Multi-sampling technique in the diagnosis of *peste des petits ruminants* virus in goats using reverse transcriptase polymerase chain reaction

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Peste des petits ruminants (PPR) remains a threat to goat production in Nigeria. The study was designed to evaluate diagnostic efficiencies using different sampling techniques in detecting PPR virus with RT-PCR. A total of 29 samples comprising nasal swabs (NS), ocular swabs (OS), and rectal swabs (RS) were obtained from 13 goats, from suspected outbreaks in three Local Government Areas (LGA), namely Igabi, Sabon Gari and Giwa LGAs, in Kaduna state. More than one sample type (from different routes) here referred to as the multi-sampling technique was obtained per goat in Igabi and Sabon Gari, while a single sample type was obtained per goat in Giwa. The overall detection rate was 31.9%. Only 2 (33.3%) instances existed where only one sample type was positive; whereas on 6 (31.9%) occasions, at least one sample type from the same goat was found positive. The detection rate was 50%, 44.4% and 30% for NS, OS and RS respectively. PPR was not detected in Giwa. In conclusion, using RT-PCR targeting PPRV nucleoprotein gene, NS appeared to have a higher positive detection rate, while RS was the least reliable. Utilizing more than one sample type or multi-sampling technique from different routes per goat enhanced the detection rate of PPRV using RT-PCR.

Keywords: Detection, Goats, Multi-sampling, Nucleoprotein gene, Peste des petits ruminants virus, RT-PCR, Swabs

Introduction

Peste des petits ruminants (PPR) also referred to as "Kata" is a highly contagious and fatal disease of goats and sheep caused by PPR virus (Baron et al., 2011). It is widespread in Africa, the Middle East and other parts of Asia (CFSPH, 2008; Abubakar et al., 2015) and, is currently emerging and re-emerging in different territories of the world (Wu et al., 2016). Goat rearing for meat production is a profitable venture in Nigeria. Nevertheless, major production constraints include inadequate credit facilities and disease outbreaks such as PPR (Baruwa, 2013). Case fatality rates have been shown to be higher in young animals (Abubakar et al., 2018). There is risk of further maintaining the virus in kids despite vaccination in adults and, this definitely threatens small ruminant population (Kock et al., 2015). Serological and antigenic evidences abound of PPR presence in arrays of animals (Balamurugan et al., 2012; Diallo, 2012). As with other Morbillivirus members such as Canine distemper virus (De Swart et
Small ruminants morbillivirus, also known as Peste des petits ruminants virus (PPRV), is a linear, single stranded, non-segmented, negative sense RNA virus belonging to the genus *Morbillivirus* of the family Paramyxoviridae. The viral genome is 15,948 nucleotides long and contains six genes encoding six major structural polypeptides: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion (F) protein, hemagglutinin (H), and large (RNA dependent) polymerase protein (L), and two non-structural C and V proteins (Bailey *et al*., 2005). The genome possesses an unusual genomic malleability which confers resistance to it against some therapeutic substances (Holz *et al*., 2012). The four distinct lineages known are I, II and III from Africa; and lineage IV from Asia (Kerur *et al*., 2008). Following reports of lineage IV in Cameroon and Central Africa Republic, investigations of outbreaks also revealed the presence of the Asian lineage IV clade-A field strains in Nigeria aside the lineage II strain (Woma *et al*., 2015).

The virus is detectable in swabs obtained from animals as early as 2 - 4 days post-infection (Couacy-Hymann *et al*., 2009; Baron *et al*., 2014) as PPRV antigens are excreted within this period. However, as time progresses antigens may be undetected in nasal and ocular swabs, though detectable in faeces up to 4 months post-infection. These results on the post-infection detection periods of PPR antigen in different sample types are of epidemiological relevance in diagnosis and disease control, since infected animals excrete virus in the environment before clinical signs appear or at early onset of clinical signs (Couacy-Hymann *et al*., 2009). With the highly contagious nature of the disease, it can easily be transmitted to healthy animals via direct contact with the faecal matters, nasal or ocular secretions, and excretions of infected animals (Ezieibe *et al*., 2008).

