



Sequence and phylogenetic analysis of a peste-des-petits-ruminants (PPR) virus isolated from central India

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Peste-des-petits-ruminants (PPR) is a highly contagious viral disease of small ruminants (Dhar *et al.* 2002) and its outbreaks were reported from many Asian countries neighboring India, including China, Bangladesh, Pakistan, Tajikistan, Iran, and Sudan. Since, its first report from southern part of India by Shaila *et al.* (1989), the disease has been recorded from various other parts of country, viz. West Bengal, Himachal Pradesh, Maharashtra, Andhra Pradesh, Odisha, Tripura, Tamil Nadu, Uttarakhand and Uttar Pradesh. The present study described the detection and genetic characterization of PPR virus isolates circulating in central part of India (Madhya Pradesh).

During the study period (2007–2008), samples were collected from affected indigenous goat flocks with a history of PPR like disease from Jabalpur, Katni, Balaghat, Dhindori, Umaria and Hoshangabad districts of Madhya Pradesh. Samples (112 blood, 90 nasal and 50 fecal) were collected. Serum was separated from aseptically collected blood samples, and stored in cryovials at –20°C after heat treatment at 56°C for 30 min. Monoclonal antibodies (MAb) based ELISAs (cELISA and sELISA) kits were used to screen samples for the presence of PPR virus antibodies and antigen (Singh 2002). The sELISA positive samples were further confirmed using N gene based RT-PCR.

The viral RNA extraction was carried out using spin viral RNA kit. The viral RNA quality and quantity was assessed by using nanodrop spectrophotometer. Purity of RNA was judged on the basis of optical density ratio at 260:280 nm and samples with the purity ratio 1.7–2.0 were accepted. RT-PCR conditions were optimized using the N gene specific primers of Kerur (2005) (N1 (F) 1208–1226bp, 5'GATGGTCAGAA GATCTGCA3' and N1(R) 1670–1652bp, 5'CTTGTCGTTGTAGACCTGA3'). One step RT-PCR kit was used for the cDNA synthesis and PCR. Master

mix and templates were mixed together with forward and reverse primers to generate a 463bp product, with RT-PCR conditions of 45 min at 45°C for reverse transcription, 5 min denaturation at 95°C and 35 cycles of 94°C for 30 sec (denaturation), 50°C for 1 min (annealing), 72°C for 1 min (extension). The amplified products were visualized as single band of expected 463bp size under UV light and documented. PCR products were purified using gel extraction kit and a selected amplicon was sequenced and analyzed.

The nucleotide sequence determined in this study was deposited in the NCBI GenBank sequence database with accession number GQ122186. The sequence chromatogram file was visualized using Chromas 2.33 version software. PPR virus sequences from different geographical locations within and outside India were downloaded from the NCBI nucleotide database. Sequence analysis was performed using MEGA4. The per cent identity and divergence of the nucleotide and deduced amino acid sequences was performed to determine the percentage homology with other PPR virus sequences. The evolutionary lineage history was inferred using the Neighbor-Joining method with the bootstrap consensus tree inferred from 1000 replicates being taken to represent the evolutionary history.

Madhya Pradesh, the central part of India is surrounded by seven other states. Majority of the state's population is rural (73.3%) and tribal (19.9%) and depends mainly on goat

Table 1. Distribution of PPR virus infection in districts of MP state

Place	cELISA results		sELISA results	
	Samples tested	Positives (%)	Samples tested	Positives (%)
Balaghat	11	02 (18.2)	07	02 (28.6)
Jabalpur	12	03 (25.0)	09	04 (44.4)
Katni	27	23 (85.2)	09	05 (55.5)
Hoshangabad	50	25 (50.0)	22	11(50.0)
Dhindori	12	08 (66.7)	11	07 (63.6)
Total	112	61 (54.46)	58	29 (50.0)

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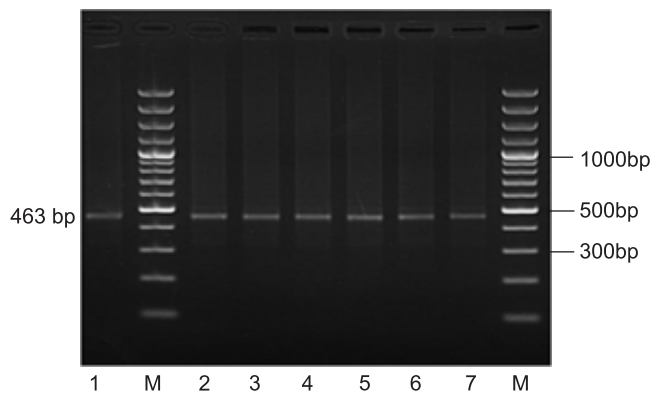


