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SHORT COMMUNICATION Regional Study

# Genetic Characterization of Peste des Petits Ruminants Virus Circulating in Different Regions of Pakistan based on Nucleocapsid Gene Sequence

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**Abstract**

**The current study was aimed at understanding the molecular epidemiology and genetic nature of *Peste des petits ruminants' virus* (PPRV) strains causing outbreaks in Pakistan by phylogenetic analysis of its nucleocapsid (N) gene. The phylogenetic analysis of N gene indicated that there are at least two different PPRV strains circulating in Pakistan at present. Phylogenetic analysis suggests that these strains are genetically similar to the strains reported from Iran and China. Moreover, Nucleotide sequence similarity among four N gene sequences analyzed in this study was found to be 80–90% when compared with sequences (available in GenBank) previously reported in the country.**

Key Words: Nucleocapsid gene, Phylogenetic Analysis, PPRV

*Peste des petits ruminants* (PPR) is a highly contagious viral disease of goats and sheep causing high morbidity and mortality. The disease is characterized by oculo-nasal discharges, erosions in mouth and clinical signs indicative of respiratory and digestive tract involvement. PPR is a transboundary animal disease (TAD) caused by *Peste des petits ruminants virus* (PPRV) belonging to the genus morbillivirus of the family Paramyxoviridae. It is included in the list of Office International des Epizooties (OIE) notifiable diseases. Historically, PPR was reported for the first time in 1942 from Cote d'Ivoire and later on various countries of Asia and Africa declared its presence[3)]. Due to the effect of PPR on small ruminant production, the disease has emerged as an issue of global concern. Food and Agricultural Organization (FAO) and OIE have launched a PPR Global Control and Eradication Strategy (PPR GCES) (FAO, 2016).

In Pakistan the disease was noted for the first time in 1991[6)] and the presence of PPRV in

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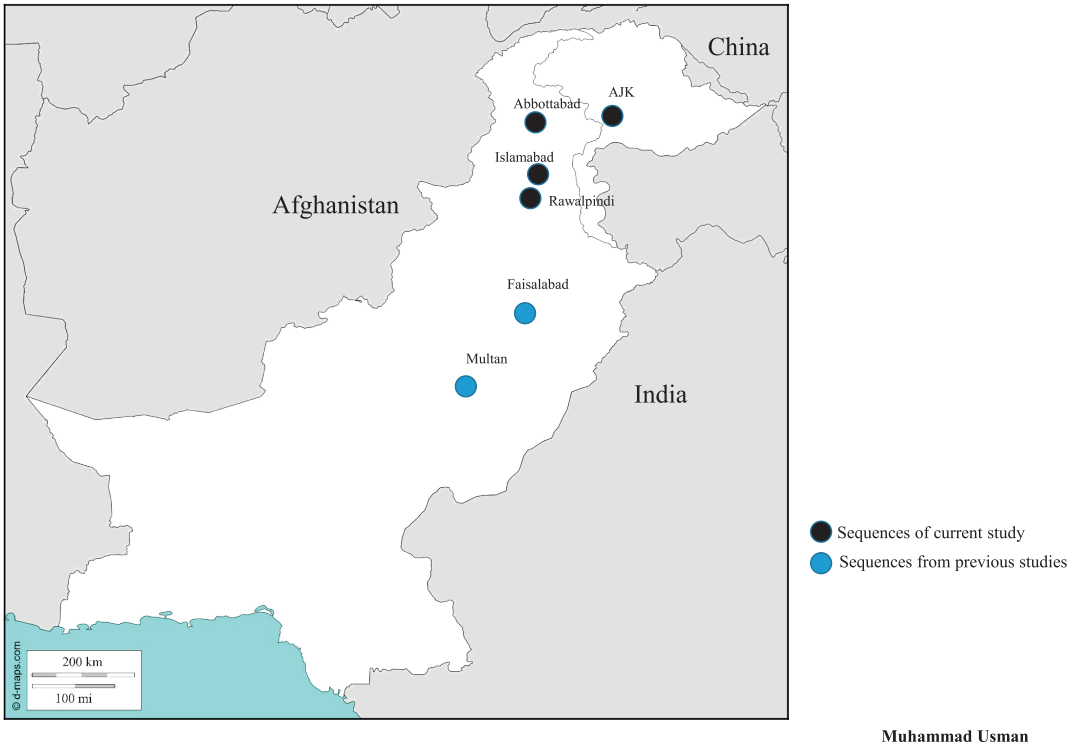


