Species	Breed	Sex	Age	Sample type collected	Clinical signs observed	RT- PCR result
Amosun Abattoir	Caprine	WAD	Male	Adult	Lung, spleen	Nasal discharge, matted eyelid	+ ve
Amosun Abattoir	Caprine	WAD	Male	Adult	Lung, spleen	Soiled hindquarters Nasal discharge	+ ve
Amosun Abattoir	Ovine	WAD	Male	Adult	Lung, spleen	Nasal and ocular discharges	+ ve
Amosun Abattoir	Caprine	WAD	Female	Adult	Lung, spleen	Nasal and ocular discharges	+ ve
Amosun Abattoir	Caprine	WAD	Male	Adult	Lung, spleen	Nasal and ocular discharges	+ ve
Amosun Abattoir	Caprine	WAD	Male	Adult	Lung, spleen	Soiled hindquarters Nasal discharge	+ ve
Akinyele Abattoir	Caprine	WAD	Male	Adult	Lung, spleen, mesenteric lymph node	Nasal discharge, matted eyelid	+ ve
Akinyele Abattoir	Caprine	WAD	Female	Adult	Lung, spleen, mesenteric lymph node	Nasal and ocular discharges	+ ve
Akinyele Abattoir	Caprine	WAD	Male	Adult	Lung, spleen, mesenteric lymph node	Nasal and ocular discharges	+ ve
Akinyele Abattoir	Caprine	WAD	Male	Adult	Lung, spleen, mesenteric lymph node	Nasal and ocular discharges	+ ve

Discussion

The results of this investigation show a 7.4% prevalence of PPRV in Ibadan, Oyo State by RT-PCR. The low prevalence can be attributed to the sample size and the season of sample collection as most of the samples were collected between August and October. However, Wosu (1994) reported more cases of PPR in South-eastern Nigeria throughout the harmattan period (from December to January) compared to the rainy season with the highest rainfall occurring in April. The prevalence of PPRV in this study is however below the prevalence (27.3%) reported in Adamawa State by Ularamu *et al*. (2002). Further, in an earlier PPR survey in the Southern States of Nigeria by Obi and Ojeh (1989) using tissue homogenate with dot Enzyme-linked Immunosorbent Assay (ELISA) and standard indirect ELISA procedures, PPR prevalence rates of 86.8%

and 81.6%, respectively were recorded. In addition, a prevalence of 51.2% was reported for samples examined in North-Central States of Nigeria using a set of primers specific for the F gene of PPRV (Luka *et al*., 2011). Previous studies in some African countries such as Morocco revealed PPR prevalence of 44.4% by RT-PCR and a greater prevalence of 80% in Sudan (Kwiatek *et al*., 2011). PPRV was also confirmed in 33.3% clinical samples tested in Algeria, with a group of primers appropriate for the F gene of the PPRV (De Nardi *et al*., 2012). In Northern and Eastern Tanzania, PPRV was reported in 29.6% and 31.1% of the goats examined, (Kgotiele *et al*., 2014). In Ethiopia, a prevalence of 46.4% has been reported (Alemu *et al*., 2019) while in India, a prevalence of 50% was recently reported in an endemic region using RT-PCR (Kerur *et al*., 2008). Variations in the prevalence of PPRV in the diverse locations reported above could be due to differences in sample size, season of sample collection, detection method used, type of sample(s) employed for PPR diagnosis, phase of infection and the gene targeted for RT-PCR procedure (Luka *et al*., 2012). Thus, the detection of PPRV in small ruminants in Ibadan, Oyo State poses a major risk to the neighbouring communities and States as there is a high chance of spread since the disease is transboundary in nature, and considering that these animals are often transported from one market to another and sometimes to farms. This could lead to further epidemics and serious economic impact on the nation.

This current investigation revealed a significantly higher rate of PPR in goats compared with sheep using the RT-PCR technique. This finding is in tandem with that reported in Ethiopia by Alemu *et al*. (2019). Similarly, Abubakar *et al*. (2008) showed PPR cases in Pakistan to be more dangerous/virulent in goats compared to sheep. A greater occurrence of PPR was detected in goats when compared with sheep in a survey by Mahajan *et al*. (2013). In a study by Abraham *et al*. (2005) in Ethiopia, it was reported that the seeming non-existence of pathogenicity in sheep might be due to a specific resistance of the indigenous species and/or an absence of virulence seen with the Ethiopian PPRV strains for sheep. Also, epidemiological findings from this study revealed that the West African Dwarf breed of goats was more predisposed to PPR in comparison with other breeds.

This observation is consistent with the position of FAO (1999), Baazizi *et al*. (2017) and Balogun *et al*. (2017).

The absence of any positive results in the swab samples collected at the live small ruminant market could be due to the method of sample collection and the consideration that despite efforts to maintain cold chain, RNA viruses do not store well and this may affect the outcome of RT-PCR which is a limitation with studies conducted in developing countries.

The detection of PPRV in goats and sheep in Ibadan, Oyo State, Nigeria in this study can probably be attributed to the continuous transportation/ movement of sheep and goats for foraging or commercial purposes from the Northern savannah area of Nigeria or neighbouring Sahelian African countries which are dry, to the Southern forest zone of the country, which is wet. Nigeria remains a focal point of animal meat consumption in West Africa owing to her huge population. In order to satisfy the continually growing local need, there is increased influx of sheep and goats from nearby countries.

The sale and transportation of animals and their products from one State to the other and from neighbouring countries into Nigeria may serve as a major route of transmission and spread of transboundary animal diseases including PPR. According to studies by Cattaneo *et al*. (1987) and Couacy-Hymann *et al*. (2002), it has been proposed that the PPRV N-gene is surplus in positive tissue samples. Therefore, aiming at the N-gene of PPRV for RT-PCR yields good outcome. It has been discovered that the N-gene is responsible for the coding of an inner structural protein.

Since mRNAs of N-gene are copies of the virus, it is thus a conducive target for the development of a diagnostic PPR test, with very high sensitivity and specificity (George, 2002). Due to the clinical signs observed in the animals screened in this study and the detection of PPRV by RT-PCR technique, it can be concluded that the disease that affected the animals that were positive in this study was caused by PPRV. The data obtained from this investigation have thus provided relevant information on the current status and epidemiology of PPR virus in Nigeria, particularly in Ibadan, Oyo State.

Limitation

As at the time of compiling this report, the result of sequencing of the PPR amplicons from the samples that tested positive is yet to be received. The delay is due to changes in protocol associated with the recent Coronavirus disease (COVID)-19 pandemic. This would have aided the molecular characterization as well as helped to determine whether there are differences between circulating field strains and the locally available vaccine.

Conclusions

The results of this study showed a prevalence of 7.4% for PPR in goats and sheep in Ibadan, Oyo State, Nigeria using RT-PCR detection method.

These findings confirm the presence of PPR virus within goat and sheep populations in Ibadan. These results show that PPR is still endemic and could be responsible for the intermittent outbreaks of pneumonia and diarrheal disease in small ruminants in Nigeria.

Considering the interstate transportation of sheep and goats in Nigeria, this further indicates the potential risk of spread of PPR to regions where the disease is presently absent in the country.

Statement of animal rights

In conducting this study, all relevant institutional, national and/or international guidelines for the care and use of animals were duly followed (World Organization for Animal Health, 2009).

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