

# Human metapneumovirus infection in South African children hospitalised with respiratory tract disease

**To the Editor:** Viruses are a common cause of respiratory tract infections in young children, and the most frequently isolated viruses from nasopharyngeal aspirates (NPA) are respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, adenovirus, cytomegalovirus, enteroviruses, rhinoviruses and coronaviruses. There is, however, a proportion of infections for which no aetiological agent can be found. Recently a novel virus, human metapneumovirus (HMPV), was isolated from young children in the Netherlands with respiratory tract disease.<sup>1</sup> Since then numerous PCR-based studies from around the world have confirmed this disease association in both adults and children, with prevalences ranging from 1.5% to 43%.<sup>2-8</sup> Seroprevalence studies have shown that all children

above 10 years old have been exposed to the virus and are seropositive.<sup>1,9</sup> The virus is a new human pathogen of the genus *Metapneumovirus*, family Paramyxoviridae.<sup>1,10</sup>

This study was undertaken to determine HMPV infection in a paediatric group hospitalised with respiratory tract infection in Cape Town, South Africa. The occurrence of HMPV was determined over two consecutive winter seasons (April - August 2001, and April - August 2002). In addition the extent of HMPV infection in the summer months (January - April 2003) was also examined. The study population consisted of children under the age of 3 years admitted to the Red Cross War Memorial Children's Hospital with respiratory disease and from whom none of the common respiratory viruses were



isolated in routine viral culture. Thus in the 2001 and 2002 winter seasons, 64 and 69 children were investigated respectively, while 51 children were investigated in the 2003 summer season. In addition possible HMPV co-infection was assessed in 20 children from the 2001 winter season. RNA was extracted from NPA using the QIAamp Viral RNA mini kit (QIAGEN GmbH, Hilden, Germany). The RNA was reverse transcribed into cDNA using random primers and Superscript III RNase H<sup>-</sup> reverse transcriptase, according to the manufacturer's instructions (Invitrogen Ltd., Paisley, UK). The quality of the cDNA for PCR amplification was assessed using  $\beta$  globin primers (GH20 and PC04). Only  $\beta$  globin-positive samples were used for HMPV detection. PCR was performed using 10  $\mu$ l cDNA and primers to the fusion (F) gene as previously described.<sup>2</sup> The sensitivity of the assay was increased with the use of nested primers (sense 5'CTGAACTAGCCAGAGCTGT) and (antisense 5'CATTGATTCTGCTGCTGTGTC) using the same

amplification conditions. Amplicons (354bp) from positive samples were cloned into pGEM-T vector (Promega Corporation, Madison, USA), and plasmid DNA was extracted using QIAprep Spin Miniprep kit (QIAGEN, GmbH, Hilden, Germany). The DNA was sequenced and sequences were aligned with HMPV fusion gene sequences from the GenBank database using the CLUSTAL\_X software package. A phylogenetic tree was constructed using the neighbour-joining method to determine the genotype clusters circulating in this paediatric group.

In the 2001 winter season 52/64 samples (81%) were  $\beta$  globin-positive, and of those 4 (7.7%) were HMPV-positive. Among the 20 children with proven infections with another respiratory virus, HMPV RNA was found in 1 child co-infected with RSV. In the 2002 winter season 91% (63/69) of the NPA samples were  $\beta$  globin-positive and in 12 (19%) HMPV RNA was detected. Eighty-eight per cent (45/51) of the summer