Fig. 1. PPR virus N gene specific RT-PCR. Arrow indicates amplicons of 463bp with primer pair N1 (F) and N2 (R). Lanes indicate M: 100bp DNA ladder, 1: vaccine virus and 2-7: PPR virus positive field samples.

husbandry for their livelihood. As per the 17th livestock census, Madhya Pradesh has 0.89% of sheep and 6.55% of goat's population (81.42 lakh) of the country. Availability of data on systematic study of PPR is very limited including characterization of local virus isolate. During the study, 54.46% goats (61/112) were found to carry PPR antibodies in cELISA (Table 1) and district-wise prevalence of antibodies to PPR virus varied from 18.2% in Balaghat (bordering Chhattisgarh state) to 85.2% in Katni (central part of state) (Table 1). Variations were noticed in the distribution of the PPR virus antibodies in the state and similar variations were reported earlier (Rengasamy *et al.* 2007). In sELISA, PPR virus antigen was detected in 29 (50%) clinical samples out of 58 nasal/faecal samples from goats showing symptoms and lesions suggestive of PPR (Table 1). The district-wise

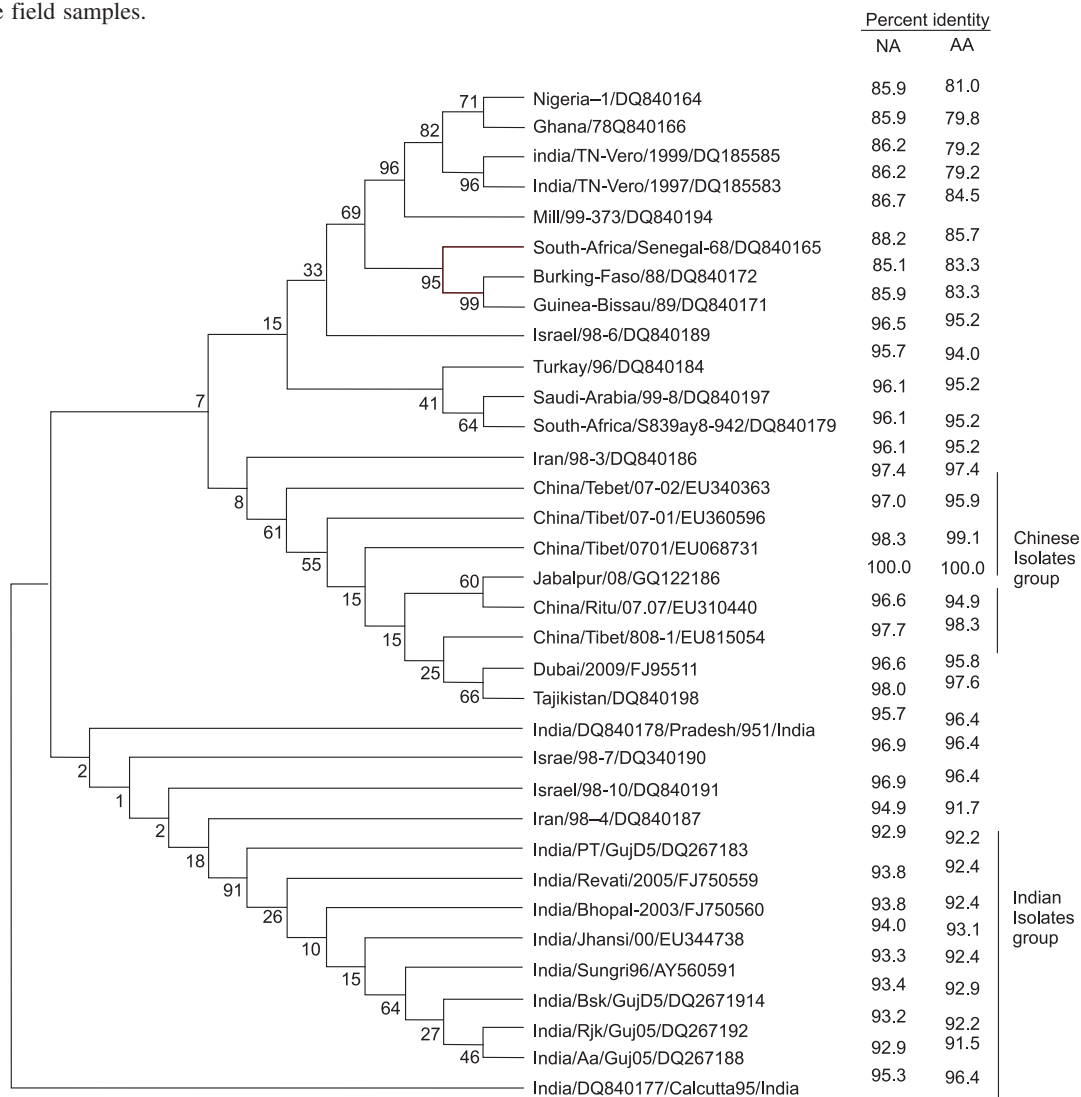


Fig. 2. Phylogenetic and percent sequence identity at nucleotide (NA) and amino acid (AA) of PPR virus isolate (Jabalpur/08) from central India with other PPR virus isolates. Jabalpur/08 isolate is highlighted in black dot. The phylogenetic analysis was completed with MEGA4 software that used a neighbor-joining algorithm and absolute distances and that followed 1,000 bootstrap replicates. The tree is based on the partial sequence of the nucleocapsid (N) protein gene.

