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Outbreak of Human Metapneumovirus Infection in a Severe Motor-and-Intellectual Disabilities Ward in Japan

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Human metapneumovirus (hMPV) was first isolated in 2001 by van den Hoogen et al. (1) and is now considered one of the major respiratory viruses affecting children and elderly persons (2) as well as immunocompromised individuals (3). Because hMPV causes community-wide outbreaks, its introduction into clinical settings could result in outbreaks in healthcare facilities (4–8), eventually with undesirable outcomes (5–7). Here we report an outbreak of hMPV infection in a ward housing patients with severe motor and intellectual disabilities in a hospital in northern Japan, utilizing a series of diagnostic tests.

A retrospective investigation of outbreak of hMPV infection was conducted upon the request of the attending clinician in the hospital, which contained 2 convalescent wards, wards A and B, each with 22 beds. Patients with severe mental retardation, with a requirement for long-term care, were admitted to the hospital. At the time of the outbreak, 44 residents were admitted to the 2 wards of the hospital. During the period April 4–26, 2008, residents having an increased body temperature of 1.1°C above the baseline were subjected to follow-up as

a “probable case.” Among these probable cases, “confirmed cases” of hMPV infection were defined by any of the following criteria: (i) hMPV positive by virus isolation, (ii) hMPV RNA positive by reverse transcription (RT)-PCR, or (iii) a 4-fold increase in IgG antibody titer against homologous hMPV using paired serum samples.

Nasopharyngeal swabs (NPS) were collected upon onset of the disease while paired serum samples were collected during the acute and convalescent phases with at least a 3-week interval. Virus isolation was performed according to the modified microplate method using human embryonic lung fibroblast, human laryngeal carcinoma (HEp-2), Madin-Darby canine kidney (MDCK), green monkey kidney (Vero-E6), and rhesus macaque kidney (LLC-MK2) cell lines (9). Samples showing hMPV-specific cytopathic effects on LLC-MK2 or Vero-E6 cells were subjected to an identification test using molecular methods, as follows. RNA was extracted from 140 µL of supernatant from NPS or the virus isolate using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). RT-PCR targeting the N gene of

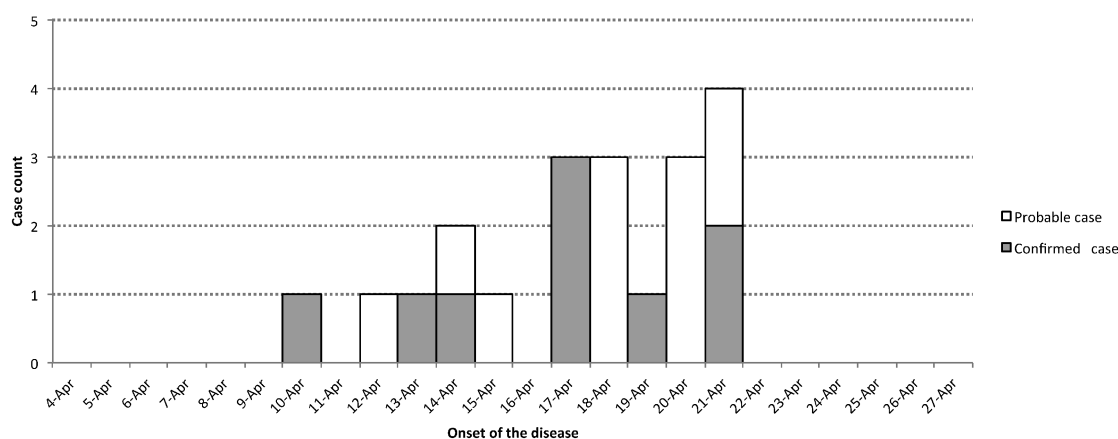


Fig. 1. Epidemic curve of probable cases in ward A.