There are arrays of diagnostic techniques of varying sensitivity and specificity that can be used in confirming the disease. Some are, however, tedious and time-consuming (Balamuragan *et al*., 2007; Osman *et al*., 2009). Proper sampling technique, type of sample/swab and primer employed are very important in the molecular diagnosis and control of PPR field virus (Luka *et al*., 2012). The right transport media is also essential as well as prompt delivery of samples to the laboratory (Bhuiyan *et al*., 2014). Using Lateral Flow test and antigen Enzyme-linked Immunosorbent Assay, it has been observed that faecal swab is least suitable for antigen detection of PPRV, followed by ocular swab, yielding false-negative results. They were all bested by nasal swab, reflecting the increased viral load in nasal swabs of PPRV infected goats compared to ocular swabs (Halecker *et al*., 2020). Given that the N gene is the most abundantly transcribed gene, sensitivity is better achieved with RT-PCR targeting its synthesized mRNA (Kumar *et al*., 2014). Characterization of PPRV based on the N gene, which is highly conserved, also tends to be better in molecular epidemiology of the virus (Kerur *et al*., 2008).

RT-PCR targeting the N gene of PPR virus has proven to be effective in diagnosis of PPR (Toukoura *et al*., 2018), however, diagnosis of PPR in Nigeria is mainly based on clinical signs, which can be somewhat limited, and control is impeded by poor veterinary management and diagnostic services (Annatte *et al*., 2006). With OIE and FAO eager to control and subsequently eradicate PPR from the globe, as has been achieved for rinderpest (Munir *et al*., 2012), development of easier and effective means of diagnosis is of the essence, especially in endemic countries like Nigeria (Herbert *et al*., 2014). The study was therefore designed to determine the best sample/technique that yields the viral antigen, out of three types of swab commonly utilized in the diagnosis of PPR namely ocular, rectal and nasal swab using RT-PCR.

**Materials and Methods**

**Study area**

The study was conducted in Kaduna State, Northwestern, Nigeria. The state lies between latitudes 6° and 11° North and longitude 7° and 44° East. It is 608m above sea level. It has distinct wet and dry seasons, and is within the Northern Guinea Savannah zone and part of the Sahel Savannah zone of Nigeria. Kaduna state shares geographical boundaries with Katsina and Zamfara States to the North, Plateau and Bauchi States to the East, Nasarawa State and the Federal Capital Territory to the South, Niger State to the West; and Kano State to the Northeast. Nigeria has a goat population of 65.65 million, about 2.5% of which are found in Kaduna State (NASS, 2012). The state comprises 23 Local Government Areas (LGA), from which Giwa, Sabon Gari and Igabi LGAs were selected based on presence of suspected on-going outbreaks.

**Sampling procedure and collection**

Thirteen goats with clinical signs suggestive of PPR from homes, pens and animal farms were used for the
study. These comprised five from Igabi, four from Sabon Gari, and four from Giwa. Employing different sampling strategies commonly employed by field veterinarians, multiple samples, triplicate and duplicate, from different routes (multi-route sampling) were obtained from each goat in Igabi and Sabon Gari, while single sample type per goat was obtained from Giwa. There were 29 samples in total: Nasal swabs (NS) (n = 10), ocular swabs (OS) (n = 9), and rectal swabs (RS) (n = 10). Swabs were collected using sterile swab sticks and inserted in viral transport media (VTM). The samples were transported to the National Veterinary Research Institute (NVRI), Vom, Plateau State and kept at -20°C until further analysis.

RNA extraction/amplification of PPR nucleic acid using RT-PCR
Total ribonucleic acid (RNA) extraction was performed from the samples using a Qiagen RNeasy Plus RNA extraction kit (Qiagen, Germany) according to the manufacturer’s instructions. Briefly, each sample was aliquoted and incubated at room temperature for about 10 min in a micro centrifuge tube containing buffer AVL. Following brief centrifugation, 96 – 100% alcohol was used for precipitation. The mixtures were decanted into QIAamp mini spin column (in 2ml collection tube) for further washing and centrifugation, before RNA elution from the mini spin column into a new collection tube.

The positive control was live-attenuated Nigeria 75/1 PPR vaccine produced by NVRI, Plateau state. The extracted RNA was stored at -80°C for RT-PCR.

PPRV nucleoprotein (N) gene was amplified using Titan One Tube One-Step RT-PCR kit (Roche Life Science, Indiana, USA) using PPRV-specific primers. The forward primer: NP3 (5'-CTCTGGAAATCGCCTACAGACTG-3'), and reverse NP4 (5'-CTCTCCTCTGTTCTCCAGAATCT-3') previously described by Coaux-Hymann et al. (2002) targeted 351bp segment of the N gene. The reaction mixture was set up thus: 12.5μl of nuclease-free water, 6μl of PCR buffer, 0.25μl of forward primer, 0.25μl of reverse primer, 0.5μl of enzyme (taq polymerase) and 5μl of extracted RNA. Nigeria 75/1 vaccine produced by NVRI, Jos, Plateau state, was used as positive control.

