Int.J.Curr.Microbiol.App.Sci (2020) *9*(9): 522-526

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 9 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.909.066

Detection and Molecular Characterization of Peste Des Petits Ruminants Virus from Goat Flocks in Ambala, Haryana

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Keywords

Goat Flocks, Peste des petits ruminant (PPR)

Article Info

Accepted:
07 August 2020
Available Online:
10 September 2020

A B S T R A C T

A Peste des petits ruminant (PPR) is a highly contagious viral disease of sheep and goats characterized by high morbidity and mortality. The present study was conducted to determine comparative efficacy of F and N gene based reverse transcription polymerase chain reaction for molecular detection of PPR virus from clinical samples from goat flocks in Ambala district of Haryana. Out of 36 clinical samples tested, 28 samples were found positive by N gene-based primers, however, 23 samples were found positive by F gene based primer set. Hence, N gene-based RT-PCR used in this study was found to be more sensitive for detection of PPRV as compared to F gene based assay. The nucleotide sequence analysis and inferred phylogenetic relationship revealed the presence of lineage IV of PPRV.

Introduction

Peste des petits ruminants (PPR), also known as'goat plague', is a viral disease of goats and sheep characterized by fever, sores in the mouth, diarrhea, pneumonia, and sometimes death. Etiologic agent has been classified as member of genus *Morbillivirus* of Family *Paramyxoviridae.* The disease is associated with high morbidity (100%) and mortality (up to 90%) (Abu-Elzein *et al.,* 1990). PPR is also classified as transboundary animal disease (TAD) (Zahur *et al.,* 2011). Animals of all ages are susceptible to the disease. The disease is characterized by pyrexia, dyspnoea, pneumonia, mucopurulent occulo-nasal discharge, erosive rhinitis, necrotic ulcers in mouth, on dental pad, tongue and lips. In early stages there is stomatitis followed by severe enteritis and diarrhoea (Taylor, 1984).

In India, PPR was first reported from Arasur, Villupuram district (Tamilnadu state) during 1987 (Shaila *et al.,* 1989). PPR is now enzootic in India causing significant economic losses, and therefore, the infection is a major constraint for small animal production. Several RT-PCR assays have been developed for the rapid and specific detection of PPRV using different sets of

primers targeting F, M or N proteins (Couacy-Hymann *et al.,* 2002 and Balamurugan *et al.,* 2006). The present study was conducted to determine comparative efficacy of F and N gene based assays for molecular detection of PPR virus from clinical samples from goat flocks in Ambala district of Haryana.

Materials and Methods

Collection of samples

A total of 36 clinical samples including 18 blood and 18 nasal swabs were collected from goat flocks in and around Ambala, Haryana. These goats were suffering from fever, respiratory distress, coughing and nasal discharge suggestive of PPRV infection. A blood sample collected from an apparently healthy goat was used as negative control.

Molecular detection of PPRV nucleic acid using RT-PCR

The collected samples were tested for the presence of genomic RNA by the reverse transcription polymerase chain reaction (RT-PCR) assay. Viral genomic RNA was extracted from collected samples using a commercial RNA extraction kit (Qiagen® RNeasy Universal Mini Kit) as per the manufacturer's instructions. RT-PCR was performed for the N-gene and F-gene of PPRV using the QIAGEN® One-step RT-PCR kit as per the manufacturer's instructions. Mastermix was prepared as per manufacturer's instructions containing 2X RT-PCR Buffer, RNA template, Enzyme Mix, RNAse Free Water and Primers with final concentration of 0.6 µM (Table 2).

Sequencing and phylogenetic analysis

Subsequently, RT-PCR amplicons of PPRV positive samples were gel purified and sequenced using genetic analyser (3130XL) in the Department of Animal Biotechnology, LUVAS, Hisar. The nucleotide sequences of 447 bp and 351 bp amplicons amplified from F and N genes, respectively were aligned with corresponding sequences available in GenBank using Clustal W (Thompson *et al.,* 1994). Phylogenetic analysis of F and N gene segments of PPRV was performed with the MEGA version 6 software. Phylogenetic analysis of the aligned sequences was performed by the Neighbor-joining method (Saitou and Nei, 1987). Bootstrap confidence intervals were calculated on 500 iterations.

