Veterinary Microbiology 174 (2014) 39–49

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Molecular characterisation of lineage IV peste des petits ruminants virus using multi gene sequence data

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form 4 August 2014
Accepted 26 August 2014

Keywords:
Peste des petits ruminants
RT-PCR
PPR outbreak
Phylogenetic analysis using N, F and H genes

ABSTRACT

Peste des petits ruminants is responsible for an economically important plague of small ruminants that is endemic across much of the developing world. Here we describe the detection and characterisation of a PPR virus from a recent outbreak in Tamil Nadu, India. We demonstrate the isolation of PPR virus from rectal swab and highlight the potential spread of disease to in-contact animals through faecal materials and use of faecal material as non-invasive method of sampling for susceptible wild ruminants. Finally we have performed a comprehensive 'multi-gene' assessment of lineage IV isolates of PPRV utilising sequence data from our study and publically available partial N, partial F and partial H gene data. We describe the effects of grouping PPRV isolates utilising different gene loci and conclude that the variable part of N gene at C terminus gives the best phylogenetic assessment of PPRV isolates with isolates generally clustering according to geographical isolation. This assessment highlights the importance of careful gene targeting with RT-PCR to enable thorough phylogenetic analysis.

1. Introduction

Peste des petits ruminants virus (PPRV), a member of the morbillivirus genus, causes an acute and devastating viral disease of small ruminants. The morbillivirus genome consists of a single strand of negative sense RNA of approximately 16 kilobases containing six genes, encoding six to 8 proteins. The first clinical description of PPR was made in West Africa in the 1940s although it was not until 1979 that the virus was characterised as a distinct entity (Gibbs et al., 1979). Since then the virus has been reported in many countries across Sub Saharan Africa, the Middle East, the Arabian Peninsula and Asia (Banyard et al., 2010). The virus exists as one serotype, but genetically divides into four distinct lineages.

Within India, the disease was first reported in Southern India (Shaila et al., 1989) before being detected in northern India during 1994 (Nanda et al., 1996) and later across the Indian sub-continent (Dhar et al., 2002; Nanda et al., 1996; Roy et al., 2010; Sreeramulu, 2000; Taylor et al., 2002).

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http://dx.doi.org/10.1016/j.vetmic.2014.08.031

Recent estimates on the potential impact of PPR have suggested that 63% of the small ruminant populations of Southern Africa, central Asia, South-east Asia, China, Turkey and Southern Europe at risk to PPR as determined by the Food and Agriculture Organisation (Libeau et al., 2014).

The present study describes an outbreak of PPR during 2011 in the Coimbatore District of Tamil Nadu, India. Further to this we have analysed the rectal swabs from the infected goats in the outbreak for the presence of both viral antigen and live virus and hypothesise that live virus may be present in faecal matter that may plays a role for spreading the diseases. Finally we have genetically analysed the sequence derived from the virus of this outbreak and shown it to be a lineage IV virus. With this new genetic data we have then performed an extensive phylogenetic comparison of available data from the GenBank and generated phylogenetic trees for global PPRV isolates (lineage IV) using partial gene sequences of the nucleocapsid (N), fusion (F) and haemagglutinin (H) genes of PPR. Finally we have compared the grouping of Asian virus isolates, focussing on those derived from Indian PPRV outbreaks to investigate differences in phylogenetic grouping of isolates. Previous phylogenetic studies of lineage IV PPR have been limited in the availability of data for comparison (Balamurugan et al., 2010; Dhar et al., 2002; Kerur et al., 2008; Shaila et al., 1996; Nanda et al., 1996). The phylogenetic analyses of the global PPRV sequences presented here benefit from the increased reporting and characterisation of PPR and, comparing multiple genetic loci give a comprehensive overview of the geographic distribution of lineage IV PPRV and the relatedness of isolates across Asia.

