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Human metapneumovirus infection among family members

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SUMMARY

The transmission of human metapneumovirus (hMPV) among family members is not well understood. We identified 15 families in which multiple members were diagnosed with hMPV infection by real-time PCR in 2008 and 2010. Index patients ranged in age from 2 years to 11 years (median 5 years), and all 15 index cases were children who attended primary school, kindergarten, or nursery school. Contact patients ranged in age from 2 months to 46 years (median 6 years). Excluding five adult cases, contact patients were significantly younger than index patients ($P = 0{\cdot}0389$). Of the 12 contact children, seven (58 %) were infants who were taken care of at home. The serial interval between the onset of symptoms in an index patient and the onset of symptoms in a contact patient was estimated to be 5 days. These results suggest that the control of school-based outbreaks is important for preventing hMPV infection in infants.

Key words: Infectious disease epidemiology, respiratory infections.

INTRODUCTION

Human metapneumovirus (hMPV), first described in 2001, is a pathogen that is associated with respiratory infections. It has been classified as a member of the genus *Metapneumovirus* of the subfamily Pneumovirinae of the family Paramyxoviridae [1]. Based on genetic and phylogenetic analyses, hMPV can be separated into two subgroups, A and B, with each subgroup divided into two genotypes (i.e. A1 and A2, B1 and B2) [2]. This virus circulates predominately in late winter and spring, and several different hMPV genotypes co-circulate in the community [3–5]. For example, in the community of Yamagata, Japan, A2 and B2 are the major types in endemic circulation almost every year [5].

Human MPV is a common cause of respiratory infections worldwide, particularly in infants and young children, and it leads to conditions ranging from upper respiratory infections to severe lower respiratory infections, such as bronchitis, bronchiolitis, and pneumonia [1, 6, 7]. Serological studies have revealed that most individuals have been exposed to hMPV by the age of 5 years, and re-infections can occur in all age groups [1, 4, 8]. Outbreaks of this virus have been reported in long-term care facilities, hospitals, primary schools, and nurseries [9–11]. In infants, hMPV infection is most likely to be acquired from

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a family member. However, there have been no reports about the household transmission of hMPV. In this study, we identified 15 families in which multiple members had hMPV infection confirmed by molecular techniques in 2008 and 2010 and retrospectively investigated the hMPV infection in members of the same families.

METHODS

During the periods of January–April 2008 and January–May 2010, a total of 675 nasopharyngeal swab specimens were collected from patients with acute respiratory infection (ARI) at the Yamanobe Pediatric Clinic collaborating with the local health authority of the Yamagata Prefecture for the surveillance of viral diseases in Japan. Informed consent was obtained from the participating patients or their guardians. Specimens were transported to the Yamagata Prefectural Institute of Public Health and grown in a virus culture as previously described [5], and were further tested for hMPV by real-time PCR. HMPV-positive patients whose specimens were confirmed by real-time PCR were included in this study. Clinical information for patients who tested positive for hMPV was retrospectively obtained from their medical records. Statistical analysis was performed using StatView J-4.02 (Abacus Concepts, USA). The Mann–Whitney *U* test was used to compare median values. *P* values of $<0{\cdot}05$ were regarded as statistically significant.

For real-time PCR, viral RNA was extracted from 200 μl specimens using the High Pure Viral RNA kit (Roche Diagnostics, Germany) according to the manufacturer's instructions and subsequently transcribed into cDNA using random primers. The resulting cDNA was used for real-time PCR with a TaqMan probe targeting the hMPV *N* gene, as previously described [12].

