Molecular characterization of peste-des-petits ruminants virus from Nepal, 2005 to 2016

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Abstract Outbreaks of peste-des-petits ruminants (PPR) has been reported regularly in Nepal since 1994. Despite this, there has been limited molecular characterization of circulating virus in the country. In this study a 351 bp segment of the nucleoprotein gene of the PPR virus (PPRV) was amplified and sequenced from ten samples collected between 2005 and 2016. Phylogenetic trees were estimated from these sequences using the maximum likelihood method confirming that all of the PPRV from the samples analysed belonged to the sub-clade IV of clade I of lineage IV and that they shared a common origin with other PPRV isolates in the region.

Keywords peste-des-petits ruminants · Nepal · Lineage IV · Molecular epidemiology

Peste-des-petits ruminants (PPR) is a highly contagious viral disease of small ruminants which was first described in Côte d'Ivoire in 1942 [7] and has now been reported in many parts of Africa, the Middle East, Eurasia and Asia [10, 12]. The causative agent of the disease is the PPR virus (PPRV) which belongs to the genus *Morbillivirus* within the *Paramyxoviridae* family. PPR is characterized by high

fever, oculonasal discharge, stomatitis, diarrhoea and pneumonia. Mortality and morbidity can be between 50–90 and 10–90% respectively, with the higher percentages recorded in naïve populations of sheep and goats [10]. The economic impact of PPR on sheep and goat production is significant and there are huge food security implications associated with the disease in many countries. For these reasons the disease has been targeted by international organizations for global eradication by 2030 [8].

Although, PPRV exists as a single serotype it can be differentiated into four genetically distinct lineages I, II, III and IV based on partial sequences of the C-terminus of the nucleoprotein (N) gene (351 bp) and the fusion protein (F) gene (370 bp) [1]. All four lineages have been identified in Africa while in Asia the majority of PPR outbreaks have been associated with viruses belonging to lineage IV. In South Asia, PPR outbreaks are reported regularly in India and Bangladesh, with strong evidence existing for cross border movement of the disease between India and Bangladesh [4, 9, 11, 13, 14]. A live-attenuated Sungri vaccine has been used in India as a control measure against PPRV since 2010 while the Titu goat-origin vaccine is used in Bangladesh [11, 13].

The goat and sheep population in Nepal is estimated to be 11 million and 800,000, respectively. Animals are imported from both India and China and internal movement of goats and sheep within the country is common particularly during festive seasons. Nepal reported PPR for the first time in 1994. A National PPR Control program was launched in 2001. Although strategic mass and ring vaccination with Nigeria 75/1 PPRV vaccine (produced locally) were carried out under this program, outbreaks continue to occur on an annual basis. In 2016 alone, 24 districts throughout the country reported PPR outbreaks. Two partial F gene sequence (FR667648 and FN996974)

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from two PPRVs identified in Nepal in 1995 and 2009, respectively, are available in GenBank. Other than this, there is very limited sequence data available for PPRV in the country. This study was, therefore, undertaken to generate molecular epidemiological data on PPRV in Nepal during the period 2005 and 2016 in order to provide comparative data for those interested in PPRV circulation in the region.

Ten PPRV suspected tissue and nasal swab samples from seven different outbreaks in four districts of Nepal representing two different administrative divisions, Karnali and Province 3 were included in the study. These sample were collected by the Regional Veterinary Laboratories and District Livestock Service Offices between 2005 and 2016 and sent to the Central Veterinary Laboratory (CVL), Kathmandu for confirmation (Table 1). The samples had been stored at -70°C at the CVL. The tissue samples and swabs were processed using standard techniques [5] and total RNA was extracted using the RNeasy kit (Qiagen). The extracted RNA samples were analysed by RT-PCR using the One-Step RT-PCR kit (Qiagen) to amplify a 351 bp fragment of the PPRV N gene with primers NP3 and NP4 [3]. PCR amplicons were purified using the Wizard® SV gel and PCR Clean-up system (Promega) and sent for sequencing using standard Sanger methods at LGC genomics (Berlin, Germany). The generated sequences have been submitted to GenBank under accession numbers (MH880864–MH880873).

