Laboratory and Epidemiology Communications

Detection of Various Respiratory Viruses in Patients with Influenza-Like Illness before and after Emergence of the 2009 Pandemic H1N1 Influenza Virus in Okinawa

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The 2009 pandemic H1N1 influenza virus (AH1 pdm) emerged suddenly in Mexico and spread rapidly throughout the world, including Japan (1). Seasonal influenza was prevalent from November to March in Okinawa before the emergence of AH1 pdm, whereas large numbers of patients with influenza-like illness (ILI) were reported from May to December in 2009 (Fig. 1). We therefore decided to analyze the trends of the various respiratory viruses before and after the emergence of an unexpected, pandemic respiratory virus such as AH1 pdm. Herein we report on the variation of the respiratory viruses detected in patients with ILI before and after the emergence of AH1 pdm.

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Nasopharyngeal swabs were obtained from 1,089 patients with ILI from January to December 2009. All patients were residents of Okinawa Prefecture, Japan. Viral RNA was purified using a commercial kit (QIAamp Viral RNA Mini kit; Qiagen, Valencia, Calif., USA), then suspended in DNase/RNase-free water. Initially we attempted to detect AH1pdm and seasonal influenza viruses (subtypes AH1, AH3, and B) using real-time RT-PCR (2) or conventional RT-PCR (3), as described previously. When the ILI sample was negative for influenza virus, we attempted to detect other viruses using RT-PCR methods for respiratory syncytial virus (RSV) (4), human parainfluenza virus (HPIV) (5), human metapneumovirus (HMPV) (6), enteroviruses (EV) (7,8), and human rhinovirus (HRV) (7,8). All amplicons were confirmed by agarose gel electrophoresis. Adenoviruses were confirmed by cell culture and immun-chromatographic methods.

The number of ILI patients in Okinawa peaked in January, August, and December 2009 (Fig. 1). The age

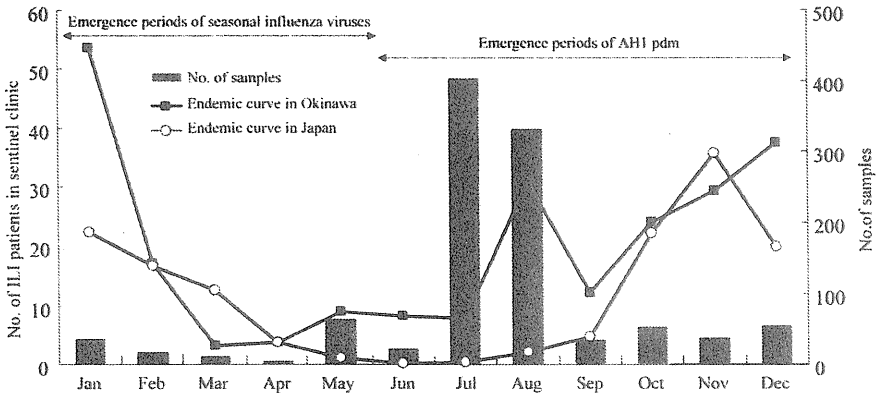

Fig. 1. The endemic curve of influenza-like illness (ILI) and monthly sample numbers in the present study.

Table 1. Monthly detection of the various viruses in the present study

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (%)
AH1	15	2											17 (1.6)
AH3	4				42	10	31						87 (8.0)
B	1	1			9	4							15 (1.4)
AH1pdm						1	292	293	27	43	36	45	737 (67.7)
HRV					1		3	3		3			10 (0.9)
RSV		1	1				5	2					9 (0.8)
HMPV			2				2	1					5 (0.5)
HPIV		2	1		1		1						5 (0.5)
AdV	8	7	4	1				1		1			22 (2.0)
EV	1	1					4						6 (0.6)
Total no. of InfV	20	3			51	15	323	293	27	43	36	45	856 (78.6)
Total no. of non-InfV	9	11	8	1	2		15	7		4			57 (5.2)
Not detected	9	5	5	5	13	8	67	33	9	9	3	10	176 (16.2)
Total	38	19	13	6	66	23	405	333	36	56	39	55	1,089

AH1, influenza virus subtype AH1; AH3, influenza virus subtype AH3; B, influenza virus subtype B; InfV, influenza virus; AH1pdm, 2009 pandemic H1N1 influenza virus; HRV, human rhinovirus; RSV, repiratory syncytial virus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; AdV, adenovirus; EV, enterovirus.