For sequencing analysis, the transcribed cDNA was subjected to PCR amplification of the fusion region, which was performed under the following conditions: 30 s at 94 °C; 35 cycles of 94 °C for 30 s, 53 °C for 30 s, and 72 °C for 2 min, with a final extension at 72 °C for 9 min. The primer pair hMPVM + 724 (5′-TGGAGYCAYCAAGGRACAAG-3′) and hMPVM2-92 (5′-GGCCAACTCCAGTAATTGTG-3′) was used to amplify the fusion region, which includes the full-length *F* gene (1620 bp). The PCR products were purified with a QIAquick PCR purification kit (Qiagen, Germany) and sequenced using a Big Dye Terminator v. 1.1 cycle sequencing kit (Applied Biosystems, USA) on an Applied Biosystems 3130 automatic sequencer. The nucleotide sequences of the primers used for sequencing were as follows: hMPVF + 75 (5′-AGARTCATGYAGYACYATAAC-3′), hMPVF + 574 (5′-AGCTTCAGTCAATTCAACAG-3′), hMPVF + 1081 (5′-TGCAAAGTYAGCACAGGAAG-3′), and hMPVF-302 (5′-GATTGTCTGGGATTYYCAATTTG-3′). Sequence data were analysed with CLUSTAL W version 1.83, and a phylogenetic tree was constructed by the neighbour-joining method using the same software. The nucleotide sequences determined in this study were deposited in GenBank under the accession numbers AB618745–AB618755, AB618758–AB618778 and AB693954–AB693960.

RESULTS

Real-time PCR for hMPV was positive in 141 of the 675 respiratory specimens. An analysis of these samples revealed 15 families in which two or more members had hMPV infection. The first hMPV-positive patient in a family was defined as the index case, and the second or third hMPV-positive patient in the same family was defined as a contact case. In 15 families, 15 index cases and 17 contact cases were identified. The demographic characteristics of the index and contact patients are shown in Table 1. Although one patient (F3, contact 1) was undergoing kidney dialysis due to chronic renal failure, no other subject had any immunosuppressive illness or other underlying disease.

The index cases ranged in age from 2 years to 11 years (median 5 years) (Fig. 1); 10 (67%) of the index patients were male. All 15 index patients were children who attended primary school, kindergarten, or nursery school. The contact cases ranged in age from 2 months to 46 years (median 6 years); seven (41%) of the contact patients were male. Of the 17 contact patients, five were parents (aged 31–46 years), two were older siblings and 10 were younger siblings. Excluding the adult cases, seven (58%) of 12 contact cases were infants who were taken care of at home. The median age of the paediatric contact patients was 2 years (range 2 months to 8 years). Statistical analysis revealed that the contact patients, excluding the adult cases, were significantly younger than the index patients ($P = 0{\cdot}0389$).

Of the 32 subjects, 27 (84%) were diagnosed with upper respiratory infection (URI). Bronchiolitis and bronchitis were diagnosed in one and four

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Table 1. *Demographic characteristics of patients with human metapneumovirus infection*

Family	Subject	Age	Sex	Diagnosis	Situation	Strain name
F1	Index	6 yr	Male	URI	Primary school	187-Yamagata-08
	Contact	8 yr	Male	URI*	Primary school	236-Yamagata-08
F2	Index	5 yr	Male	URI	Kindergarten	234-Yamagata-08
	Contact	34 yr	Female	URI*	Index's mother	331-Yamagata-08
F3	Index	11 yr	Female	URI	Primary school	428-Yamagata-08
	Contact 1	46 yr	Male	URI	Index's father	495-Yamagata-08
	Contact 2	41 yr	Female	URI	Index's mother	484-Yamagata-08
F4	Index	2 yr	Male	Bronchitis	Nursery school	489-Yamagata-08
	Contact	31 yr	Female	URI	Index's mother	560-Yamagata-08
F5	Index	5 yr	Female	URI	Kindergarten	497-Yamagata-08
	Contact	7 yr	Male	URI	Primary school	562-Yamagata-08
F6	Index	3 yr	Male	URI	Nursery school	535-Yamagata-10
	Contact	2 mo.	Male	URI*	Taken care of at home	618-Yamagata-10
F7	Index	10 yr	Female	URI	Primary school	543-Yamagata-10
	Contact	7 yr	Male	URI*	Primary school	636-Yamagata-10
F8	Index	5 yr	Male	URI	Kindergarten	553-Yamagata-10
	Contact	1 yr	Female	Bronchiolitis	Taken care of at home	626-Yamagata-10
F9	Index	4 yr	Male	Bronchitis	Kindergarten	640-Yamagata-10
	Contact	39 yr	Female	URI*	Index's mother	748-Yamagata-10
F10	Index	6 yr	Female	URI	Kindergarten	629-Yamagata-10
	Contact	2 mo.	Female	URI*	Taken care of at home	783-Yamagata-10
F11	Index	10 yr	Male	URI	Primary school	630-Yamagata-10
	Contact	2 yr	Female	URI*	Taken care of at home	764-Yamagata-10
F12	Index	8 yr	Female	URI	Primary school	651-Yamagata-10
	Contact 1	5 yr	Female	URI	Kindergarten	737-Yamagata-10
	Contact 2	1 yr	Female	URI	Taken care of at home	738-Yamagata-10
F13	Index	5 yr	Male	URI	Kindergarten	776-Yamagata-10
	Contact	2 yr	Male	URI	Taken care of at home	858-Yamagata-10
F14	Index	5 yr	Male	Bronchitis	Kindergarten	869-Yamagata-10
	Contact	6 mo.	Female	Bronchitis	Taken care of at home	923-Yamagata-10
F15	Index	7 yr	Male	URI	Primary school	1119-Yamagata-10
	Contact 1	6 yr	Male	URI	Primary school	1158-Yamagata-10