The Staden Package (<http://staden.sourceforge.net/>) was used to assemble the generated sequences. Multiple sequence alignment was performed using MUSCLE (<http://www.ebi.ac.uk/Tools/msa/muscle/>) with default settings, incorporating all the sequences generated here combined with those available in GenBank (accession numbers are shown in the tree figures). Phylogenetic trees were estimated using the Maximum likelihood (ML) method available in MEGA 6 [16], employing the Kimura

2-parameter model of nucleotide substitution with uniform rates among sites and 1000 bootstrap replications.

From the phylogenetic analysis (Fig. 1) it can be clearly seen that the PPRVs analysed in Nepal from 2005 to 2016 all belong to lineage IV. Some nucleotide diversity between the N gene sequences was detected and is reflected in the phylogenetic tree. NPL01, NPL02, NPL03 and NPL05 (all from Nuwakot, Province 3) were identical to each other while the percentage of nucleotide differences between the other viruses ranged from 1.9 to 5.2%. Although samples NPL11 and NPL12 were both collected from Dhadhing, Province 3, they do not cluster together. The nucleotide difference between these two samples might be explained by the fact that there was a 4 year period between sampling and that RNA viruses like PPRV are constantly evolving. Likewise, although NPL07 and NPL09 are from the same province (Lalitpur, Province 3) and time period (September to November 2005), they are only share 96.7% identity and, therefore, cluster separately on the tree. Further sequence analysis of these samples is required in order to fully understand the relevance of these differences.

In 2014, Kumar et al. [9] performed a comprehensive assessment of lineage IV isolate and were able to further subdivide viruses based in the N gene within the lineage and according to geographical location. Two clades (CI and CII) and four subclades (SCI to SCIV) were identified. SCI consists of PPRVs from India, SCII consists of viruses from Africa, Pakistan, Saudi Arabia and Israel, SCIII includes viruses from Iran, Iraq, Turkey and Israel and SCIV includes PPRVs from Pakistan, Bangladesh, Tibet, India and the UAE identified from 2004 outwards. The viruses identified in Nepal belong to CI and SCIV (Fig. 1).

Based on the molecular epidemiological analyses presented here, some transboundary movement between Bangladesh, India, Nepal is the most probable explanation for the shared origin of the virus presently circulating in

Table 1 Samples analysed in this study

District, administrative region	Flock size	Sample	Species	Sample type	Collection date	GenBank #
Lalitpur, Province 3	25–30	NPL09	Goat	Lung	September 2005	MH880864
Lalitpur, Province 3	25–30	NPL07	Goat	Lymph node	November 2005	MH880865
Dhadhing, Province 3	30–50	NPL11	Goat	Nasal swab	October 2010	MH880866
Dhadhing, Province 3	30–50	NPL12	Goat	Nasal swab	November 2014	MH880870
Nuwakot, Province 3	20–30	NPL01	Goat	Nasal swab	September 2013	MH880867
Nuwakot, Province 3	20–30	NPL02	Goat	Nasal swab	December 2013	MH880868
Nuwakot, Province 3	20–30	NPL03	Goat	Nasal swab	October 2014	MH880869
Nuwakot, Province 3	20–30	NPL05	Goat	Nasal swab	May 2015	MH880871
Humla, Karnali	4500	NPL08	Sheep	Spleen	May 2016	MH880872
Humla, Karnali	4500	NPL06	Sheep	Lung	October 2016	MH880873

Fig. 1 ML phylogenetic tree of partial N gene sequence (219 bp) from the PPRV sampled in Nepal combined with similar sequences available in GenBank. The virus from Nepal are shown by filled black circles. The numbers indicate the bootstrap values calculated from 1000 bootstrap replicates

Nepal. Suspected transboundary movement of PPRV has been reported in several countries both in Asia [11, 15] and Africa [2, 6]. Indeed, it has been observed that PPR outbreaks in Nepal occur more often during the festive season of September to October believed to be due to the import of approximately five hundred thousand goats from India and approximately thirty thousand mountain goats from the Tibet Autonomous Region.

In summary, this study has generated comparative data on PPRVs present in Nepal between 2005 and 2016 and shows that the viruses belong to sub-clade IV of clade I of lineage IV. These data will be of use to those interested in the circulation of PPRV in Nepal and neighbouring countries and will assist veterinarian authorities in the reassessment of PPR disease control programmes in the country.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests regarding the publication of this study.

Human and animal rights No animal work was performed for this study.

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