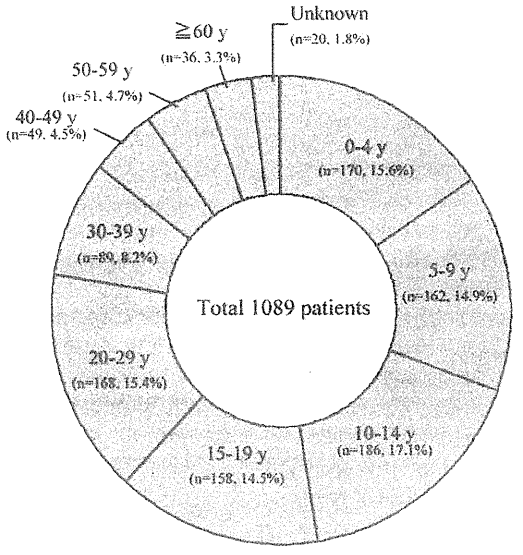

Fig. 2. The age groups of the patients who provided the samples collected in the study.

groups of the patients from whom samples were obtained, over 50% of whom were under 20 years of age, are shown in Fig. 2. The monthly detection rates of the various viruses are shown in Table 1. AH1 pdm and seasonal influenza viruses were detected in about 80% of patients, with AH1 pdm (68%) being the most prevalent virus detected. Non-influenza respiratory viruses were detected in 5% of patients, although none of the viruses tested were detected in about 16% of patients. Seasonal influenza viruses (subtype AH1, AH3, and B) were the most prevalent between January and June. AH1 pdm, together with AH3 and other viruses, was first detected in July, after which AH1 pdm was predominant. Non-influenza viruses were detected from January to October. Our previous report suggested that RSV circulated in Okinawa during the summer, whereas in other regions of Japan RSV is detected in autumn and winter (9). In addition, the endemic curve of ILI in Okinawa Prefecture in 2009 differed from the pattern observed in other regions of Japan (Fig. 1). A possible reason for this could be that Okinawa is located in a subtropical region, thus suggesting that the epidemiology of some respiratory viruses in Okinawa may be unique (10). Our findings suggest that the ILI outbreak and AH1 pdm occurred together with other non-influenza viruses, with non-influenza viruses being

detected throughout the year. The epidemiology of the various respiratory viruses in Okinawa is not currently known. However, these findings show the sustained impact of non-influenza viruses on ILI even during the outbreak of the newly emerged pandemic influenza virus, and provided useful information to allow clinicians to make an accurate diagnosis. Further epidemiological studies based on systematic virus surveillance may, however, still be needed.

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Conflict of interest None to declare.

