JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2004, p. 5961–5962 Vol. 42, No. 12
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.12.5961–5962.2004

Letters to the Editor

First Detection of Human Metapneumovirus in Children with Acute Respiratory Infection in India: a Preliminary Report

In 2001, van den Hoogen et al. first reported the discovery of human metapneumovirus (HMPV) in respiratory patients from The Netherlands. The disease spectrum ranged from mild upper respiratory tract disease to severe bronchiolitis and pneumonia. Serological studies documented circulation of HMPV for at least 50 years (8). Subsequently, this virus has been recognized as a significant cause of acute respiratory illness (ARI) in infants, children, and adults worldwide (1, 2, 5). Though respiratory diseases are highly prevalent in India (7), the role of HMPV has not been examined so far.

We analyzed 17 nasopharyngeal aspirate specimens collected from the pediatric inpatient department of Sassoon General Hospital, Pune (Maharashtra state), India, and 12 throat swab and 15 nasal swab specimens from outpatient departments during July-August 2003 for detection of HMPV by reverse transcription-PCR. These were stored in transport medium at −70°C till being tested. Total RNA was extracted from 250 μl of the respiratory specimens using the TRIZOL (LS) reagent (GIBCO BRL, Life Technologies) according to the manufacturer's instructions and used for nested reverse transcription-PCR according to the published protocol and primer sequences (6).

Stringent precautions were taken to avoid false positivity in the PCR. Negative controls were included between two samples and subjected to the entire PCR protocol. Pre- and post-amplification procedures were performed on the different floors of the laboratory. All five HMPV-RNA-positive PCR products were sequenced directly on both strands. The phylogenetic status of HMPV-positive specimens was assessed by employing the software MEGA (3). For analysis in MEGA, the Jukes-Cantor distance was utilized, employing the neighbor-joining algorithm. The reliability of different phylogenetic groupings was evaluated by using the bootstrap test (1,000 bootstrap replications) available in MEGA.

Patients with severe ARI (17 children) admitted in the inpatient department had fever and severe lower respiratory tract infection and were diagnosed as having pneumonia or bronchopneumonia. Patients with mild ARI attending outpatient departments had symptoms of common cold, pharyngitis, laryngitis, tracheitis, bronchitis, body ache, fever, and weakness in different combinations. These included 9 children and 18 adults. HMPV RNA was detected in 19.2% (5 of 26) of the children and 0 of 18 adults. Importantly, four out of five patients in whom HMPV RNA was detected were children below the age of 1 year, and one was from the age group 1 to 5 years.

The GenBank accession numbers for the sequences generated during this study are AY532377 to AY532381. Phylogenetic analysis based on a 216-nucleotide fragment of the M gene (Figure 1) demonstrated genetic heterogeneity of the Indian HMPV viral isolates with 100% bootstrap support. Four isolates (INDHMP1 and INDHMP2, both from mild cases, and INDHMP4 and INDHMP5, both from severe cases) clustered with several Canadian strains isolated in 1997, 1998, and 2000. Isolate INDHMP3 (from a severe case) belonged to a distinct second lineage comprising isolates from The Netherlands (2000) and Canada (1997, 1998, 1999, and 2002). In the second lineage, the Indian isolate (INDHMPV3) was separated from other isolates, forming a separate subgroup.

The results of this preliminary study showed that during the 2-month study period, HMPV was detected in nearly five children with respiratory infections. Though none of the 18 adults were positive, unless a larger sample size is screened, infrequent infection of adults with HMPV cannot be concluded. The fact that four of the five PCR-positive patients were infants suggests importance of HMPV in this vulnerable age group. Importantly, both severe and mild cases yielded PCR positivity, demonstrating the clinical spectrum of the disease. The presence of viral RNA could be demonstrated in nasopharyngeal aspirates ($n = 3$) as well as nasal swabs ($n = 2$). The latter specimens are easy to collect. Reports from Italy have shown similar observations (4).

M-gene-based phylogenetic analysis demonstrated cocirculation of distinctly different HMPV strains in Pune in 2003. The four isolates belonging to a lineage represented both mild and severe cases, whereas the only isolate belonging to the other lineage came from a severe case.

