

Original Research Article

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Molecular Detection of Peste des-petits Ruminant Virus (PPRV) by RT-PCR

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ABSTRACT

Keywords

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Peste des petits ruminants is one of the important transboundary, highly contagious and infectious viral disease of small ruminants especially sheep and goats caused by *Small Ruminant Morbillivirus* alias *Peste- des- petits ruminants virus*. In the current study, PPRV was detected in clinical samples of suspected goats as well as post vaccinated samples in nasal swabs and PBMC by conventional RT-PCR. Out of the 23 nasal swabs (12 infected and 11 vaccinated) 11 infected and one vaccinated samples was found positive. Out of 16 (5 infected and 11 vaccinated), all five infected and one vaccinated PBMC samples were found positive. Thus, the present study delineated the presence of PPRV in clinical samples as well as viaremic stages of PPRV. Further sequencing and characterization will warrant lineage of PPRV circulating in Nagpur district of Maharashtra.

Introduction

Peste des petits ruminants is a distinguishable and economically important transboundary disease of sheep and goat. Peste des petits ruminants (PPR), is a highly contagious and infectious viral disease that led the high morbidity and mortality caused by *Small Ruminant Morbilli Virus* (SRMV) also known as *Peste- des- petits ruminants virus* (PPRV). SMRV is an enveloped, single-stranded negative-sense RNA virus, belonging to the

genus morbillivirus within the family *Paramyxoviridae* along with other member including Rinderpest (Gibbs *et al.*, 1979; ICTV report 2019). The PPRV disease exhibits clinical signs such as fever, oculo-nasal discharge, watery blood-stained diarrhea, dyspnea, sloughing of the epithelium of oral and nasal mucosa, which later becomes mucopurulent giving fetid odor. PPRV infection may end with high morbidity (up to 100%) and mortality of (80%) and close contact between animals is highly risk factor

to transmission of disease (Nanda *et al.*, 1996; Dhar *et al.*, 2002; Balamurugan *et al.*, 2012; Truong *et al.*, 2014). PPR has been reported in Africa, as well as in Middle East and Asia (Banyard *et al.*, 2010). Many reports have been recorded in India since 1994 in southern and northern parts of India (Nanda *et al.*, 1996). An outbreak of PPR was reported in Coimbatore, India in 2011, where the samples were investigated using RT-PCR targeting nucleocapsid (N), fusion (F) and haemagglutinin (H) genes (Kumar *et al.*, 2014).

The genome of PPRV contains, 3'-N-P-M-F-H-L-5' which encodes for six structural proteins namely, nucleocapsid, phosphoprotein, matrix, fusion, haemagglutinin and large polymerase (Bailey *et al.*, 2005; Nanda *et al.*, 2006; Chard *et al.*, 2008). The matrix protein (M protein) is located on the inner surface of the envelope which bridges the ribonucleoprotein and cytoplasmic tails of two membrane glycoproteins, H and F proteins (Parida *et al.*, 2015).

It is known to play a significant role in the virus assembly and budding by concentrating F and H proteins as well as ribonucleocapsid at virus assembly site. It may improve the growth characteristics of the virus, incorporating M protein. The diagnosis of PPRV is basically based on serological techniques and on viral isolation from clinically suspected samples.

These techniques are time-consuming, laborious and insensitive as well for PPRV detection (Libeau *et al.*, 1994; Couacy-Hymann *et al.*, 2002; Parida *et al.*, 2015; Barrett *et al.*, 1994; Singh *et al.*, 2004). On the other hand, reverse transcription polymerase chain reaction (RT-PCR) considered as a diagnostic tool for PPR detection (Toplu *et al.*, 2012). The present study was conducted to

confirm the PPRV circulating in the Nagpur district as well as to ascertain the viaremic stages in the disease conditions or post vaccination using RT-PCR targeting M gene.

Materials and Methods

Sample collection

A total of 39 samples (nasal swab and blood) were collected from goats suspected for PPR disease, PPR vaccinated from different locations at Aptur village Umred Tahsil Dist. Nagpur and Nagpur city in year 2018-19 as detailed in table 1.