Results and Discussion

Out of 36 clinical samples tested 28 samples were found positive by N gene-based primer set producing an expected amplicon of 351bp. F gene-based primers detected 23 samples positive for PPRV producing desired amplicon of size 447 bp. The causative agent of the disease PPR; is a member of genus morbillivirus in the family Paramyxoviridae (Barrett *et al.,* 2005). It has a single strand negative sense RNA genome that encodes eight proteins in the order of 3'- N-P/C/V-M-F-H-L-5' (Bailey *et al.,* 2005).

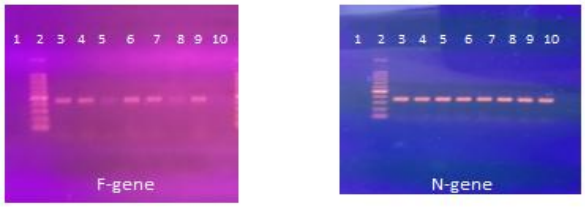
Table.1

Sr. no.	Primer	Primer sequence	Reference
1	NP3	5'-GTC-TCG-GAA-ATC-GCC-TCA-CAG-ACT-3'	Couacy-Hymann *et al.,* (2002)
	NP4	5'-CCT-CCT-CCT-GGT-CCT-CCA-GAA-TCT-3'	
2	F1b	5'- AGTACAAAAGATTGCTGATCACAGT-3'	Dhar *et al.,*(2002)
	F2d	5'- GGGTCTCGAAGGCTAGGCCCGAATA-3'	

Table.2 Cycling conditions used in one step RT PCR to amplify N and F gene

S. No.	Step	Temperature	Time
1.	Reverse Transcription Step	50	30 min (1 cycle)
2.	Inactivates RT and activates polymerase	95	15 min (1 cycle)
3.	Denaturation	94	1min (35 cycles)
4.	Annealing	60 (N-gene) 55 (F-gene)	1 min (35 cycles)
5.	Extension	72	1 min (35 cycles)
6.	Final Extension	72	7 min (1 cycle)

Fig.1



Agarose gel electrophoresis of amplified 447 bp product and 351 bp by PCR using F- gene and N-gene specific primers for PPR virus; Lane 1 :Negative control, Lane 2:DNA marker, Lane3-10: Samplestested

Fig.2 Phylogenetic analysis of nucleotide sequence from the amplified product of N gene of PPRV with different lineages occurring worldwide