In summary, the present study suggests the importance of HMPV in causing mild and severe respiratory infections among children, especially infants in India. Multicentric, long-

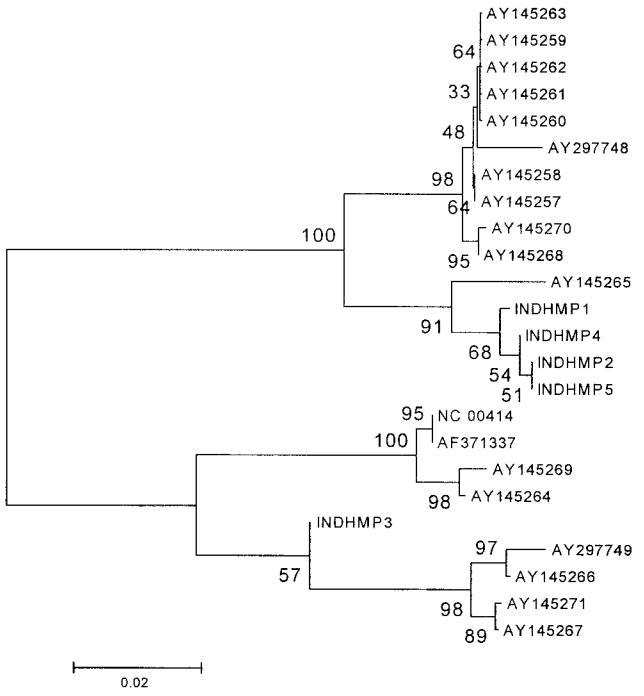

FIG. 1. Phylogenetic analysis of partial M gene (216-base fragment) sequences of 24 HMPV isolates. See the text for details of the isolates and abbreviations used. Percent bootstrap support is indicated by the values at each node.

term studies from different parts of India are needed to assess the disease burden.

We thank Dr. A. C. Mishra, Director, National Institute of Virology, Pune, India, for critical review of the manuscript.

REFERENCES

1. **Falsey, A. R., D. Erdman, L. J. Anderson, and E. E. Walsh.** 2003. Human metapneumovirus infections in young and elderly adults. J. Infect. Dis. **187:** 785–790.
2. **Freymouth, F., A. Vabret, L. Legrand, N. Eterradossi, F. Lafay-Delaire, J. Brouard, and B. Guillois.** 2003. Presence of the new human metapneumovirus in French children with bronchiolitis. Pediatr. Infect. Dis. J. **22:**92–94.
3. **Kumar, S., K. Tamura, and M. Nei.** 1993. MEGA. Molecular evolutionary genetic analysis version 1.0. Pennsylvania State University, University Park, Pa.
4. **Maggi, F., M. Pifferi, M. Vatteroni, C. Fornai, E. Tempestini, S. Anzilotti, L. Lanini, E. Andreoli, V. Ragazzo, M. Pistello, S. Specter, and M. Bendinelli.** 2003. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. J. Clin. Microbiol. **41:**2987–2991.
5. **Osterhaus, A., and R. Fouchier.** 2003. Human metapneumovirus in the community. Lancet **361:**890–891.
6. **Peiris, J. S., S. T. Lai, L. L. Poon, Y. Guan, L. Y. Yam, W. Lim, J. Nicholls, W. K. Yee, W. W. Yan, M. T. Cheung, V. C. Cheng, K. H. Chan, D. N. Tsang, R. W. Yung, T. K. Ng, K. Y. Yuen, and the SARS Study Group.** 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet **361:**1319–1325.
7. **Pio, A.** 1988. W.H.O. Programme on acute respiratory infections. Indian J. Pediatr. **55:**197–205.
8. **van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier, and A. D. Osterhaus.** 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat. Med. **7:**719–724.

B. L. Rao
S. S. Gandhe
S. D. Pawar
V. A. Arankalle*
National Institute of Virology
20 A, Dr. Ambedkar Rd., Post Box No. 11
Pune-411001, India

S. C. Shah
A. A. Kinikar
Department of Pediatrics
B. J. Medical College
Pune-411001, India

*Phone: 91-20-26127301
Fax: 91-20-26122669
E-mail: Varankalle@yahoo.com