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Table 1. Demographics, clinical features, and laboratory investigation results of the probable cases

Demographic information			Clinical information							Laboratory test						Case classification	
Case ID	Age (yr)	Gender	Clinical diagnosis	Fever °C		Cough		Wheeze		Rhinorrhea	Virus isolation	PCR	Antibody:Subtype A		Antibody:Subtype B		
				Maximum	(Days)	Presence	(Days)	Presence	(Days)				Acute	Convalescent	Acute		Convalescent
1	21	Male	Acute bronchitis	40	(4)	+	(4)	—		—	—	—	1:1600	1:6400*	1:1600	1:6400*	Confirmed
2	51	Male	Acute bronchitis	38.6	(4)	+	(6)	—		—	—	—	1:800	1:800	1:1600	1:800	Probable
3	39	Female	Acute bronchitis	39.5	(10)	+	(10)	—		+	—	—	1:400	1:12800*	1:400	1:12800*	Confirmed
4	46	Female	Acute bronchitis	40.1	(11)	+	(12)	—		—	—	—	1:400	1:6400*	1:800	1:12800*	Confirmed
5	60	Male	Acute exacerbation of CB	39.4	(3)	+	(2)	+	(2)	—	—	—	1:1600	1:3200	1:800	1:1600	Probable
6	27	Male	Acute bronchitis	39.6	(6)	+	ND	—		—	—	—	1:6400	1:6400	1:12800	1:12800	Probable
7	51	Male	Acute bronchitis	38.4	(2)	+	(2)	—		+	—	—	1:400	1:6400*	1:400	1:6400*	Confirmed
8	43	Male	Acute bronchitis	37.9	(4)	+	(8)	—		+	—	+	1:400	1:1600*	1:400	1:1600*	Confirmed
9	31	Male	Acute bronchitis	40	(4)	—		—		+	—	+	1:400	1:1600*	1:400	1:800	Confirmed
10	49	Female	Acute bronchitis	39.1	(3)	+	(8)	+	(8)	—	—	—	1:3200	1:3200	1:3200	1:3200	Probable
11	44	Female	URTI	37.4	(3)	—		—		+	—	—	1:400	1:400	1:400	1:400	Probable
12	39	Male	Acute bronchitis	37.7	(2)	+	(1)	—		+	—	—	1:800	1:400	1:800	1:400	Probable
13	39	Male	Acute bronchitis	40	(5)	—		—		+	—	+	1:200	1:6400*	1:200	1:6400*	Confirmed
14	30	Female	Acute bronchitis	38.5	(3)	+	(5)	+	(4)	—	—	—	1:1600	1:800	1:800	1:800	Probable
15	41	Male	Acute exacerbation of CB	38.3	(5)	+	(> 23)	—		—	—	—	1:400	1:400	1:400	1:400	Probable
16	17	Female	Acute bronchitis	39.4	(3)	—		+	ND	—	—	—	1:400	1:400	1:400	1:400	Probable
17	45	Male	Acute bronchitis	40.2	(5)	+	(8)	—		—	+	+	1:200	1:6400*	1:200	1:6400*	Confirmed
18	31	Male	Acute bronchitis	39.3	(2)	—		—		—	—	+	1:400	1:12800*	1:400	1:12800*	Confirmed
19	54	Female	Acute bronchitis	38.6	(2)	+	(8)	—		—	—	—	1:200	1:200	1:200	1:200	Probable
20	49	Male	URTI	37.6	(2)	—		—		—	—	—	1:400	1:400	1:400	1:400	Probable

*, 4-fold rise in IgG between acute and convalescent serum.

CB, chronic bronchitis; URTI, upper respiratory tract infection.

hMPV was performed using the One-Step RNA PCR kit (TaKaRa Bio Inc., Shiga, Japan) (10). The PCR products were sequenced using the ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA, USA), and homology search was conducted using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Antibodies to 2 hMPV subtypes (subtypes A and B) were quantified using the whole virus-based enzyme-linked immunosorbent assay (ELISA) (11). Informed consent was obtained verbally from the legal guardians of the residents, and all study procedures were performed in accordance with the Helsinki Declaration. This study was reviewed and approved by the Ethics Committee of Sendai Medical Center, Japan.

