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Geographic distribution and epidemiology of peste des petits ruminants viruses

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Abstract

Peste des petits ruminants (PPR) is an important viral disease of goats and sheep prevalent in West Africa and the Middle East. In recent years, PPR has emerged in India, first in the South India and later in North India. To study the genetic relationships between viruses of distinct geographical origin we have sequenced a 322 nucleotide cDNA fragment of the fusion protein gene generated using reverse transcription followed by polymerase chain reaction (PCR) amplification. Viruses from nineteen independent PPR outbreaks were compared; these included the prototype African strain from Senegal and viruses from disease outbreaks which have occurred at different times and locations across Africa, Arabia, the Near East and the Indian subcontinent. Four separate lineages of the virus were identified and the virus isolates from Asia over the past 2 years were all of one lineage which had not previously been identified in Africa or Asia.

Peste des petits ruminants (PPR) is a highly contagious viral disease affecting sheep and goats, widely distributed in sub-Saharan Africa and the Arabian Peninsula (Taylor, 1984; Abu-Elzein et al., 1990; Roeder et al., 1994). The virus is antigenically related to rinderpest virus which infects cattle and other large ruminants (Barrett, 1994). It is a member of the morbillivirus genus, which also includes measles, canine distemper and viruses of marine mammals, in the family Paramyxoviridae

Abbreviations: PPR; Epidemiology; Fusion protein; PCR sequence.

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(Barrett et al., 1993a). PPR is considered to be one of the main constraints to improving productivity of small ruminants in the regions where it is endemic (Stem, 1993). It was first discovered in West Africa in 1942 (Gargadennec and Lalanne, 1942) and was later found in Arabia (Abu-Elzein et al., 1990), the Middle East (Lefevre et al., 1991) and in southern India, where it was identified using virus-specific cDNA probes (Shaila et al., 1989). At the present time outbreaks of the disease are being reported in northern India (Nanda et al., 1995) and also in Bangladesh, Pakistan, Nepal, Israel and Saudia Arabia. In order to obtain epidemiological information on these recent virus outbreaks, we have studied the genetic relationships between the viruses from different geographical regions by sequence comparisons of a segment of the virus fusion (F) protein gene. We have also analyzed a number of PPR virus isolates collected over the past two decades from Africa (Nigeria, Ivory Coast, Senegal, Sudan) and Arabia (Oman).

The isolation and characterisation of the older PPRV strains has previously been described (Senegal, Nigeria 75/1, 76/1, 76/2: Taylor and Abegunde, 1979; Sudan 72/1 and 72/2: El Hag Ali and Taylor, 1984; Oman 83/2: Taylor et al., 1990). Viruses were grown on Vero cells maintained in MEM supplemented with antibiotics and 5% foetal calf serum. Confluent cells were infected at a low multiplicity of infection and cells were harvested when extensive cytopathic effects had developed. RNA was extracted from either the infected Vero cell monolayers or, in the case of the recent virus outbreaks, from post-mortem tissues (spleen or lymph nodes) supplied to the Pirbright World Reference Laboratory for confirmatory diagnosis, using the method described by Chomczynski and Sacchi (1987). Total extracted RNA (5 μg) was transcribed into cDNA using reverse transcriptase and random oligonucleotide primers (75 μg) and amplified using F gene-specific primers in a polymerase chain reaction (PCR) described previously (Forsyth and Barrett, 1995). Two primer sets were used for PCR amplification. The first set, F1 (mRNA sense) and F2 (vRNA sense) amplifies a region between nucleotides 777 and 1148 in the PPR F gene sequence (Meyer and Diallo, 1995). The second set F1A (mRNA sense) and F2A (vRNA sense) is a nested set which amplifies a region between nucleotides 802–1110 in the gene. The sequences of the primers are given below:

F1	5′	ATCACAGTGTTAAAGCCTG-TAGAGG
F2	5′	GAGACTGAGTTTGTGACCTA-CAAGC
F1A	5′	ATGCTCTGTCAGTGATAACC
F2A	5′	TTATGGACAGAAGGGACAAG

cDNAs were subjected to a 30 cycle amplification (denaturation at 95° for 1 min, annealing at 50° for 1 min and elongation at 72°C for 2 min) by PCR using 10 p/mol of each primer. The amplified DNA was purified from 2% low melting point agarose and ligated to pGEM-T PCR cloning vector DNA and used to transform competent *Escherichia coli* JM 109 cells. The recombinant plasmid DNA was isolated from representative clones, digested with restriction enzymes flanking the cloning site to release the inserts and checked for correct size of the inserts by electrophoresis on an agarose gel. DNA from clones carrying the correct sized inserts was then subjected to sequencing using dideoxynucleotide chain termination method as previously described (Chamberlain et al., 1993).