Briefly, a reverse transcription was executed at 45°C for 30 minutes before initial denaturation of DNA at 95°C followed by 40 cycles of denaturation at 95°C for 30s. Annealing was at 55°C for 30 s, elongation at 72°C for 30s, and a final extension at 72°C for 10 min. Fragments of cDNA thus amplified were in resolved 1% agarose gel stained with ethidium bromide and the pictomicrogram was visualized accordingly.

Results
Clinical samples from Igabi LGA were obtained in May, 2018. In Sabon Gari, and Giwa LGAs sampling was done in the month of July, 2018. The following clinical signs were observed in the sampled animals: fever, lethargy, coughing, ocular and nasal discharges, dypsnoea, conjunctival congestion, diarrhoea and death. With the commonest clinical signs being abnormal nasal discharge and diarrhoea. The animals were both male and female between 2 months and 1.5 years old. The most severe clinical manifestations were observed in kids or young goats (approximately 2-3 months old).

RT-PCR using NP3 / NP4 primers pair yielded the anticipated 351bp of segment of the N gene as indicated by distinct band picture using 100bp molecular marker on the gel picture (Figure I). The positive detection of PPR from all the sampled locations was found to be 31.9%. In Giwa LGA, where multi-sampling was not employed, evaluating the effectiveness of single sample type in

![Figure 1](image-url)
diagnosis, PPRV was not detected, in nasal, ocular or rectal swabs using RT-PCR (Table 1). 50% of total NS showed positive results when compared with 44.4% for total OS and 30% for total RS. In Igabi and Sabon Gari, one goat from each LGA (22.2%) showed positive result to all three swabs. In two goats (22.2%), NS yielded positive results to PPR, though OS and RS were negative. Similarly, only 4 (44.4%) goats from both LGAs were positive with at least two swabs. NS was one of the samples in both cases.

NS yielded positive results to 3 cases (33.3%) for which OS produced negative results; whereas, only 1 case (11.1%) existed in which OS was positive and NS negative. Also, on 3 (33.3%) occasions, PPRV was detected in NS, and undetected in RS. It is noteworthy that there was no instance when RS was positive and NS was negative concurrently. PPRV antigens were detected in OS on 2 (22.2%) occasions when RS yielded negative results; and undetected in OS on 1 (11.1%) occasion when RS was positive. All the goats sampled in Sabon Gari LGA were positive to at least one swab, except 1 goat (25%) which was negative to all 3 samples (Table 1).

Of all the positive samples, only in two scenarios from both Igabi and Sabon Gari was only one sample found positive representing 33.3% of total positive samples (Table 2).

**Discussion**

PPR is still an endemic crisis in Kaduna state, Nigeria affecting small ruminant populations in both rural and urban areas, undermining the economy of poor farming communities. Effective diagnosis is of utmost importance in check-mating the menace of this disease.

RT-PCR using NP3/NP4 primers pair detected the presence of PPRV in all the outbreaks except Giwa LGA implying that it remains a very reliable technique in the detection of PPRV, though only available in few laboratories across the country. Its increased availability along with diagnostic techniques like real time RT-PCR will play significant role in the control of the disease. The observed outbreak in Giwa could be due to a different disease such as contagious caprine pleuropneumonia (CCPP), foot-and-mouth disease (FMD) and bluetongue due to presence of pyrexia, nasal discharge, general malaise and death (Balamurugan *et al*., 2014). As previously reported,

<table>
<thead>
<tr>
<th>Location</th>
<th>Animal No.</th>
<th>Age (month)</th>
<th>NS</th>
<th>OS</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igabi</td>
<td><strong>1</strong></td>
<td>≤6</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><strong>2</strong></td>
<td>≤6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>≥12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>≥12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5*</td>
<td>≤12</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sabon Gari</td>
<td><strong>1</strong></td>
<td>≤12</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>≤6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3*</td>
<td>≤6</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><strong>4</strong></td>
<td>≤6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Giwa</td>
<td>1</td>
<td>≤6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
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<td></td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>≤6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n/N (x%) 5/10 (50.0) 4/9 (44.4) 3/10 (30.0)