distribution of PPR virus was 28.6% (2/7) in Balaghat, 44.4% (4/9) in Jabalpur, 55.5% (5/9) in Katni, 50% (11/22) in Hosangabad and 63.6% (7/11) in Dindori (Table 1). Tiwari (2004) reported an incidence of 60% PPR in Gujarat, the neighboring state of MP, using sELISA. Later, Kerur (2005) reported incidence of 81.3% in Gujarat through sELISA.

N gene sequence based classification of PPR virus into lineages gave better picture of molecular epidemiology for PPR virus (Kerur 2005). In the present study, nucleocapsid 'N' gene based RT-PCR results confirmed the PPR infection in all sELISA positive goats (Fig.1). N gene partial product (463bp) sequences of the Jabalpur/08 PPR virus were further compared with PPR virus isolates from India, Eastern Asian countries, Middle East and Central Asian countries. The sequence analysis revealed relatively higher identity (95.8% to 99.1%) at amino acid level with PPR virus isolates from China-Tibet (Acc. no. EU340363, EU068731, EU360596, EU815054) and 97.6% with Tajikistan strain (Acc. No. DQ840198) (Fig. 2). Wang *et al.* (2009) reported that sequences of a PPR virus from Tibet had high level of nucleotide identity with India/Bsk/Guj/05 strain isolated in India in 2005 from Gujarat and the Tibet PPR virus isolate has a probable origin from India. However, our results showed that China-Tibet strains makes a different group and the India/Bsk/Guj/05 isolate falls in separate Indian group (Fig.2).

The homology at amino acid level of Jabalpur/08 isolate with majority of the Indian PPR virus isolates was between 91.5 and 93.1%, except Calcutta95 strain (Acc. no. DQ840177) which showed sequence similarity of 96.4%. Jabalpur/08 isolate showed similarity of 92 to 93% at nucleotide and amino acid level with isolates from neighboring areas of Jabalpur, viz. Bhopal and Jhansi, submitted during 2003 and the sequence similarity with Sungri96 (a vaccine strain) was 93.3% at nucleotide level and 92.4% at amino acid level (Fig. 2). The divergence from vaccine strain could be the probable reason of number of outbreaks occurring in field in spite of vaccination. However, further studies are warranted to confirm this assumption. The sequence comparison with international strains showed highest sequence homology of 99.1% with Tibet strain (Tibet0701) followed by 98.3% with China-Tibet B08-1 strain. Sequence homology with Tajikistan strain was 98 and 97.6% at nucleotide and amino acid levels. The phylodynamic analysis confirmed that the PPR virus strain from central India (Jabalpur/08) is closely related to viruses currently circulating in neighboring Southeast Asian countries. The phylogenetic tree clustered the Jabalpur/08 isolate with the international PPR virus strains from Tibet, China, Dubai, Iran and Tajikistan (Fig.2), whereas majority of the Indian PPR virus isolates clustered in a separate group. These findings suggested that the Jabalpur/08 isolate might have an origin distinct from the majority of Indian isolates and might have either entered by crossing the neighboring international boundaries or co-evolved over the time, which need further confirmation.

Overall, our results confirmed the circulation of PPR virus in indigenous goat population in central India through cELISA, sELISA and RT-PCR. The virus that circulates in this region of central India is closely related to PPR virus isolates from China-Tibet (2007) and Tajikistan. Our results on spread of virus warrants further elaborative studies at molecular level to understand the evolution of PPR virus circulating in central India. Our data provides valuable information on PPR virus infection in small ruminants in central India.

SUMMARY

Peste-des-petits-ruminants (PPR), is an acute and highly contagious viral disease of small ruminants. The present study described distribution of PPR infection in central India (MP) with molecular characterization of selected PPR virus isolate. Sequence comparison showed highest homology of 99.1% with Tibet strain (Tibet0701) followed by 98.3% with China-Tibet B08-1 strain. Phylogenetic analysis clustered central Indian isolate with PPR virus isolates from neighboring Asian countries, mainly China-Tibet and Tajikistan. The result confirmed the occurrence of PPR in indigenous goats in central India with circulation of a virus strain with more similarity with cross border PPR strains.

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