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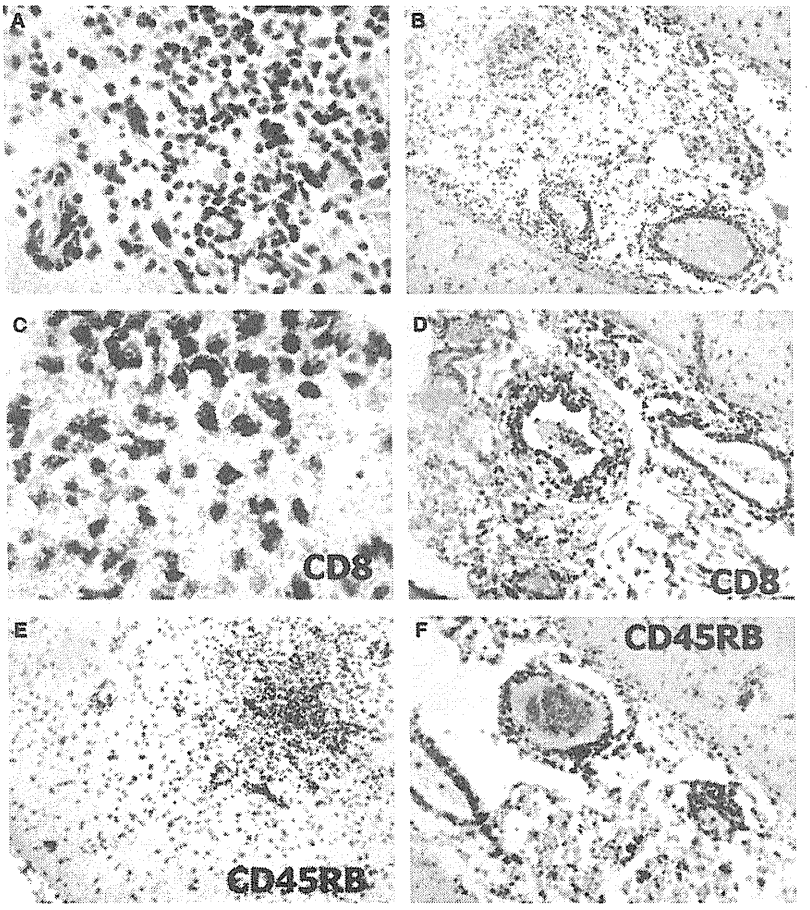

Figure 1 Brain histopathology shows multifocal perivascular infiltrations of lymphoplasmacytic cells in the cerebral cortex (A) and leptomeninges (B). The majority of the cells are $CD8^+$ lymphocytes (C and D), with a smaller component of CD45RB (E and F) and $CD3^+$ cells (not shown). On the H&E images (A and B), there are many cortical neurons that are surrounded by lymphocytes and show features of acute neuronal injury.

$CD8^+$ lymphocyte infiltration in the cerebral cortex and leptomeninges (Fig. 1). Cortical neurons were surrounded by lymphocytes but not neutrophils and showed the features of both neuronal injury and areas of astrogliosis. The majority of infiltrating lymphocytes expressed granzyme B, which may have mediated T-cell-dependent brain injury. The patient has become cachectic, immobile and unable to drink and eat and died at age 7.

PCR-based assays (serum and cerebrospinal fluid) failed to unveil potential causal agents for the disease. Unbiased pyrosequencing (4) used in Patient 2 failed to define virus-specific genetic markers in brain biopsy material. These findings provide further evidence that cytotoxic T cells play a critical role in the pathogenesis of PNG in patients with XLA (5). Viral sequences could not be identified in brain biopsy by metagenomic analysis, suggesting that causative agents other than astrovirus are to be defined. We propose that prospective, multicenter analysis by using standard high-throughput assays is needed to define the role of astroviruses or other potential agents that may cause this formidable neurological complication of patients with XLA.

Conflict of interest

The authors declare that they have no conflict of interest.

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Enterovirus 68 infection in children with asthma attacks: virus-induced asthma in Japanese children

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Keywords: asthma attack; bronchial asthma; children; Enterovirus 68.

Enterovirus (EV) 68, which belongs to the genus *Enterovirus*, a member of the Picornaviridae family, is associated with respiratory illnesses such as upper respiratory infections and bronchiolitis (1). However, the relationship between EV68- and other virus-induced asthma is not clear. In this study, EV68 was the only virus detected in a group of hospitalized patients suffering from asthma attacks, suggesting a strong association between EV68 infection and exacerbation of asthma.

Enterovirus 68 infection induces asthma attack in Japanese children with bronchial asthma.

