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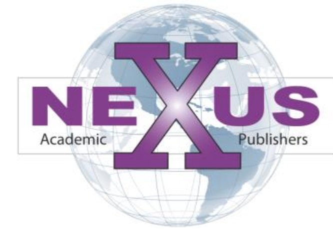

Short Communication

Peste Des Petits Ruminants in Pakistan

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ABSTRACT

Peste des petits ruminants is a highly contagious disease of domestic and wild small ruminants and camels. Despite continuous occurrence of the disease in Pakistan, limited information is available on the genetic diversity of the peste des petits ruminants' virus (PPRV) in small ruminants. This study was performed to provide information on the molecular characteristics, genetic diversity and the phylogenetic relationship of PPRV strains causing continuous outbreaks in Punjab, Pakistan. The causative agent of two different outbreaks was confirmed to be PPRV using real–time PCR against the nucleoprotein (N) gene of the virus. Sequence analysis of the N gene of PPRV indicated that representative isolates were identical to each other. Notably, these PPRV strains were significantly distinct from previously characterized PPRV strains from Pakistan. The results indicate that there are at least two different genetic variants of PPRV circulating in Pakistani small ruminants. These findings are fundamental in developing national disease control policies, domestic vaccine strain, and improved disease diagnosis.

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Peste des petits ruminants virus (PPRV), a *Morbillivirus* within the family *Paramyxoviridae*, causes a highly contagious disease in both domestic and wild small ruminants, and in camels. It is characterized by high fever, oral ulceration, diarrhea, and pneumonia, hence characterized as stomatitis pneumo–enteritis complex (Shaila et al., 1996; Bunyard et al., 2010; Munir et al., 2012a). A number of disease outbreaks are being reported annually to World Organization for Animal Health from different parts of the world. Owing to its high morbidity (20–80%) and mortality (80–100%), the disease is considered a major constraint to productivity in small ruminants industry particularly in African, Asian and Middle East countries (Munir et al., 2012).

Peste des petits ruminants virus (PPRV) has negative sense single stranded RNA genome of 15,948 nucleotides in length that follows the so called "rule–of–six" (Gibbs et al., 1979; Bailey et al., 2005; Munir et al., 2012). The genome encodes six structural proteins that include nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin (H) and large polymerase (L) (Bailey et al., 2005; Munir et al., 2012). Based on sequence analysis of the F and N genes, the PPRV has been classified into four lineages distinct to different geographical areas; however, the N gene is considered most divergent and appropriate for molecular characterization of closely related isolates (Shaila et al., 1996). The PPRV isolates of lineage I and II are found in Western and Central Africa, lineage III is most prevalent in Eastern Africa and the southern part of the Middle East, whereas, lineage IV is most prevalent in Asian countries (Shaila et al., 1996; Bunyard et al., 2010; Munir et al., 2012b).

Since first documentation of PPR in Pakistan in 1994 (Amjad et al., 1997), the disease is considered endemic and large numbers of outbreaks are being reported from different parts of the country (Munir et al., 2012). Most of these reports are based on clinico–epidemiology observations as evidenced by annual reports of Directorate of Animal Disease and Surveillance System in Punjab province of Pakistan[1]. In Pakistan, the disease is considered endemic and vaccination is being performed with live–attenuated virus (Nigeria/75) belonging to the lineage I. In spite of this, PPRV outbreaks are not uncommon in both vaccinated and unvaccinated small ruminants and are generally associated with respiratory and enteric ailments (Munir et al., 2012c). This raises concerns to determine the genetic nature of circulating strains of PPRV particularly the strains that are causing outbreaks even in vaccinated animals.

The aim of this study was to provide information on the molecular characteristics, genetic diversity and the phylogenetic relationship of PPRV strains causing continuous outbreaks in Punjab, Pakistan.

