Detection of Human Metapneumovirus Genomes during an Outbreak of Bronchitis and Pneumonia in a Geriatric Care Home in Shimane, Japan, in Autumn 2009

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Human metapneumovirus (HMPV), which belongs to the family Paramyxoviridae, genus Metapneumovirus, is an important causative agent of acute respiratory infections (ARIs) in humans (1). Despite this, the molecular epidemiology of HMPV in Japan is not well understood. We described herein an outbreak of HPMV infection in a geriatric care home in Shimane, Japan in autumn 2009 and the results of genetic analyses of the HMPV detected in samples obtained from residents of this home. An epidemiological investigation in late September 2009 found that 2 of the 99 residents of this home exhibited symptoms such as high fever (≥38°C), cough, and inflammation of the lower respiratory tract. Other residents were identified with similar symptoms up until late October 2009. The overall prevalence during this outbreak was around 30% (27/99 persons), although the infection route could not be determined. Nine throat swab samples were collected from these patients after obtaining verbal informed consent and attempts made to detect and/or isolate influenza virus subtype A, human rhinovirus, enteroviruses, respiratory syncytial virus, parainfluenza viruses, and/or adenoviruses using previously reported reverse transcriptase-polymerase chain reaction (RT-PCR) and cell culture methods (Vero E6, RD, MDCK, and HEP-2 cells) (2–5). Viral nucleic acid was extracted from the samples using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany) and suspended in DNase/RNase-free water. After RNA extraction, RT-PCR was performed as described previously (6,7). Amplicons were purified using a QIAquick PCR Purification Kit (Qiagen, Germantown, Md., USA) and the nucleotide sequences were determined by direct sequencing (6). Phylogenetic analysis based on the fusion (F) gene of HMPV strains was then performed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 4 (8). Evolutionary distances were estimated using Kimura’s two-parameter method and a phylogenetic tree was constructed using the neighbor-joining method (9,10). The reliability of the tree was estimated on the basis of 1,000 bootstrap replications.

A summary of patient and viral data is shown in Table 1. HMPV was detected in samples from 7 patients; no other viruses were detected. In addition, serum IgG against HMPV was detected in 2 patients using an indirect immunofluorescence assay (11), with significantly higher levels being found in the convalescent phase. Nucleotide sequence analysis of different HMPV genes, with F gene being the most common, allows the virus to be divided into two major genetic groups (A and B) and four subgroups (A1, A2, B1, and B2) (12,13). The phylogenetic tree determined here showed that all strains detected in the patient samples were clustered in subgroup B2 (Fig. 1). The nucleotide identity among the present strains was 100%, with a nucleotide identity of 99.7% with respect to the Yamaguchi 09-15 strain detected in Yamaguchi Prefecture during the same season. A very recent study suggested that HMPV subgroups A2 and B2 are the major types circulating in Japan (14). Indeed, subgroups A2, B2, and B1 were found in 3, 4, and 2 strains, respectively, of the 9 HMPV strains detected by the sentinel surveillance system for viral diseases in Shimane Prefecture from March 2009 to January 2010. Furthermore, a high degree of nucleotide identity (98.7–100%) was seen between the subgroup B2 strains.

It is suggested that HMPV infection mainly occurs in children, although recent reports indicate that outbreaks of HMPV infection also occur in the elderly (15). Indeed, a similar outbreak to the present case occurred in another geriatric care home in Japan (16). However, despite these occurrences, the epidemiology of HMPV infection still remains unclear. A high prevalence (around 30%) of HMPV infection was seen in the present study, with some patients presenting with severe infections such as pneumonia. HMPV infection should therefore be considered in outbreaks among elderly peo-
Table 1. Patient and human metapneumovirus data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Onset date</th>
<th>Sampling date</th>
<th>Strain</th>
<th>Subgroup</th>
<th>GenBank accession no.</th>
</tr>
</thead>
</table>

Fig. 1. Phylogenetic tree constructed on the basis of partial sequences of the human metapneumovirus F gene. Distance was calculated using Kimura’s two-parameter method, and the tree was plotted using the neighbor-joining method. Numbers above the branches are bootstrap probabilities (%). Reference strains were NL/00/1 (AF371337), CAN99-81 (AY145294), JP/02/10190 (AB113377), JP/03/11015 (AB113372), JPS03-180 (AY530092), CAN97-83 (AY145296), NL/17/00 (AY304360), JPS03-1 (AB533251), JP/03/11011 (AB533251), Yamaguchi 09-03 (AB533245), Yamaguchi 09-03 (AB533239), NL/1/99 (AY304361), Yamaguchi 09-17 (AB533244), JPS02-76 (AY530089), JPS03-194 (AY530094), JPS03-194 (AY530094), JP/03/10016 (AB126611), JTY06-1 (EU127917), JP/03/10023 (AB126608), JP/03/20034 (AB119493), CAN98-75 (AY297748), JP/03/10016 (AB126607), CAN00-13 (AY145298), NL/1/94 (AY304362), and Yamaguchi 09-15 (AB533243). Avian metapneumovirus type C (AMPV-C, AY579780) was also included as an outgroup.

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Conflict of interest None to declare.

REFERENCES