Isolation of PBMC from blood

Five milliliter blood was collected in the sterile vacutainer with pretreated anticoagulant from PPRV suspected infected, PPRV vaccinated goats. PBMC were isolated using Histopaque-1077 (Sigma) by density gradient centrifugation according to manufacturer's protocol. Briefly, the blood was diluted with sterile cell culture grade PBS in equal proportion. Histopaque reagent (3ml) was loaded on to 9 ml of the diluted blood sample in a ratio of 1:3 which was then centrifuged at 2000 g for 25 minutes at room temperature. Various components in the blood could be seen as separate layers with plasma at top followed by a buffy coat of monocytes, histopaque and lymphocytes in the middle and RBC's at bottom. The buffy coat was collected carefully without disturbing any another component. The cells were then washed using 10 ml isotonic phosphate buffer and centrifuged at 2000 g for 10 minute at room temperature. The pellet was obtain and resuspended in RNA later and stored in -70 °C.

Isolation of RNA

RNA isolation from PBMC was done by miRCURYTM RNA Isolation kit (Exiqon) and

RNA isolation from Nasal swab was done by TRI reagent (Cat # 15596026, Invitrogen). The isolated RNA was quantified by Nanodrop (NP 1000, Thermo fisher Scientific, USA). The RNA was used for PPRV confirmation as well miRNA/mRNA analysis (data not shown).

cDNA synthesis

The cDNA synthesis was carried out as per SuperScript® III First-Strand Synthesis System for RT-PCR kit (Invitrogen, USA Cat. No. 18080-051) according to manufacturer's instructions in 20µl reaction volume containing the following reagent in labeled microcentrifuge tubes. Briefly, 10µl of reaction I was prepared by adding upto 5µg of total RNA, 1µl of random hexamer primers, 1µl of 10mM dNTP mixture and DEPC water was make up to 10µl reaction volume. The prepared reaction mixture I was incubated at 65°C for minutes and then it was snap chilled on ice for at least 1minute. Reaction II of 10µl was prepared by adding 2µl of 10X RT buffer, 4µl of 25mM Magnesium chloride, 2µl of 0.1M DTT, 1µl of RNase Out (40U/µl) and 1µl of Superscript III Reverse Transcriptase (200I/µl). The reaction I & II was mixed and briefly centrifuged and incubated in PCR thermo-cycler (Prima Trio, High-Media, India) at 25°C for 10 min, 50°C for 50 min and followed by 85°C for 5 minutes. The synthesized cDNA was used immediately/ stored at -20°C until used.

Amplification of M gene by RT- PCR

Polymerase chain reaction for amplification of M gene from samples was carried out using published primers PPR-M-FOR 5'-TGTGTACATGAGCATAACTAGATT ATCA-3' and PPR-M-REV 5'-ACTTTCAATTCTTAGTAGTGTAACCAA GATG-3' (Balamurugan *et al.*, 2006). The reaction mixture (25 µl) was prepared

containing 12.5 µl of 2X PCR master mix (Purigene), 1 µl each of 10 pmol of forward and reverse primers, 1µl of template and 9.5µl of nuclease free water. The reaction was carried out in PCR thermo-cycler (Prima Trio, High-Media, India) with cyclic conditions: initial denaturation at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 56°C for 1 minute, extension at 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. The PCR products were analyzed on 2% agarose gel with 50bp DNA ladder (GeneRuler 50 bp cat # SM0372 Thermo fisher Scientific, USA) for expected amplicon size of 124 bp.

Results and Discussion

The clinical signs of PPR suspected animal exhibits off feed, high body temperature with approximately 105-106°F, coughing, semi solid/profuse diarrhea, mouth ulcers were observed which were typical symptoms of PPRV infection (Fig. 1), whereas, none of the vaccinated animals showed any symptoms. The clinical symptoms indicated suspected for PPR disease outbreaks in Nagpur district. In different parts of the world, variable percentage of mortality and morbidity involving PPRV in both sheep and goat populations have been reported. The status of PPR by serology sheep and goats in southern India with a prevalence of 41.35% and 34.91% respectively have been reported (Raghavendra *et al.*, 2008). All the studies showed that the prevalence of the disease might have been attributed due to agro-climatic conditions, socioeconomic factors and the pattern of migration of small ruminants in relation to season, flock size and population density of the animals.