During the observation period, 20 of the 44 residents were identified as probable cases, and all of them had been staying in ward A. Onset of symptoms was observed in the index and last cases on April 10 and 21, respectively (Fig. 1). Among the 20 probable cases, 16 were clinically diagnosed as acute bronchitis, and 2 were diagnosed as acute exacerbations of chronic bronchitis and upper respiratory tract infection (Table 1). Fatalities related to the outbreak were not observed.

We applied 3 methods for the diagnosis of hMPV infection (Table 1). hMPV was isolated using Vero-E6 cell lines from only 1 probable case, and positive results for hMPV RNA using RT-PCR were observed for 5 probable cases, including the isolation-positive case. Phylogenetic analysis resulted in the classification of 3 of the above-mentioned 5 cases as hMPV subtype B, namely genotype B2 (data not shown). Paired serum samples were collected with a time interval of 24–32 days. All probable cases had at least 1:200 antibody titers against the homologous subtype B as well as heterologous subtype A. The use of paired serum samples revealed that 8 cases showed a 4-fold increase in IgG titers against both subtypes, with the exception of 1 case (Case 9) where lower reactivity was obtained to circulating subtype B than to subtype A. As a consequence, 9 of the 20 (45%) probable cases were confirmed to have had a recent hMPV infection.

In summary, we confirmed the hMPV outbreak in a severe motor-and-intellectual disabilities ward in Japan. Interestingly, hMPV activity was also high in Japan during the month corresponding to the outbreak event (12). Because the residents were usually bedridden, hMPV was likely introduced to and transmitted within the ward by non-residents.

Three different methods were used for the diagnosis of hMPV infection. While the molecular detection methods had the advantage over virus isolation methods, the serological test provided more insight into the nature of hMPV infection. ELISA revealed that all confirmed cases had a baseline antibody titer against hMPV of at least 1:200, which is in line with our previous report using the same methodology (11,13). This finding supports the concept that hMPV reinfection is a common occurrence (13,14). Moreover, Case 1 showed an increase in antibody titer from 1:1600 to 1:6400, suggesting that such a high titer of antibody remains insufficient to offer protection against reinfection (13). Notably, one of the drawbacks of the serological test employed in the present study is the cross-reactivity between the 2 hMPV subtypes (11,15); Case 9 is an

example, where a stronger reaction against subtype A in the serological test but positive results for subtype B using PCR were observed. This result, in fact, indicates the relevance of virus detection for subtype-specific laboratory confirmation. All the aforementioned diagnostic methods may not be practically applicable in several clinical settings; the point-of-care (POC) test can be employed as an alternate and better option in such situations. The POC test for hMPV is commercially available in Japan for diagnostic purposes. Hence, the application of the POC test can be more useful in early intervention against hMPV infection (8) and may facilitate the epidemiological study of hMPV in clinical settings in the near future.

There are several limitations of the present study. First, the impact of bacterial pathogens, which could affect the clinical outcome of the disease course (16) or could be the causative agents for hMPV negative probable cases, could not be assessed in the present study. Second, asymptomatic cases were likely overlooked, which might have an impact on infection control (8). Third, certain non-isolatable strains of the virus may have been missed; the isolation system employed in the present study, however, is capable of isolating the major respiratory viruses (9). Despite the above limitations, the results of the present investigation suggest that adopting multiple methods is invaluable for the proper diagnosis of hMPV during outbreaks.

To conclude, clinicians should keep in mind regarding hMPV as a probable causative agent during the outbreak of any respiratory disease in a healthcare setting. Moreover, the presence of hMPV in suspected hMPV-infected cases should be investigated using appropriate laboratory methods.

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Conflict of interest None to declare.

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