URI, Upper respiratory infection.
* Indicates that the onset symptom is cough.

children, respectively. Fever (body temperature $>38\ ^{\circ}C$) was noted in all of the index patients and in 10 of the 17 contact patients. Seven of the contact patients had no fever during the period of illness, and their onset symptom was cough. Of these patients, two were 2-month-old infants (contact patients of F6 and F10), and two were adults (contact patients of F2 and F9). The symptom onset interval was calculated as the number of days from the onset of fever in the index patient to the onset of fever (or cough, if fever was not present) in the household contact. This interval ranged from 3 days to 7 days (median 5 days) (Fig. 2). When the seven cases for which the onset symptom was cough were excluded from the analysis, the median interval was 4 days (range 3–7 days).

As shown in Figure 3, phylogenetic analysis revealed that hMPV recovered from members of the same family in 2008 belonged to A2 (F1, F2, F4, F5) or B2 (F3) lineage and all isolates recovered in 2010 belonged into B2 (F6–F15) lineage. The nucleotide sequences of the hMPV isolates recovered within each family were completely identical to the 1620 bp of the *F* gene. A comparison of the interfamily sequences showed that the *F* gene sequences of hMPV from F2, F3, F6 and F9 differed from those of the viruses recovered from other families by at least one nucleotide. Furthermore, compared to the *F* gene of hMPV circulating within this community, they differed from those of viruses recovered from families identified in the same season by 0–21 bp.

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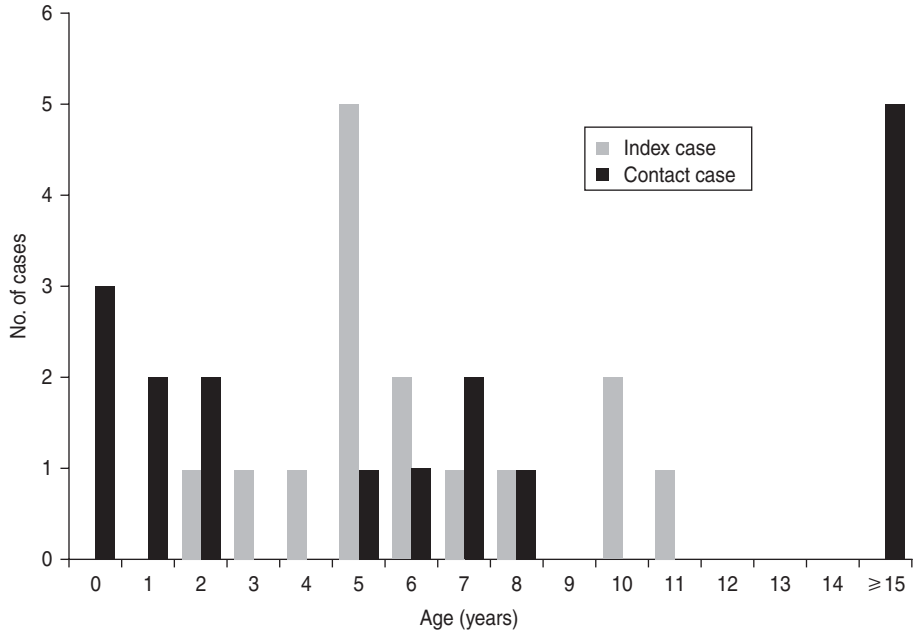

Fig. 1. Age distribution of human metapneumovirus-positive index cases and contact cases.