A total of 39 samples (23 nasal swabs and 16 PBMC) were screened by M gene RT-PCR. Out of 39 samples, 12 nasal swabs and 16 PBMC were found to be positive. The

expected 124 bp amplicons was detected in nasal swabs and PBMC samples (Fig. 2A, 2B and Fig. 3).

In the present study, 11 infected samples out of 12 infected samples and one vaccinated out of 11 vaccinated nasal swab sample were found positive for the PPRV. The 124bp expected amplicons indicated the presence of PPRV in 91.66% positivity in PPR suspected goats whereas, 9.09% in nasal swabs of vaccinated goats. For the diagnosis of PPR several methods such as ELISA, conventional PCR were employed, however RT-PCR allows efficient virus detection in clinical samples such as nasal swabs (Forsyth and Barrett 1995; George *et al.*, 2006; Bao *et al.*, 2008). High sensitivity, specificity and rapidity of PCR makes it, the first choice of diagnostics in comparison to virus isolation, immune fluorescence. Since the detection of PPRV from clinical samples is difficult to assess by other diagnostic tools, RT-PCR is recognized as the most sensitive tool for the detection of viral products. The virus was readily detected in nasal swabs, makes it an optimal clinical sample from suspected animals for PPRV (Parida *et al.*, 2019). Apart from nasal swabs, other samples can also have

the potential for demonstrating the presence of virus in suspected samples. The detection was also done in PBMC isolated from blood. In the present study, 16 PBMC samples (5 infected and 11 vaccinated) were screened for PPRV, out of which 5 from infected and only one from the vaccinated was found positive for the PPRV. The PBMC samples from the suspected samples showed 100% positivity, however 9.09 % in vaccinated samples. Studies have shown that PPRV could be detected from blood samples not only at late stages but also at early stage of infection. It was also showed that PPRV was successfully recovered from PBMC of sub clinically infected cattle (Sen *et al.*, 2014). The present study delineated the presence of PPRV in clinical samples as well as viaremic stages of PPRV in clinical and post vaccinated sample. Further sequencing and characterization will warrant lineage of PPRV circulating in Nagpur district of Maharashtra. The study confirmed the circulation of PPRV in the different locality of Nagpur city and Umred tahsil, Nagpur district, Maharashtra. The trade of animals/ migration of animals, mixing of animals for market from different location might be responsible for regular outbreaks in Nagpur region of Maharashtra.

Table.1 Details of sample collection

Place	Types of Samples	Number of Samples
Aptur village, Umred Taluka, District. Nagpur, Maharashtra	Nasal Samples	4 (suspected for Infection) 11 (6-8 days post Vaccination)
	Blood Samples	2 (suspected for Infection) 11 (6-8 days post Vaccination)
Telankhedi, Mominpura, Jafar Nagar, Sadar Nagpur, Maharashtra	Nasal Samples	8 (suspected for Infection)
	Blood Samples	3 (suspected for Infection)
Total		23 (Nasal Samples), 16 (PBMC)