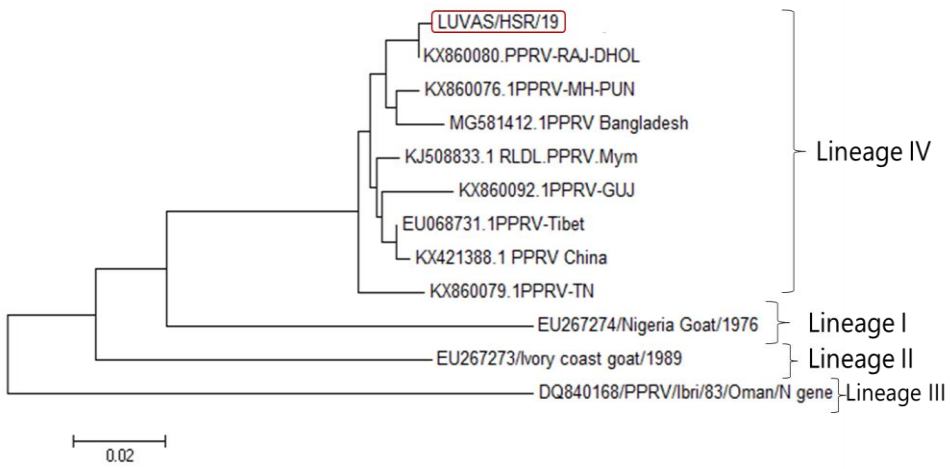
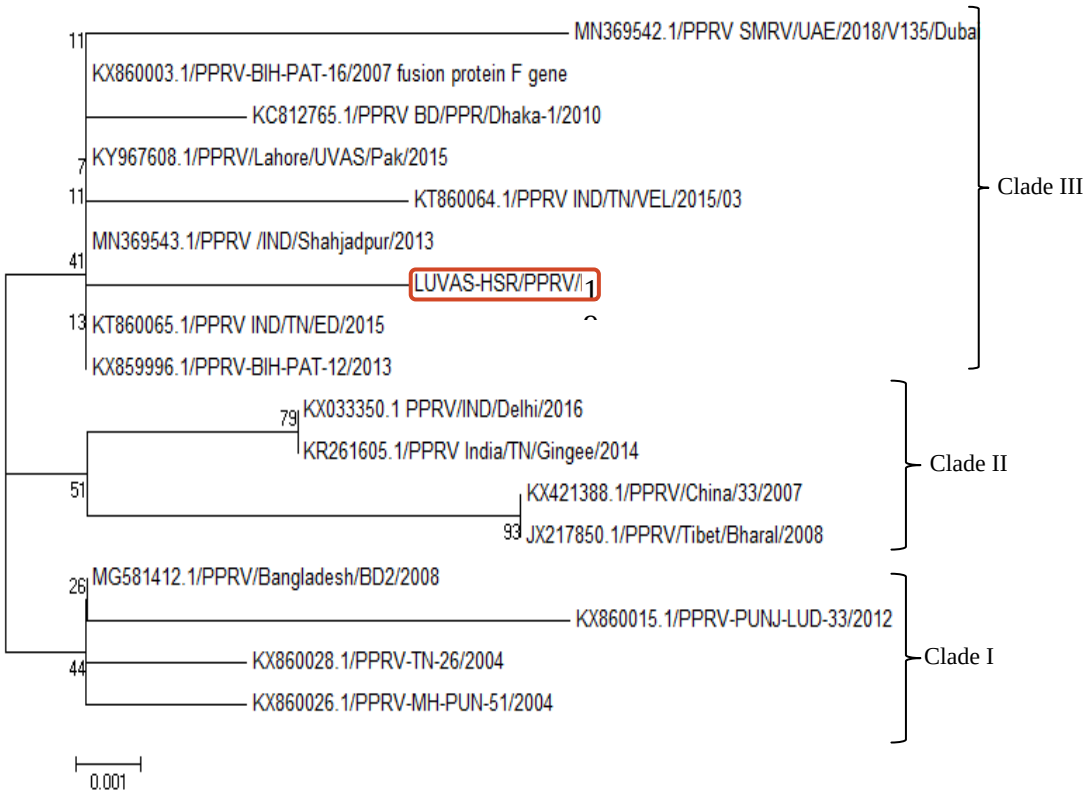


Fig.3 Phylogenetic analysis of nucleotide sequence from the amplified product of F gene of PPRV with different global isolates



Among them, the nucleocapsid protein (NP) is the major viral protein. It has been the target for developing diagnostic tests that can be used to identify PPRV (Couacy-Hymann *et al.,* 2002). The results of the present study are in accordance with study conducted Kerur *et al.,* (2008) who reported N gene primers are more sensitive than F-gene based primers. The evident reason for higher sensitivity of the N gene based primers than F gene based primers can be attributed to the fact that N gene are most abundant transcripts of the virus and is the first protein to be produced, (Ghosh *et al*., 1995) thus making N gene more suitable target for improving the sensitivity of RT-PCR for detection of PPRV from clinical samples. The results are also in agreement with those of Mahajan *et al.,* (2014) and Manar *et al*., (2017) who stated that F gene primers could give false negative results and suggested that F gene primers could be easily replaced by the highly sensitive and specific N gene primers for the detection of PPRV nucleic acid.

Phylogenetic analysis

The phylogenetic tree (Fig. 2) based on the 351 bp sequence of N gene clustered all the PPRVs of India including those from the present study, isolates of turkey, an isolate of Pakistan and Iran into a separate branch from Nigerian isolate. While the phylogenetic analysis (Fig. 3) based on the 447 bp F gene sequences out-rooted the Nigerian isolate, while the Turkey and Indian isolates were grouped into two clusters, although with low bootstrap values. The inferred phylogenetic relationship among the PPRV strains taken in this study vis-a-vis other PPRVs sequence showed that the PPRV of this study belonged to PPRV lineage IV (Figs. 2 & 3).

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How to cite this article:

Vandna Bhanot, Aman Kumar and Naresh Jindal. 2020. Detection and Molecular Characterization of Peste Des Petits Ruminants Virus from Goat Flocks in Ambala, Haryana. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 522-526. doi: https://doi.org/10.20546/ijcmas.2020.909.066