

Full Length Research Paper

Molecular diagnosis of recent suspected outbreaks of peste des petits ruminants (PPR) in Yola, Adamawa State, Nigeria

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Peste des petits ruminants (PPR) is an economically important viral disease of goats and sheep. It is endemic in Nigeria and was first described in West Africa in the 1940s. Clinically, PPR can be confused with other infections such as pasteurellosis or contagious ecthyma and mild strain of rinderpest in small ruminants. Effective implementation of control measures for PPR requires that a proper and rapid diagnosis of the disease is made. Therefore, the use of reverse transcriptase polymerase chain reaction to analyze suspected field samples collected from sheep and goats in Yola, Adamawa State has helped to give urgent diagnosis that was needed to effectively control the spread of the disease. The samples collected on filter paper were suitable source of the viral RNA.

Key words: Peste des petits ruminants, *Morbillivirus*, reverse transcriptase polymerase chain reaction, diagnosis.

INTRODUCTION

A Peste des petits ruminant (PPR) is a highly contagious viral disease of sheep and goats that causes high morbidity and mortality in these species (Couacy-Hymann et al., 2002). Due to the endemic nature of the disease in Nigeria, it is considered to be one of the main constraints to the productivity of small ruminants in the country (Obi and Patrick, 1984). Clinically, the disease is characterized by severe pyrexia, catarrhal ocular and mucopurulent nasal discharges, erosive stomatitis in early stages followed by severe enteritis and pneumonia. The disease is of high economic importance because of its high mortality rates, especially among young animals, with serious effect on animal production and trade (Nduaka and Ihemelandu, 1973).

PPR was first recognized as a disease entity in Côte

d'Ivoire (Gates, 1952) and has since been described in Senegal (Shaila et al., 1989), Nigeria (Singh et al., 2009; Gates et al., 1950-1951) and Ghana (Bonniwell and Gibbs, unpublished results, 1976). In Nigeria, the disease was described under such names as "Kata" (catarrh) by Whitney et al. (1967); stomatitis pneumoenteritis complex (Forsyth and Barrett, 1995) and pseudo-rinderpest (Durtnell and Eid, 1973). However, earlier reports of goat catarrhal fever (Campbell, 1958) and goat pneumonia (Whitney et al., 1967) may have referred to the same disease.

PPR is a disease caused by a pleomorphic, single stranded negative sense RNA virus known as PPR virus (PPRV). The virus belongs to the *Morbillivirus* genus in the family *Paramyxoviridae* (Obi and Patrick, 1984). PPRV is closely related antigenically to human measles virus (MV), canine distemper virus (CDV), rinderpest virus (RPV) and to the marine *Morbilliviruses* (Gibbs et al., 1979; Barret et al., 1993). Genetically, PPRV isolates can be grouped into four distinct lineages on the basis of

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partial sequence analysis of the fusion (F) protein gene (Dhar et al., 2002).

PPR has emerged as an endemic disease in Nigeria and other parts of Africa, the Middle East, Bangladesh, Pakistan and India (Choi et al., 2003; Wosu, 1991), but up till now, no large scale vaccination programmes have been established to eradicate the disease which is still widespread (McCulloch, 1951). Studies carried out in western Nigeria showed that PPR may be encountered almost all round the year but with peak period in the wet months of June to August (Obi et al., 1983). Also, introduction of new stock into the farm was associated with PPR outbreaks as reported by Obi et al. (1983).

According to report by the Federal Livestock Department (FLD) on PPR/Kata outbreaks in Nigeria (1977-1978), 191 outbreaks of PPR occurred mainly in goats in Oyo and Ondo State, with Oyo state having the highest incidence of 104 outbreaks. A total of 115 animals died of the 2,701 that were affected (Taylor, 1984). Wosu et al. (1991) in a study around the Nsukka area reported that PPR incidence were higher during the dry harmattan season (December to January) than in the rainy season with a peak in April.

The diagnosis of PPR was mainly based on conventional assays such as agar gel immunodiffusion test, counter immunoelectrophoresis (Mathew, 1980), haemagglutination test (Brindha et al., 2001), immunocapture enzyme linked immunosorbent assay (ELISA) (Dhar et al., 2002; Gargadenc and Lalanne, 1942; Libeau et al., 1994) or virus isolation (Diallo et al., 1995). These assays are mostly replaced by genome-based detection techniques such as reverse transcription polymerase chain reaction (RT-PCR) (McCulloch, 1951; Diallo et al., 1995; Mornet et al., 1956; Couacy-Hymann et al., 2002) and nucleic acid hybridization (Wosu, 1991; Nduaka and Ihemelandu, 1973) by virtue of their high sensitivity and specificity. However, whatever the qualities of all the new techniques is, it is important to know though not necessarily used for all outbreaks, that virus isolation still remains the gold standard diagnostic technique.

Thus, the objective of this work was to have prompt, specific and sensitive techniques for the effective diagnosis and implementation of the control measures on PPRV in Yola, Adamawa State, Nigeria. More so, the use of filter paper to collect sample in the field to detect virus-specific RNA for ease of collection and transportation to the laboratory was investigated.