**Fig. 1. Geographical distribution of PPRV strains used in the phylogenetic analysis.**

the blood and tissues of diseased goats was confirmed through laboratory procedures in 1994[4]. In Pakistan PPR disease is endemic in most of the areas of the country and its cases increase in number rapidly at the start of the winter season[12]. In a recent baseline information study, the sero-prevalence of the infection was found to vary from 9.93% to 38.16% in the various provinces of the country[2]. Few studies have been conducted to know the genotypes of PPRV prevalent in Pakistan. Pakistani PPRV isolates belong to lineage IV[1]. Though, only genotype IV is prevalent in the country, the virus can mutate to form groups within a lineage. To establish epidemiological links, it is important to do genetic analysis of viruses especially in face of new outbreaks[10].

Results of recent studies have revealed that there is more variability in the N gene compared to the H and F genes among isolates within a lineage, which makes classification based on N gene a more accurate way of genotyping new isolates of virus[8,11]. Very few genotyping studies based on the N gene have been conducted on Pakistani PPRV isolates[5]. The present study was aimed at N-gene based genetic characterization of the PPRVs involved in new outbreaks.

The study includes twenty (20) outbreaks of PPR in different regions (Islamabad, Rawalpindi, Abbottabad and Barnala, Azad Jammu & Kashmir) of the country during 2016 as shown in Fig. 1. A total of twenty swab samples were collected during the outbreaks from the affected animals (sheep and goat) showing the clinical signs and symptoms suggestive of PPR like pyrexia, sneezing, coughing, oculo-nasal discharges, conjunctivitis, erosive mouth lesions and severe diarrhea (Supplementary Table 1). Fourteen tissue samples including lymph nodes, lungs and spleen were collected by conducting postmortem of animals died during the outbreaks. These samples were kept at −20°C for subsequent processing (Supplementary Table 2).

The samples were processed as per OIE instructions and kept at −80°C for RNA extraction. RNA was extracted by using Invitrogen

Purelink Viral RNA/DNA Kit (Carlsbad, CA 92008 USA) following manufacturer's instructions. Then, extracted RNA was confirmed for PPRV by Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) using Verso 1-step RT-PCR Kit (Thermo scientific) and already published primers (NP3 and NP4)[7].

Following sequences were downloaded from National Center for Biotechnology Information (NCBI) (accession no: FJ905304, JF939201, JX217850, KM091959, KM089831, KM089830, KP260624, KM089832, AY560591, KJ867542, KF727981, KJ867544, NC_006383, AJ849636, KJ867545) and then aligned by Geneious (Version10.1.3.). The complete N gene was amplified by PCR using the forward primer- 5' ATG GCK ACT CTY CTY AAA AGC 3' and reverse primer- 5' TYA RCY GAG GAG ATC CTT GTC 3' designed using the Geneious software. These primers were used to amplify the N gene of already detected positive samples of PPRV.

A total of 25% (4/16) representative samples were randomly selected for sequencing on the basis of the area of the outbreaks. The resultant PCR products of these samples were then purified by QIAquick Gel Extraction Kit following manufacturer's instructions. The purified PCR products were sent to the Macrogen (Korea) for sequencing. The sequences were edited and assembled by using Geneious. The assembled sequences were then submitted to NCBI to get accession numbers. The obtained sequences were aligned with the sequences retrieved from GenBank depicting all the lineages of PPRV. The Neighbour joining method in Mega7 (Version 7.0.26) was used to construct the phylogenetic tree by applying Kimura two-parameter model.