This study included 35 children [male:female (M:F) = 23 : 12, mean age ± standard deviation (SD): 3.5 ± 2.6 years] with history of asthma who were admitted to the Department of Pediatrics, Yamaguchi University Hospital, between July and September 2010. Nasopharyngeal samples were collected from all patients and examined by reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify specific genes followed by nucleotide sequence determination. The RNA of EV68 was detected in the nasopharyngeal samples of 26 (74.3%) patients with asthma attacks. Table 1 provides a summary of the clinical features of the children who presented with asthma attacks and EV68 infections. Among these children, the severity distribution of the asthma attacks was as follows: mild, 1 (3.8%); moderate, 14 (53.8%); severe, 11 (42.3%). Among 17 children (65.4%) with history of asthma or wheezing, the severity distribution was as follows: severe persistent, 1 (3.8%); mild persistent, 1 (3.8%); intermittent, 15 (57.7%). In addition, nine children (34.6%) had no history or diagnosis of wheezing or asthma. Furthermore, only two children with long-term management of bronchial asthma were included in the study. Children who had asthma attacks during EV68 infection presented with low-grade fever, low SpO_2, elevated peripheral blood WBC count, and slightly elevated serum C-reactive protein (CRP) (Table 1). All 26 children showed elevated levels of serum total IgE (27–5840 IU/ml, mean: 1117.2) (Table 1).

The results of our previous study, which characterized all children with asthma attacks admitted to our hospital, were as follows: mild, 9.7%; moderate, 75.7%; severe, 14.3%; and respiratory failure, 0.3% (2). Among the children with EV68 infection and asthma attacks in the present study, a significantly high percentage of children experienced a severe attack (42.3%). These results suggest that EV68 infection is more likely to induce a severe asthma attack than other infections or allergens, similar to previous findings with pandemic H1N1 2009 (3). EV68 infection may induce severe asthma attacks regardless of pre-existing asthma severity.

The results of our study suggest that untreated asthmatic children may be at higher risk than children who receive long-term asthma management. However, a limitation of this study was the inclusion of only those patients who had EV68 infection and were admitted to the hospital. Further assessment of the effect of EV68 infection on asthma attacks should include outpatient evaluations. An accurate evaluation of asthma severity and its correlation with the effect of long-term treatment on decreasing the risk of EV68 infection-induced severe asthma attacks should be performed.

Two reports have shown that EV68 induced severe pneumonia in Osaka, Japan, and the Philippines (4, 5). Findings from these studies and our study suggest that EV68 infection may induce a small-scale epidemic, but not a worldwide pandemic such as that caused by certain influenza viruses.

In conclusion, EV68 infection can easily induce a severe asthma attack in atopic children without a history of asthma or treatment. Our results also suggest that the severity of bronchial asthma does not correlate with the severity of EV68 infection-induced asthma attacks.

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Table 1 The summary clinical features of children with EV68 positive asthma attacks

Age (years)	Gender	Severity of attack	History of asthma	Long-term treatment	Body temperature (°C)	SpO_2 (%)	WBC (per mm^3)	CRP (mg/dl)	IgE (IU/ml)	Abnormal CX-P
4.0 ± 2.7	M: 19 F: 7	Mild: 1 Moderate: 14 Severe: 11	None: 9 Intermittent: 15 Mild persistent: 1 Severe persistent: 1	2/26 (7.7%)	37.6 ± 0.6	91.4 ± 3.1	13 850 ± 4804	1.76 ± 2.26	1117.2 (27–5840)	4/26 (15.4%)

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Storage mites are the main sensitizers among adults in northern Vietnam: Results from a population survey

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Keywords: Allergic sensitization; skin prick tests; storage mites; Vietnam.

The prevalence of allergic diseases has increased in the Western world over at least the past 50 years (1). An increasing trend in allergic diseases has been observed also in Southeast Asia (2). The profile of sensitization and the association with allergic diseases are different among areas of the world and reflect the allergen exposure. Worldwide, mites are the most common sensitizers (3, 4). Cockroach is another important sensitizer and its impact on disease may be increasing (3, 4). Still few studies have investigated the prevalence, sensitization profile and risk factors for allergic sensitization in Southeast Asia, and no study has been performed in the northern part of Vietnam. We thus conducted a study with the aim to assess the prevalence of allergic sensitization and its association with asthma and rhinitis in northern Vietnam.

The most common airborne sensitizer in northern Vietnam is Blomia Tropicalis.