MATERIALS AND METHODS

Sample collection

In April 2010, a suspected outbreak of PPRV in Yola, Adamawa State, North Eastern Nigeria was reported to the National Veterinary Research Institute Vom, Plateau State Nigeria. A team

from the research group was dispatched for investigation and collected samples from sick animals manifesting typical clinical signs of the disease and animals found around the area of the outbreaks. 30 samples (nasal and lachrymal swabs) and 3 organs (lung, spleen and lymph node) were taken from clinically sick sheep and goats. The swabs were taken in 1 ml virus transport medium containing penicillin, streptomycin, gentamycin and amphotericin-B (PSGA) and some were collected on filter paper instead of using dry cotton wool as reported by Forsyth and Barret (1995). The tissue samples (lung, spleen and mesenteric lymph node) were collected from a goat dying of a PPRV-like disease. The samples were transported to PPRV/Mucosal disease unit of the Virology Research Division, National Veterinary Research Institute Vom, on ice for processing and analysis.

RNA extraction

Reverse transcriptase polymerase chain reaction was used to investigate the field samples. 20% tissue homogenate was made by weighing 1.0 g of the tissue in 4 ml of 5X PSGA and was clarified at 3000 rpm for 10 min. Total RNA was extracted from the samples using Qiagen viral purification kits according to the manufacturer's instruction.

First strand cDNA synthesis

Reverse transcription reaction (RT) was carried out by taking 8 µl of each RNA sample into a sterile 1.5 ml eppendorf tube, heat denatured at 65°C for 10 min and chilled immediately on ice. To each sample, the following were added: 5 µl of bulk first strand cDNA reaction mix, 1 µl of DTT solution (200 mM) and 1 µl of random primer pd(N)6 primer (0.2 µg/µl) and incubated at 37°C for 1 h. The corresponding cDNA products were stored at -80°C.

PPR specific PCR

The master mix contained the following reagents: 32 µl of nuclease free water (Promega), 5 µl of 10x PCR reaction buffer, 5 µl of dNTP mixture (200 mM) (Fermentas), 2.5 µl of each primer; NP3: (5'-TCT CGG AAA TCG CCT CAC AGA CTG-3' and NP4: (5' -CCT CCT CCT GGT CCT CCA GAA TCT -3') (Inqaba Biotech, Pretoria, South Africa) at 5 pmol/µl, 5 units of Taq DNA polymerase (Qiagen) and 2 µl of cDNA. This mixture was submitted to a thermal cycling profile of initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s in an AppliedBiosystem 9700 PCR machine.

10 µl of each PCR product were analysed by electrophoresis on a gel which was of 1.5% agarose gel in Tris-borate-ETDA buffer (0.089 M Tris base, 0.089 M boric acid and 0.002 M EDTA, pH 8.3). The gel was stained with ethidium bromide in the presence of 2 µl of gel loading buffer (Fermentas) and the DNA was visualised by UV fluorescence and photographed.

RESULTS

Specific detection of PPRV RNA

A total of 33 samples were tested and 9 tested positive for PPRV. RNA was extracted from organs, nasal and

Table 1. The list of suspected field samples tested species, sample type and number positive by RT-PCR.

Species	Tissue/swabs sample	Number	PPRV positive by RT-PCR
Ovine	Nasal swab	8	0
Caprine	Nasal swab	18	3
Caprine	Lachrymal swab	4	3
Caprine	Lung	1	1
Caprine	Spleen	1	1
Caprine	Lymph node	1	1