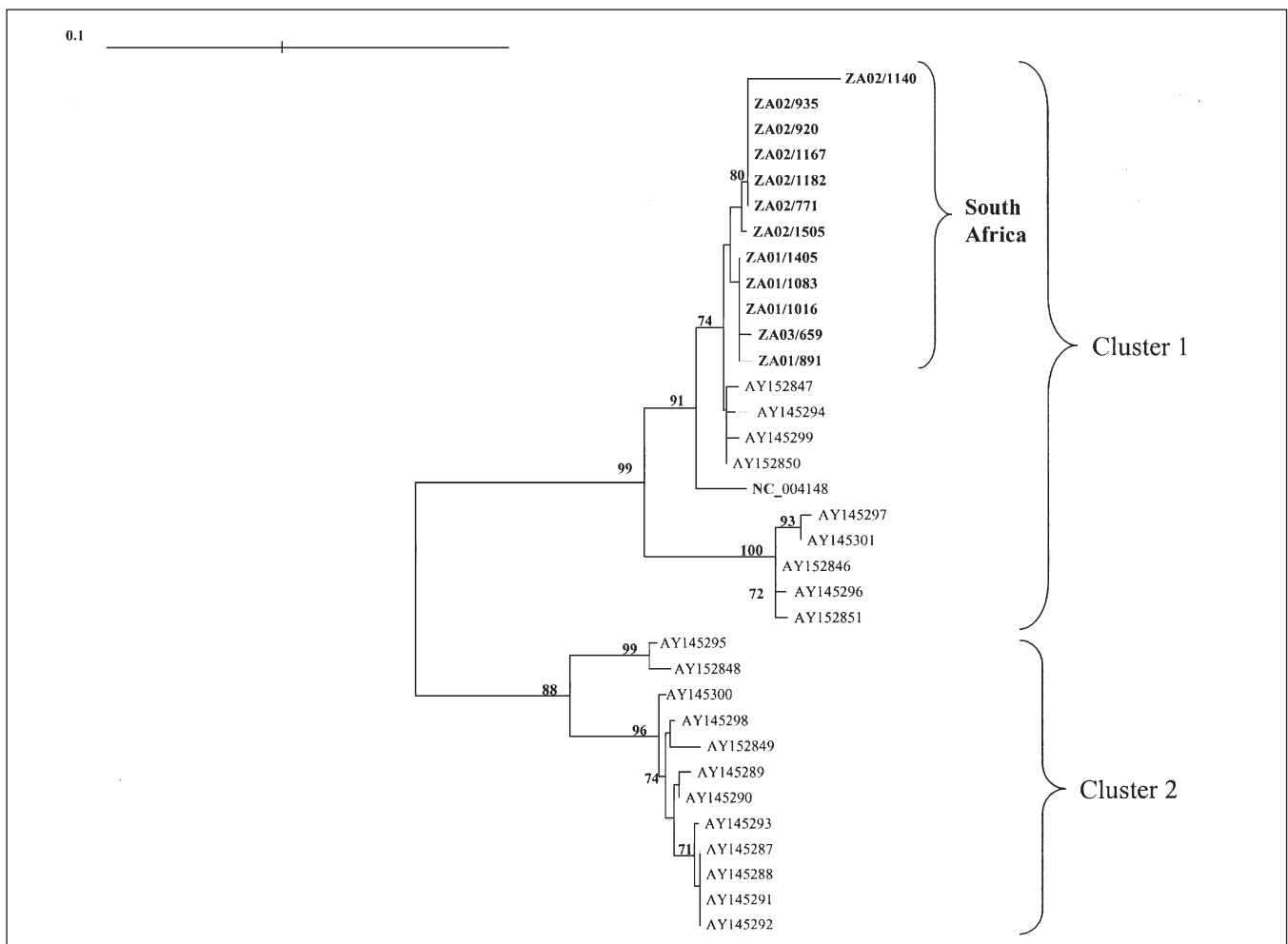


Fig. 1. Phylogenetic tree generated by neighbour-joining analysis of the fusion gene of HMPV. Bootstrap values of greater than 75% are indicated on the nodes of the tree. The branch lengths are proportional to the evolutionary distances as shown on the scale. Sequences from this study are represented in bold type. Representative sequences from GenBank are indicated by accession numbers.



2003 season NPA samples were used to detect HMPV and in 2.2% (1/45) the viral RNA was found.

Phylogenetic analysis of worldwide isolates of HMPV confirmed two genetically distinct clusters as previously described.<sup>1,2,4</sup> In both the 2001 and 2002 winter seasons HMPV from only one cluster was circulating in this study population and this cluster included AY152847 (Spain), NC\_0041489 (Netherlands) and AY145301 (Canada). The single summer 2003 sequence clustered with the 2001 sequences (Fig. 1).

The results of this study confirm the association of HMPV with respiratory diseases in children and indicate that up to 19% of children hospitalised with respiratory symptoms in Cape Town are infected with HMPV in the absence of any of the other more common viral respiratory pathogens. The prevalence differed significantly in the two winter seasons under study, with a figure of 7.7% for 2001 and 19% for 2002. The latter prevalence figure is one of the highest reported in the literature, although a recent study from Italy<sup>8</sup> has shown that over 40% of nasal swabs from infants with acute respiratory tract infections were positive for HMPV. The HMPV was found more frequently in the winter months than in summer (19% versus 2.2%); however, the virus in circulation in summer also appeared to be responsible for respiratory symptoms requiring hospitalisation. Although only a small group was examined for HMPV co-infection, it was infrequently detected and is in line with previous reports.<sup>4,7</sup>

Only one of the two major HMPV genotypes was identified in these patients, but with continued surveillance the circulation of the other major group may be found.

The high prevalence of HMPV in children hospitalised with acute respiratory tract disease indicates that HMPV is an important respiratory pathogen in the Cape Town area and should be considered a causative agent in the absence of detection of the other more commonly associated respiratory viruses.

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