A total of 47.06% (16/34) samples from suspected outbreaks were found positive for PPRV by RT-PCR through NP3 and NP4 primers. All the positive samples were also detected positive for PPRV using newly synthesized complete N gene based primers.

All the sequences obtained were submitted to GenBank under accession numbers MF574747, MF574748, MF574749 and MF574750. Nucleotide sequence similarity among four N gene sequences analyzed in this study was found to be 80–90% when compared with sequences (available in GenBank) previously reported in the country. Analysis of N gene sequences in the current study revealed that the hypervariable region of N gene was highly conserved in PPRV strains circulating at present in different regions of the country.

Phylogenetic analysis revealed that the viruses circulating in the area of Islamabad (MF574748) and Rawalpindi (MF574749) clustered closely with viruses from Multan (2010-JN009674) and Faisalabad (2010-JN009674). All these viruses are similar in genetic character to the viruses circulating in China and clustered far from the vaccine strain (Nigera/75/1) as shown in Fig. 2. Currently, Nigeria/75/1 strain of PPRV is being used in Pakistan for the formulation of its vaccine. The viruses isolated from Abbottabad (MF574747) and Azad Jammu and Kashmir (MF574750) are also closely related to previously reported isolates from Pakistan and are closer in genetic character to the viruses from Iran and India as shown in Fig. 2. These two viruses sequenced in the current study showed more similarity to vaccine strain (Nigeria/75/1).

For the best eradication and control of PPR, proper understanding of genetic nature of all strains is important in the countries where PPR is endemic in nature. So, the current study was designed to understand the molecular epidemiology of PPRV circulating in different parts of the country. As N gene depicts better picture of PPRV epidemiology and is preferred over F gene targeted analysis of PPRV strains[5]. So, complete N gene was amplified and phylogenetic analysis was carried out in this study.

Sequence analysis in this study proved that the sequence of N gene is highly conserved and low substitution rate was present. So, this study corroborates the fact that N gene should be preferred for better understanding of PPRV