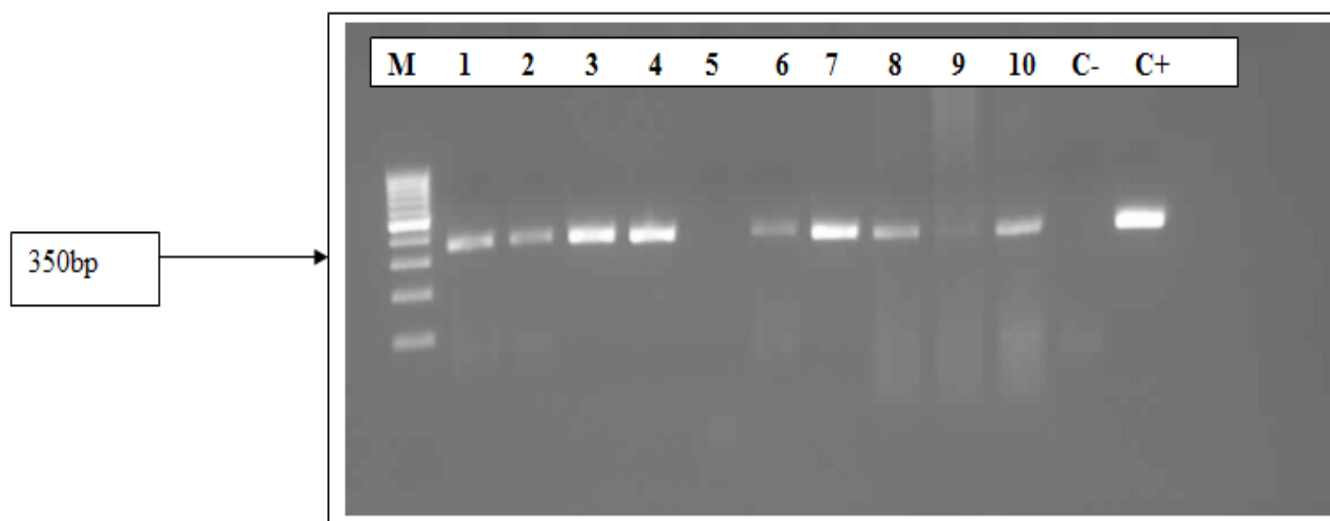


Figure 1. Specific PCR amplification of the NP gene fragment of different PPRV suspected samples with primers NP3/NP4. The amplified products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide. Lane M, 100 bp molecular weight marker; lane 1, NSF1; lane 2, NSF2; lane 3, LSF 3; lane 4, LSF 4; lane 5, LSF 5; lane 6, LSF 6; lane 7, NSF 7; lane 8, SP 8; lane 9, LN 9; lane 10, LN 10; C-, negative control; C+, positive control; M, molecular weight marker; NSF, nasal swab on filter paper; LSF, lachrymal swab on filter; SP; spleen, LN, lymph node ; LN, lung.

lachrymal swabs of animals that manifested clinical signs, indicative of PPRV. All the samples contained PPRV as demonstrated by RT-PCR test using the PPRV specific primers NP3 and NP4. The results were presented as the list of suspected field samples tested species, sample type and number positive by RT-PCR (Table 1). Of a total of 3 organs (lung, spleen and lymph node), 26 nasal swabs and 4 lachrymal swabs, 9 were positive for PPRV as indicated in the list of suspected field samples tested species, sample type and number positive by RT-PCR.

Primers NP3 and NP4 were used to amplify specifically, a fragment of the PPRV NP gene. The extracted RNA from suspected samples was tested with RT-PCR and the products read on gel. Figure 1 shows the photograph of the gel electrophoresis of the PCR products that was analyzed. The fragment size of the amplified products was 350 bp, a length that agrees with the expected band size of 351 bp as reported by Couacy-Hymann et al. (2002).

DISCUSSION

PPR can be confused clinically as rinderpest, and hence the clinical observations for both diseases should always be confirmed by a sensitive laboratory test. Such a diagnosis could be made now by competitive ELISA (antibody detection), or by immunocapture (antigen detection). It is important therefore to have laboratory confirmation of the infectious agent using a technique that is less time consuming and labour intensive. With the advent of nucleic acid techniques like PCR, rapid and specific diagnosis of PPR has become possible (Raj et al., 2008; Mornet et al., 1956). RT-PCR is a valuable method for the detection and differentiation of RPV and PPRV from pathological samples.

According to the work by Shaila et al. (1996), the target gene is that of the fusion proteins, one of the external viral proteins adopted in this study to amplify a fragment of the nucleoprotein (NP) gene. This protein is the major

viral internal protein. The primers were designed for use in RT-PCR test after the alignment of the second half of the NP gene of 10 PPRV (nine wild type strains plus the vaccine strain) as reported by Couacy-Hymann et al. (2002). RNA viruses are known to be subject to high nucleotide substitution error frequencies (Steinhauer et al., 1989). Therefore, for routine diagnosis of the virus infection from different origins, there is a risk of a false negative result if a PCR is carried out with two primers that were designed according to the gene sequence of only one virus strain (Couacy-Hymann et al., 2002). This risk might be minimized if two sets of primers are used to amplify two different fragments or if a set of primers is synthesized after taking into consideration the sequences from several strains of different origins (Couacy-Hymann et al., 2002). This latter strategy has guided the design of primers NP3 and NP4 for PPR diagnosis by RT-PCR. In the early 1990s, for the diagnosis of PPR by RT-PCR, two primers were synthesized after comparison of the NP sequence between PPRV Nigeria 75/1 and RPV lapinized strain d of one RPV strain (unpublished data).

One important practical result of the investigation was the finding that virus-specific RNA was detected in lachrymal and nasal swabs on filter paper as reported by Forsyth and Barrett (1995). This type of sample can be readily obtained, and can easily be transported to the diagnostic laboratory. In countries such as Nigeria where the disease is endemic, this sampling method is recommended since mortality rates are much lower because of vaccination, and postmortem samples may not be readily available in areas where these vaccinations are practiced (McCulloch, 1951). The swabs taken from the infected animals in the field not only provided a suitable source of viral RNA, but also was not subject to the same storage and transport problem associated with postmortem tissue samples which is in agreement with the work by Forsyth and Barrett (1995).

The recognition of the mild strains of RPV and the co-circulation of closely related PPRV, have created a situation in which the laboratory has assumed an even more vital role for confirmation of a provisional diagnosis. Therefore, the use of molecular diagnosis in the recent suspected outbreaks of PPRV in Yola, Adamawa state, Nigeria was necessary to monitor the success of measures implemented for disease control or as follow-ups to vaccination campaigns in the state. Also, the ease of sample collection, transportation and storage using filter paper was a great achievement to generate samples from all over the country.

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