The nucleotide sequences were aligned with each other using the GCG PILEUP sequence alignment programme and phylogenetic analysis was carried out using the PHYLIP programmes DNADIST and KITSCH. This method has previously been found useful for analyzing the genetic relationships between rinderpest virus (RPV) isolates and supported epidemiological investigations on the origin and spread of RPV in new areas of the world in recent years (Barrett et al., 1993b; Chamberlain et al., 1993; Wamwayi et al., 1995). Phylogenetic analysis on the 322 long DNA sequence (primer sequences were omitted from the comparisons) amplified from the F genes from 19 different PPR viruses produced a dendrogram showing four distinct lineages (Fig. 1). PPR viruses from Africa were

found to group in three distinct lineages with a maximum of 12% nucleotide sequence divergence. Viruses isolated from Africa in the early 1970s (Senegal, Nigeria and Sudan) formed one lineage (group 1), viruses isolated from the Ivory Coast and Guinea in the late 1980s formed a second (group 2) and the third group included viruses from Sudan, Oman and southern India over a 20 year period. The two virus isolates from Sudan were both isolated in 1972, however, one (Sudan/Sinnar/72/1) showed a close relationship to Nigerian viruses (group 1) while the other (Sudan/Meiliq/72/2) was genetically related to viruses from the Oman and southern India (group 3). This probably reflects the routes and ease of trade in small live animals across continents. The most recent virus outbreaks which occurred across Asia (Israel, Saudi Arabia, Pakistan, Bangladesh, Nepal and northern India) during the past two years all fell into one lineage (group 4) with very little sequence divergence between them ($< 2\%$). It is interesting to note the geographical continuity of the countries in which the recent outbreaks have been studied but the data has shed no light on the possible epizootic centre of the virus in Asia. Although the disease has been described in the Arabian Peninsula, the disease probably existed for a long time in other parts of Asia but was not diagnosed or was confused with other diseases such as rinderpest and pasteurellosis. No recent virus isolates or tissue samples have been obtained from Africa, although the disease continues to be reported in many African countries.

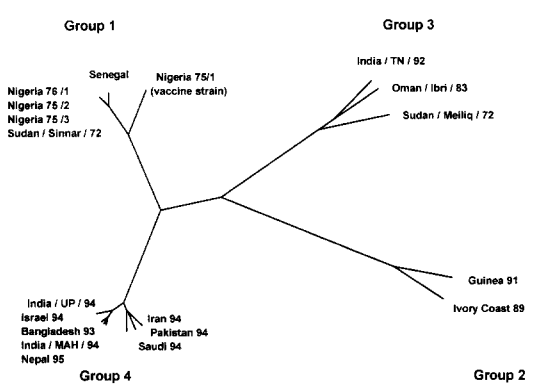

Fig. 1. Phylogenetic analysis of pest des petits ruminants virus isolates from different geographical locations. The relationships were determined using sequence data derived from part of the fusion protein gene. The relationships are presented as unrooted trees with branch lengths being proportional to the estimated mutational distances between the virus sequences and the hypothetical common ancestor that existed at the nodes in the tree. The PHYLIP programmes DNADIST AND KITSCH were used for the analysis (Felsenstein, 1989).

The occurrence of genetically distinct virus groups within the same continent, three in Africa and two in Asia, is indicative of independent evolutionary processes and suggests that there must have been physical isolation of the viruses to enable this to occur. The outbreaks of PPR in North and South India caused by two distinct viruses offers a unique epidemiological situation calling for a more detailed molecular analysis of current and future virus outbreaks. It is possible that the two viruses were independently introduced into India in the past decade, or it could be that the South Indian virus has been an enzootic infection in that region for many decades and was confused with RPV. Before 1988 all morbillivirus disease in small ruminants in India was ascribed to RPV infection and, until the introduction of specific hybridisation probes (Diallo et al., 1989), the methods used in India to identify RPV could not distinguish it from PPRV. Unlike African strains of RPV, Asian strains of RPV can cause mild to severe clinical disease in small ruminants which can then be transmitted as a more serious disease to cattle (Couacy-Hymann et al., 1995). This was the route by which RPV was transmitted to Sri Lanka in 1987 when troops traded small ruminants brought from India (Anderson et al., 1990). The occurrence of two related but different disease-causing viruses in small ruminants in South India resulted in an epidemiologically confusing situation where PPRV could have circulated for many years without being identified.

The data presented here has broadened our understanding of the epidemiology and worldwide movement of PPR viruses. The virus has undergone independent evolution which has resulted in four genetic subtypes and, in future,

more exact classification of virus causing PPR outbreaks will be possible. One lineage of the virus (group 3) was found both in Asia and Africa. In contrast, grouping according to continent is strictly maintained in the case of rinderpest virus which mainly infects large ruminants (Barrett et al., 1993a; Chamberlain et al., 1993; Wamwayi et al., 1995; Forsyth and Barrett, 1995). This is probably because of the greater trade in small ruminants over long distances and across continents. However, in spite of the genetic variation shown here, there remains only one serotype of the virus and the strong antigenic relationships that exist within the morbillivirus group means that heterologous RPV vaccines will solidly protect small ruminants against PPRV (Taylor, 1979; Romero et al., 1995) and PPRV vaccine will protect cattle from RPV (Couacy-Hymann et al., 1995).

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