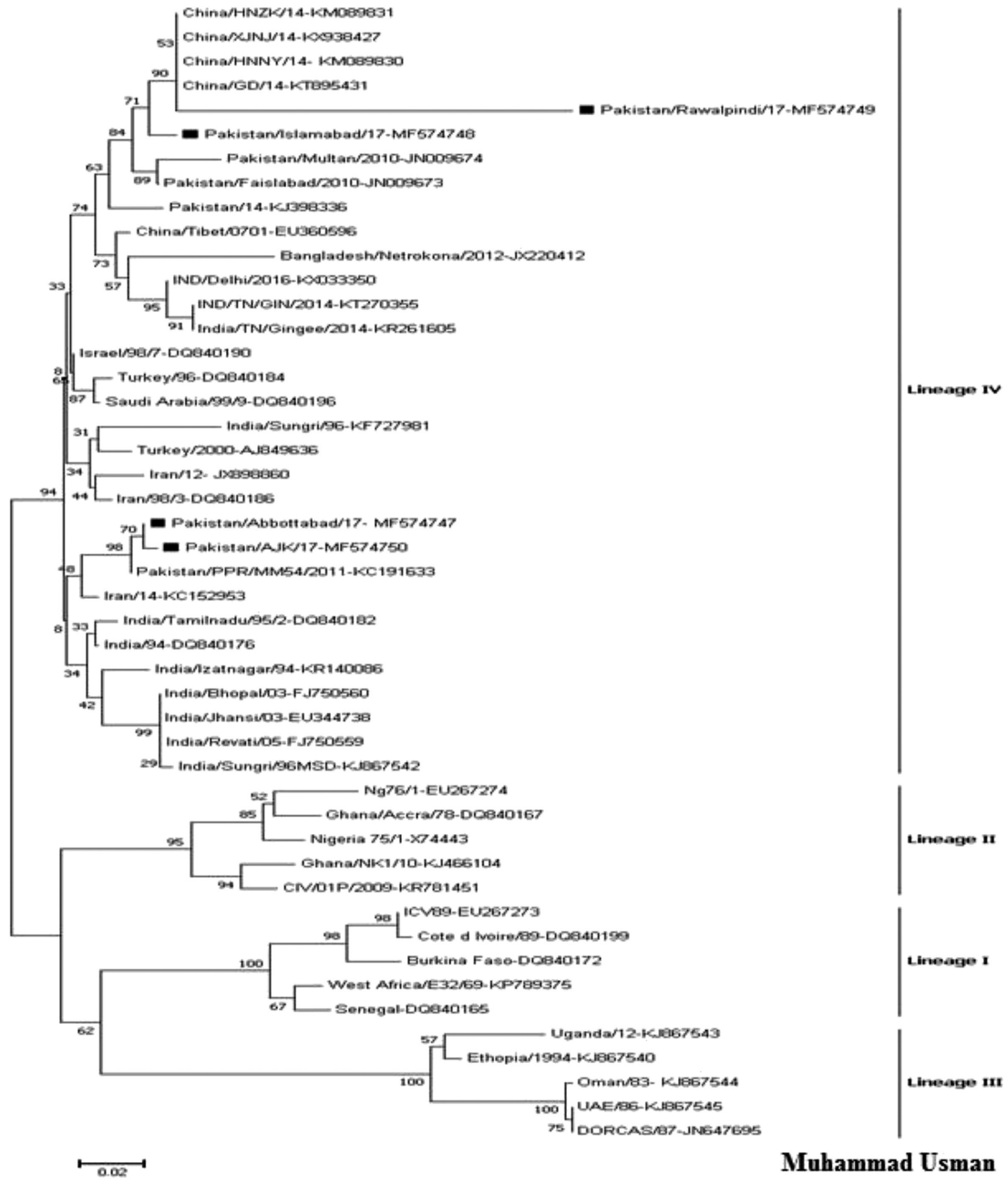


**Fig. 2. Phylogenetic analysis of the PPRV:** Neighbour joining method from sequences N gene constructed with Kimura 2 parameter model in mega 7 version at bootstrap value 1000 replicates. The sample characterized in this study were marked with Black Square (■).

epidemiology. The sequence homology of the viral strains circulating in the region of Rawalpindi and Islamabad are closer to each other and with the viruses sequenced previously from the area of Multan and Faisalabad. On the other hand sequence homology of the viral strains circulating in the region of Abbottabad and Azad Jammu and Kashmir are more relevant to each other and with the viruses previously sequenced from Multan and Faisalabad regions of Pakistan.

So, the gene sequences identity indicated that there are at least two different strains of PPRV circulating in Pakistan at present. The virus strain causing outbreaks in Islamabad and

Rawalpindi regions were clustered closer to the viruses circulating in China as reported by Li *et al.*, 2015[9]. The possible relevance of Pakistani strains to that of Chinese strains of PPRV could be due to the fact that both these countries share their border and PPR is a transboundary disease. Moreover, grazing system in the mountainous regions at the border of China and Pakistan makes animal movement across the border unrestricted.

However, the virus circulating in Rawalpindi (Pakistan/Rawalpindi/17-MF574749) showed large difference (Long Branch) in phylogenetic tree. This difference is may be due to an accelerated rate of molecular evolution in this virus in relation to all others or its source was different.

Similarly, viruses circulating in the region of Abbottabad and Azad Jammu and Kashmir were clustered closer to the viruses from Iran and India. This possible relationship of Pakistani strain to that of Iranian and Indian strains is because of the fact that all these countries share their border and have similar environmental conditions. The grouping of the four different sequences (in this study) at two distinct places in the phylogenetic tree indicates that PPRV isolates may have experienced evolutionary pathway or introduced from different regions since PPR is transboundary animal disease.

To our knowledge, this is the first study of complete N gene sequence of PPRV in the country. This information is pivotal in understanding the genetic nature of the virus and to devise appropriate mitigation strategies to cope with the virus. However, the interpretations made in this study needs to be corroborated with a wide range of research study as a small number of outbreaks were studied in the current study.

## Supplemental data

Supplemental data associated with this article can be found, in the online version, at http://dx.doi.org/10.14943/jjvr.67.1.139

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