Fig.1 Clinical signs shown by PPRV infected animals



C. Showing ulcerative ulcers of mouth

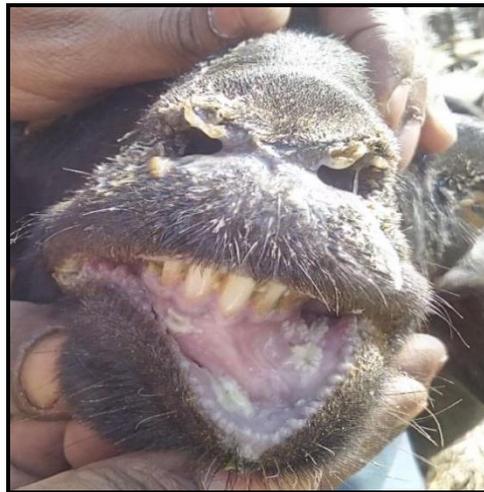
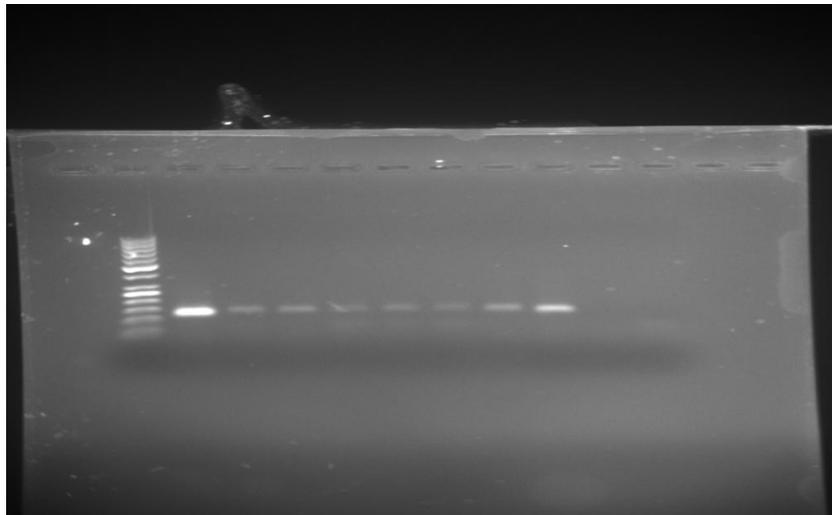


Fig.2



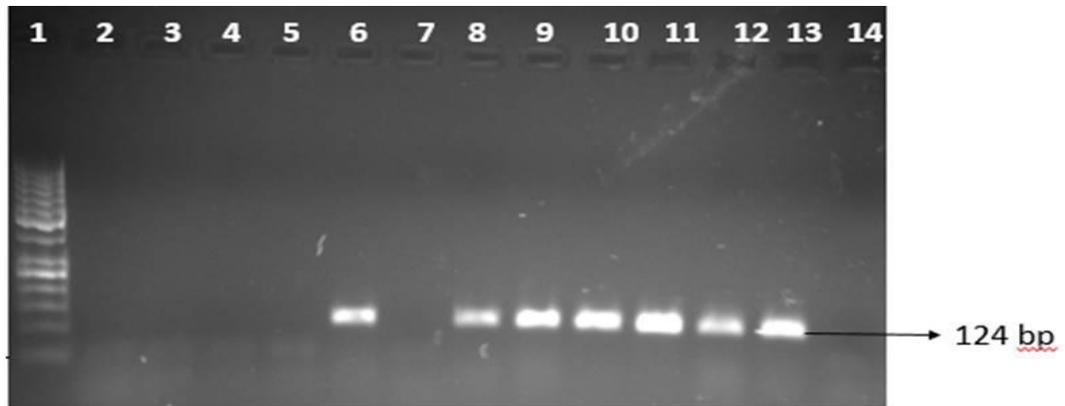
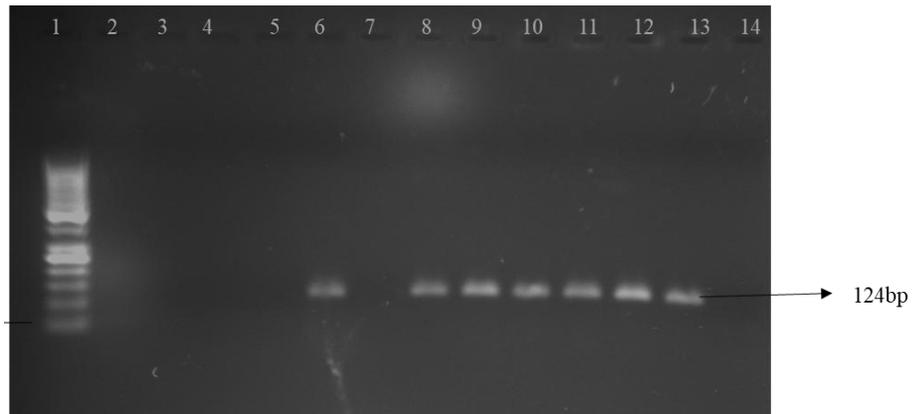


Figure 2.B: Agarose gel electrophoresis showing Mgene positive samples in nasal swabs with a product size of 124bp

Lane1: 50bp ladder
Lane2-12: Samples suspected for PPRV
Lane 13: Positive Control
Lane 14: Negative Control

Fig. 3



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