2. Material and methods

2.1. Outbreak information and clinical disease

In the Coimbatore District of Tamil Nadu, India, a total of 40 non-descript goats were purchased from a local market at Coimbatore and after one and half months the animals developed clinical illness consistent with infection with PPRV. After the onset of clinical signs specific to PPR (Pope et al., 2013) 18 goats died within a period of six days and another 18 were ailing. A detailed post mortem examination was conducted to assess the cause of death. The gross lesions were recorded and both clinical signs and post mortem lesions were suggestive of PPR. Faecal swabs, ocular swabs, nasal swabs and faeces were collected from sick animals whereas tissues were collected from the spleen and mesenteric lymph nodes from dead animals. All samples were stored in phosphate buffered saline (PBS) and maintained at 4 °C during transport to the laboratory.

2.2. Virus isolation in cell culture

Vero cells were grown in tissue culture flasks (25 cm^2) and on cover slips. For virus isolation from post mortem tissue, homogenate was filtered through a 0.22 μm membrane and inoculated onto a confluent monolayer. For detection of viral material in faecal matter, rectal swabs were transported to the laboratory on ice. The faecal material and rectal swab samples were clarified by centrifugation before being filtered and inoculated onto a confluent monolayer.

For all samples, the inoculated monolayers were incubated at 37 °C and checked regularly for the detection of cytopathic effect (CPE). In the absence of CPE, and where monolayers required splitting, cells were passaged until CPE consistent with morbillivirus infection was seen. Uninoculated Vero cells were treated in the same way and acted as a negative control. The Vero cells propagated on cover slips that developed CPE were fixed and stained using standard techniques (Pope et al., 2013).

2.3. Reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing

The presence or absence of PPRV nucleic acid in tissue samples, oculo and nasal swabs and faecal swabs was assessed by RT-PCR. RNA was extracted using Trizol (Invitrogen) from all samples collected from the outbreak. Isolated RNA was subjected to RT-PCR using PPRV partial N (Couacy-Hymann et al., 2002) and F (Forsyth and Barrett, 1995) gene primer sets. The partial H gene was amplified by PCR using the forward primer—5′ CCGGCATGGATCTTTACAACAAC 3′ and the Reverse primer—5′ ATGACGCCAAGGGAAACACTCTAT 3′ designed using the DNAstar software.

Alongside this, to provide genetic material for H gene phylogenetic analysis, historical PPRV samples from lineage III isolates (Oman 1983 and UAE 1986) were analysed by RT-PCR to determine H gene data for lineage III. The cDNA was prepared using Revert Aid First strand cDNA synthesis Kit (Fermentas, USA). Briefly, 10 μl of RNA was mixed with 1 μl of random hexanucleotide primers (50 pmol/μl), 1 μl 5× reaction buffer, 1 μl RNAse inhibitor, 1 μl Moloney's Murine Leukaemia Virus (MMLV) Reverse transcriptase and 2 μl 10 mM dNTP mix and the first strand synthesis reaction was carried out as per the manufacturer's instructions. The cDNA was stored at −20 °C until use. PCR reaction mixes were made as follows: 5 μl cDNA, 1 μl forward primer (10 pmol/μl), 1 μl reverse primer (10 pmol/μl), 10 μl Amplicon red dye Master mix (2×) and 5 μl nuclease free water. The PCR reaction was carried out in an Applied Bio system thermo cycler with the following thermal cycling conditions: 95 °C for 3 min for initial denaturation, 94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s for 30 cycles and final extension at 72 °C for 5 min. The PCR product was analysed on a 2% agarose gel containing ethidium bromide (2.5 μg/ml) at 100 mV for 45 min in 1× TAE buffer along with 100 bp DNA marker. The amplicons were viewed under a UV transilluminator.

The resulting PCR products were purified using gel extraction kit (RBC Real Biotech, USA) according to the manufacturer's instructions. The eluted DNA was stored at −20 °C until use. The purified products were then sequenced using Big Dye-Terminator® v3.1 Cycle Sequencing Reaction Kit on ABI-3730 DNA Analyzer (Applied Biosystems) following the manufacturer's instructions.