In May–June 2012, blood samples were collected from sheep (n = 5) and goats (n = 8) suffered from a respiratory disease, suspected for PPR, in a village close to Lahore, Punjab Pakistan. The major clinical observations include high fever (40.5–41.5 °C), nasal discharges, sneezing, coughing, sticking of mucus to nostrils, and erosions in the oral mucosa. The temperature dropped to 40 °C after onset of severe diarrhea. All animals (sheep = 14, goats = 21) died after 5–10 days of the clinical disease.

The whole blood (200µL) from individual animal was spotted onto QIAcard FTA Indicator Four Spots (Qiagen, Hilden, Germany), which preserve genomic material and lysed the cells and viruses. The elution of the total RNA from QIA

[1]http://www.livestockpunjab.gov.pk/View.aspx?Type=TopMenu&itemId=44

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card was performed as we described before (Munir et al., 2012c). A real-time RT-PCR, targeting the viral N gene (Kwiatek et al., 2010), was performed to screen the presence of PPRV genome in a Rotor-Gene 6000 real-time analyzer (Qiagen) using AgPath ID one-step RT-PCR kit (Applied Biosystem, Foster City, CA, USA). The reporter dye (FAM) signal was measured at the annealing step of each cycle, and the threshold cycle (Ct) for each sample was calculated. The samples that had a Ct value <35 were considered positive. A total of four samples (sheep = 2, goats = 2) appeared positive and were used in conventional PCR for the amplification of the hypervariable 3´-end of the N gene of PPRV using NP3 (5´-TCTCGG AAA TCGCCTCACAGACTG-3´) and NP4 (5´-CCTCCTCCTGGTCCTCCAGAATCT-3´) primers (Couacy-Hyaman et al., 2010) for phylogenetic purposes.