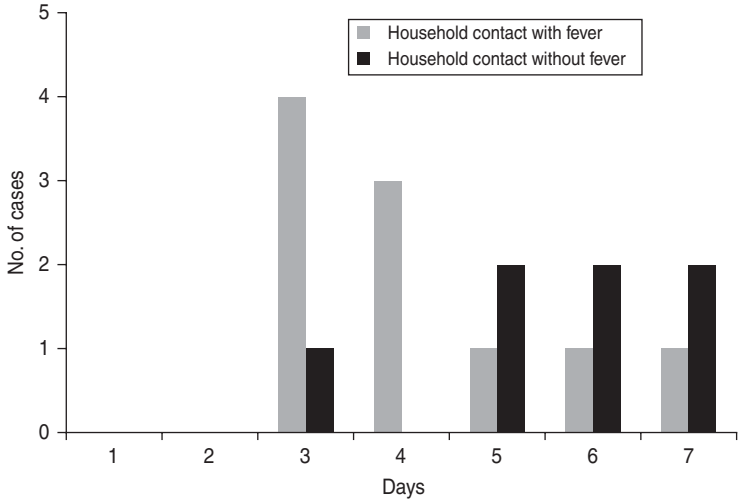

Fig. 2. Interval from symptom onset in index cases and household contacts with or without fever.

DISCUSSION

The present study shows that the nucleotide sequences of hMPV isolates recovered within each family were identical. Because viruses with identical nucleotide sequences were circulating elsewhere in this community, it is possible that in some families, the virus was acquired from a community contact rather than a family contact. However, the short intervals between symptom onset in index patients and contact patients suggest that secondary transmission most likely occurred within families.

Several studies have revealed that children aged <5 years are most susceptible to hMPV infection [3, 4, 6]. All of the index patients were children who attended primary school, kindergarten, or nursery school, and 58% of the contact children were infants who were taken care of at home. Thus, preschool- or school-aged children appeared most likely to introduce hMPV into the family, and infants are likely to acquire hMPV infection from their older siblings.

Knowledge of the incubation period, which is the time between infection and symptom onset, is useful