*= 1 positive sample; ** = 2 positive samples; *** = 3 positive samples; + = Nucleic acid detected; - = Nucleic acid undetected; n = Number of positive; N = Number of samples; % = Percentage

<table>
<thead>
<tr>
<th>Location</th>
<th>Only 1 sample type positive</th>
<th>At least 1 sample type positive</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igabi</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sabon Gari</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total (%)</td>
<td>2 (33.33)</td>
<td>4 (100)</td>
<td>6</td>
</tr>
</tbody>
</table>

%= Percentage
negative finding, however, may be related to the timing of sample collection, as sampling was done
weeks after the onset of clinical signs, as well as small
sample size (Ezeibe et al., 2008). Most of the goats
with positive results were between three months old
and one year old. This is in agreement with Woma et
al. (2015).
Sampling in Igabi was done in May, 2018. In Sabon
Gari, the positive samples were sourced in July, 2018.
Kaduna state has two distinct seasons: Dry harmattan
season (April – November) and Rainy season (May –
October) (Ajala et al., 2008). This is indicative that PPR
also affects goats during the wet season in Kaduna
state, Northern Nigeria. This contravenes the position
of the study conducted in South-eastern Nigeria. It
had been reported previously that the disease mainly
affects ruminants during harmattan season in
Southern Nigeria (Wosu, 1994). The most severe clinical manifestations were
observed in goats not older than three months,
similar to that of Abubakar et al. (2018) who believed
that case-fatality rates were higher in young animals.
This may be due to poor immune status especially in
kids from unvaccinated does that have inadequate
maternal antibodies against the disease.
A nasal swab was found to have a higher positivity rate in the confirmation of PPR using RT-PCR targeting the
N gene, followed by an ocular swab. This may be
directly related to the pathogenesis of the disease, as
inhalation is a very common mode of transmission,
and the respiratory tract is probably the site of
primary viraemia. Due to tropism, a large amount of
the virus undergoes replication at this site within the
epithelial and immune cells. Rectal swabs are the
least reliable of the three, which could be due to the
relatively low levels of SLAM and PVRL4 receptors in
the intestine. Parida et al. (2019) also reported that
detection in faecal samples is intermittent. This is in
line with Mahajan et al. (2012) who reported nasal
swabs as first among the three, closely followed by
ocular, and ahead of rectal swabs using c-ELISA.
In conclusion, RT-PCR confirmed PPRV to be
responsible for the outbreaks in goats in Igabi LGA
and Sabon Gari LGA of Kaduna state. In Giwa LGA, PPR
was not confirmed. With RT-PCR, nasal swab was
observed to have a higher PPRV nucleic acid detection
rate (50.0%) than ocular swabs (44.4%), while rectal
swabs (30.0%) were the least reliable of the three.
From the variability observed among swab samples, a
more efficient protocol for diagnosing PPR using RT-
PCR is to utilize multiple swabs from different routes
from a given animal (multi-sampling technique), this
enhances PPR detection rates and reduce the
incidence of false negative conclusion which may be
deleterious to poor farmers as accurate diagnosis is
key to accurate therapy and care.

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

References
role of vaccine to combat peste des petits
ruminants outbreaks in endemic disease
situation. Journal of Animal Science and
Technology, doi:10.1186/s40781-014-0036-
y.
Abubakar M, Zahr AB, Naeeem K, Khan MA & Qureshi
S (2018). Field and molecular epidemiology
of Peste des petits ruminants in
Pakistan. Pakistan Journal of Zoology, 50 (2):
559-566.
small ruminant production in northern
Guinea savanna, Nigeria. Asian Journal of
Animal and Veterinary Advances, 3(3): 138 -
146
Practical Extension Problems Associated
with Peste des petit ruminants (PPR)
Vaccination in Goats in LAGOS, Nigeria:
Proceedings of the eleventh International
Symposium on Veterinary Epidemiology and
Bailey D, Banyard A, Dash P, Ozkul A & Barrett T
(2005). Full genome sequence of peste de
petite ruminants virus, a member of
morbillivirus genus. Virus Research, 110(1-
Balamurugan V, Hemadri D, Gajendraagad MR, Singh
RK & Rahman H (2014). Diagnosis and
control of peste des petits ruminants: a
comprehensive review. Virus Disease, 25(1):
39 – 56.
Balamurugan V, Sen A, Venkatesan G, Bhanot V,
Peste des petits ruminants virus detected in

Sokoto Journal of Veterinary Sciences, Volume 22 (Number 1). March, 2024

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