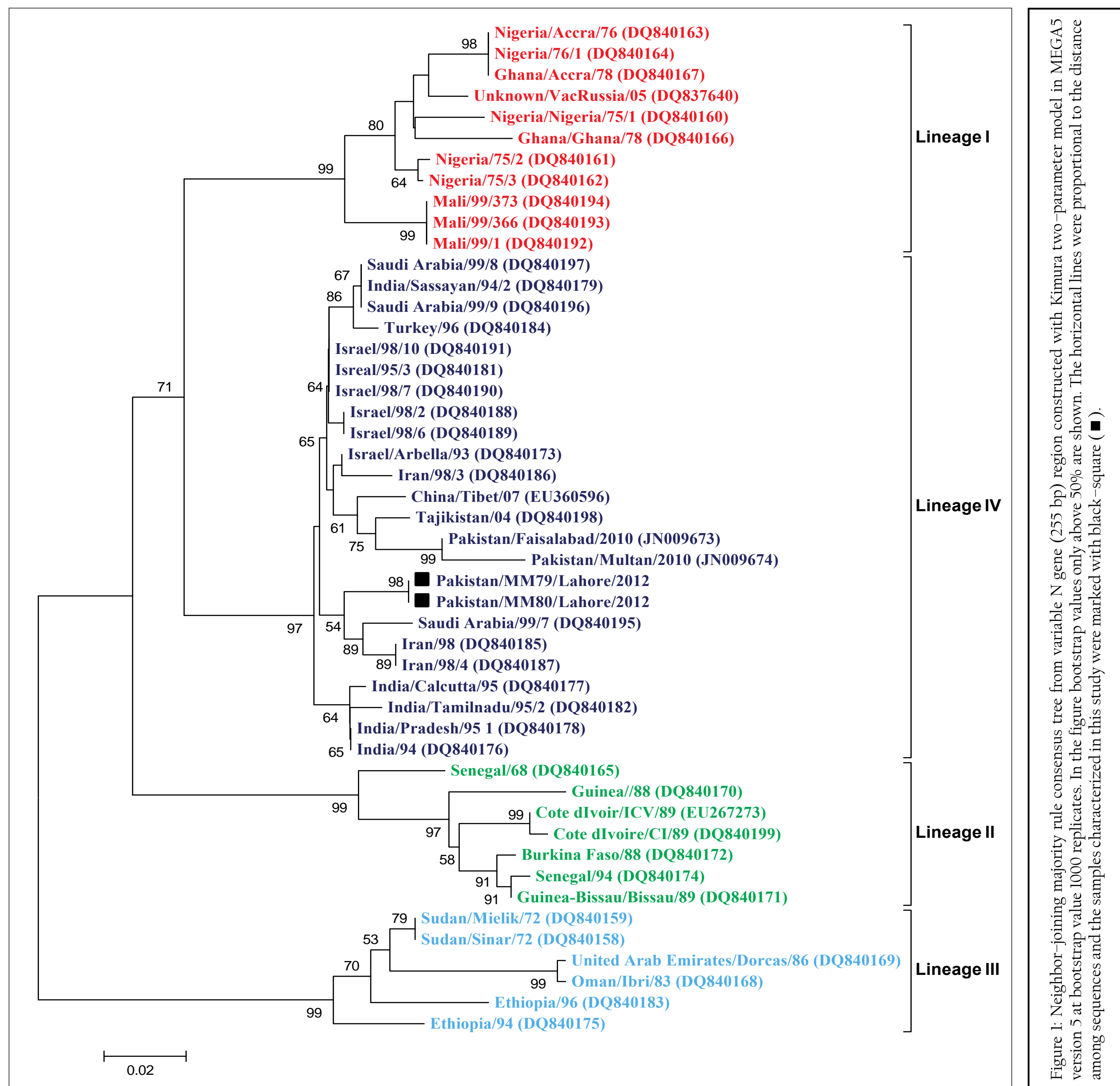

Figure 1: Neighbor-joining majority rule consensus tree from variable N gene (255 bp) region constructed with Kimura two-parameter model in MEGA5 version 5 at bootstrap value 1000 replicates. In the figure bootstrap values only above 50% are shown. The horizontal lines were proportional to the distance among sequences and the samples characterized in this study were marked with black-square (■).

The two N gene sequences, each representing one individual outbreak, obtained in this study were completely identical (100 per cent) to each other. The sequences (GenBank accession number KC249964 and KC249965) were aligned and compared with publicly available sequences representing all the lineages of PPRV. A phylogenetic tree was constructed using the neighbour-joining method and Kimura two-parameter model in MEGA5 (CEMI, Tempe, AZ, USA). Tree topology indicated that Pakistani viruses were closely related to Saudi Arabian (96.9 per cent) (Saudi Arabia/99/7, DQ840195) and Iranian (97.3 per cent) (Iran/98, DQ840185) strains of PPRV collected between 1998-99. It is noteworthy that the PPRV strains identified in this study were significantly distinct (4.5 per cent nucleotide diversity) from our previously characterized PPRV strains from Pakistan. These strains of PPRV were characterized from Faisalabad (Pakistan/Faisalabad/2010, JN009673) and Multan (Pakistan/Multan/2010, JN009674), cities in the same province, two years ago from sheep and goats (Munir et al., 2012c) and were clustered with strains from Tajikistan (Tajikistan/04, DQ840198). Taken together, the

results demonstrated that there are at least two different genetic variants of PPRV circulating in Pakistan. Despite PPRV is endemic in Pakistan and outbreaks are regularly occurring, information on the genetic nature of PPRV is scarce. There are only four sequences of N gene (two sequenced in this study) from Pakistan are available which is a main hurdle in establishing any epidemiological link between the outbreaks of PPR.

Owing to the transboundary nature of PPRV, it is likely that immense trade between Middle East and Pakistan might remain an importance source of disease transmission. Moreover, the genetic diversity of PPRV in the same region of the country highlights the importance of the market especially at *Eid–ul–Adha* to allow mixing of the flocks harboring different population of viruses. Further studies are needed to highlight the epidemiological link existing between these geographic areas, and to clarify migration patterns of these PPRV variants. These findings are fundamental in developing national disease control policies, domestic vaccine strain, and improved disease diagnosis.

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