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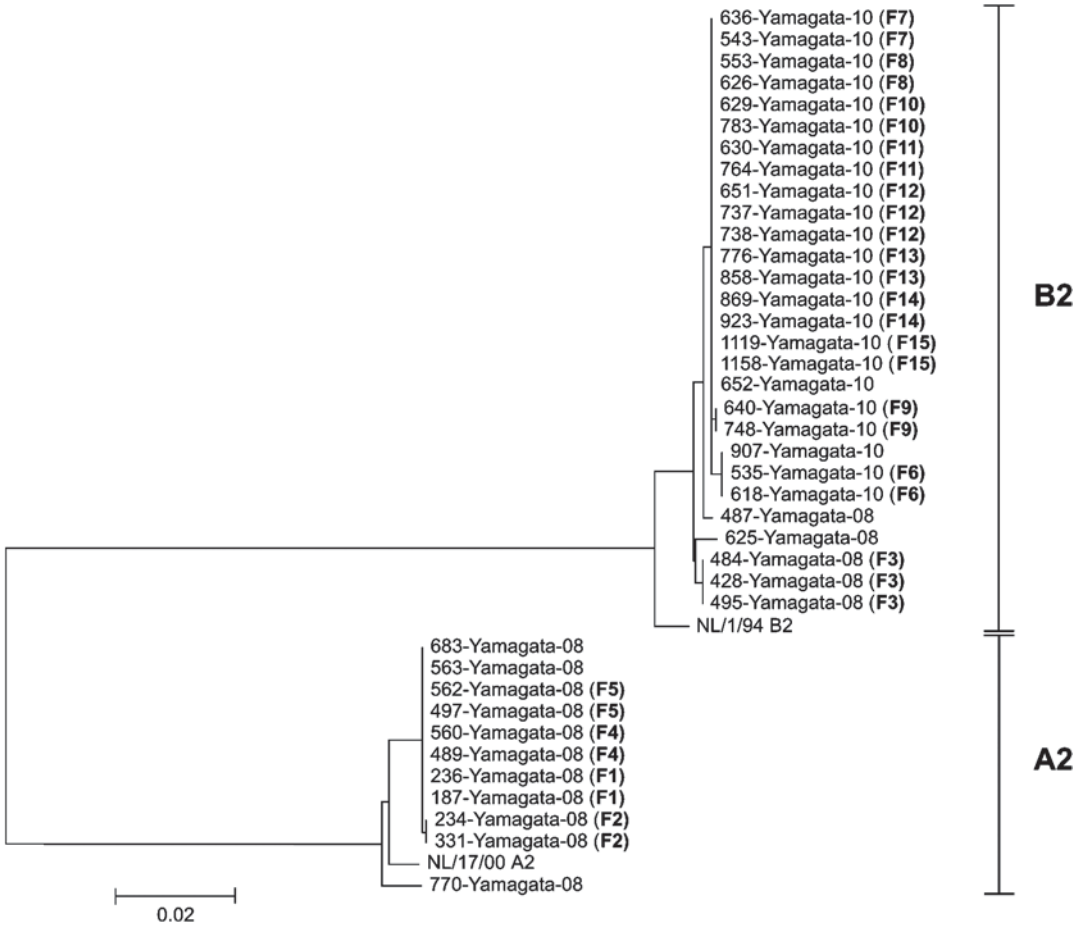

Fig. 3. Comparison of the nucleotide sequences of the full-length *F* gene (1620 bp) of human metapneumovirus recovered from the members of the 15 studied families and other residents of Yamagata, Japan. Family identification numbers are shown in parentheses.

for infectious disease control [13]. The incubation periods of several infectious diseases, such as influenza virus and respiratory syncytial virus, have been estimated by studying the experimental infection of volunteers or based on the observational study of secondary infections in families or facilities [13–15]. However, there have been only a few reports about the incubation period of hMPV infection. Based on a single case of nosocomial infection, two reports have suggested incubation periods of 5–6 days and 4–6 days [16, 17]. In another case of a paediatric haemato-oncology patient, the incubation period was estimated to be 7–9 days [10]. In this study, the serial interval for household transmission of hMPV was calculated to be 4–5 days (range 3–7 days). Our result is comparable to the incubation period of previous reports. However, in cases of transmission within families, the serial interval could not be considered to be the exact incubation period for a secondary case because it is possible that the contact patient was infected before or after the onset of symptoms in the associated index patient. In a previous study [18], we suggested that virus shedding in children with hMPV decreases by 4 days after the onset of fever. Therefore, it is likely that household contact between family members and the infected individual within 1–3 days after the onset of fever in the index patient is responsible for virus transmission.

One limitation of this study is the study design. The contact cases were retrospectively identified in patients diagnosed with hMPV infection by a molecular method in our surveillance work. Therefore, the total frequency of hMPV infection in household contacts of hMPV-positive index patients could not be estimated. There were five cases of transmission from the index child to the parent. Of these adult contacts, two had no fever during the period of illness. Because hMPV infection is associated with very mild symptoms of ARI in adults, they may not visit the clinic. If the index patient is confirmed as

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hMPV positive in a clinical setting, all household contacts should be prospectively investigated further. Infections in adults and the elderly are probably underreported [7]; in this group there may be more cases of hMPV infection acquired from a family member.

In conclusion, infants are likely to acquire hMPV infections from their older siblings. The control of hMPV outbreaks in nursery schools, kindergartens, and primary schools may be an important strategy for preventing hMPV infection in infants.

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DECLARATION OF INTEREST

None.

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