The study was performed in two areas of northern Vietnam: urban Hoankiem, Hanoi, and rural Bavi 60 km west of central Hanoi. A sample of 1500 subjects aged 21–70 years was randomly selected from all 5782 responders of a questionnaire survey conducted in 2007–2008 (5). The subjects underwent a structured interview using a modified GA^{2}LEN study questionnaire and skin prick test (SPT) with 10 common local allergens. SPTs were performed following the standards developed by the EAACI (6) using a lancet on the volar aspect of the forearm. A wheal size ≥ 3 mm was regarded positive. Definitions of asthma, rhinitis, symptoms and possible risk factors have been published previously (5). The study was approved by the Medical Ethics Research Committee of Hanoi 'Medical University and was performed from March 2009 to April 2010.

Data from the initial questionnaire survey (5) were used for evaluating the representativeness of the participants of the clinical study. PASW version 18.0 (Chicago, IL, USA) was used for statistical analysis. Difference in prevalence was calculated using chi-squared test, and a P-value < 0.05 was considered statistically significant. Risk factors for allergic sensitization were expressed as odds ratios (OR) with 95% confidence intervals (CI) and calculated by multiple logistic regression analysis.

Of the invited subjects, 684 (46%) participated in the clinical examinations. There were no significant differences in symptoms or diagnosed asthma or allergic rhinitis between those who had participated in the questionnaire survey and those who attended the clinical examinations.

Of the 533 subjects who underwent SPT, 33.8% had a positive SPT to at least one allergen, similar in men and women, and 29.8% were sensitized to any mite or cockroach. Sensitization to any animal, any pollen or moulds contributed with only 4% units to the total prevalence of allergic sensitization. The most common sensitizers were the storage mite *Blomia tropicalis*, *Dermatophagoides pteronyssinus*, cockroach and *Dermatophagoides farinae* (Table 1). Allergic sensitization to at least one allergen was strongly associated with allergic rhinitis and less so, however significantly, with asthma and recurrent wheeze.

In multivariate analysis, young age was associated with a significantly increased risk of having a positive SPT to any mite, and to any allergen; further, male sex was associated with an increased risk of having a positive SPT to cockroach. Occupational exposure to gas, dust or fumes was associated with a significantly increased risk of being sensitized to any mite. However, although significant, the associations were generally weak (mostly OR < 2). In the multivariate setting, sensitization was not dependent on urban living.

In conclusion, mites, particularly *Blomia tropicalis*, and cockroach were

Table 1 Prevalence (%) of allergic sensitization to common airborne allergens in northern Vietnam

Allergen	Prevalence (%)
Blomia tropicalis	22.9
Dermatophagoides pteronyssinus	13.3
Cockroach	13.1
Dermatophagoides farinae	10.5
Cat	4.5
Dog	3.8
Grass mix	2.1
Tree mix	1.1
Cladosporium	0.9
Alternaria	0.8

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First Detection of Measles Virus Genotype G3 in a Japanese Woman: an Imported Case

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Since 2007, the number of measles patients in Japan had continued to decrease because of regular and widespread measles immunization program (1). However, 450 cases of measles including the suspected cases were reported in 2010 (1). Epidemiological data suggests that most of these cases were imported into Japan, but domestic cases have also been reported (1). Recent molecular epidemiological studies reported the detection of measles virus (MV) genotypes D3, D4, D5, D9, and H1 in Japan (2–4). The D4 and D9 genotypes have usually been detected in imported cases, while the D3, D5, and H1 genotypes have been detected in domestic cases (2–4). Here, we describe the detection of another genotype, G3, in an imported case of measles in a Japanese woman. To the best of our knowledge, this is the first report on the detection of MV genotype G3 in Japan.

The patient was a 28-year-old Japanese woman who resided in Chiba Prefecture, Japan. She did not have a history of measles and had not been immunized against measles. She had visited Indonesia for 10 days (from January 31 to February 9, 2011) with six colleagues. On February 14, she developed common cold-like symptoms such as cough and shivering, and consulted a local physician, who made a diagnosis of common cold. On February 22, she developed clinical symptoms including high fever (39°C), cough, conjunctivitis, Koplik's spots, and rashes on the face and neck. She then consulted another physician at a general hospital. The physician suspected her to have contracted measles, and suggested that she got admitted to the hospital. Informed consent was obtained, and her whole blood sample was collected on the next day. Viral RNA was extracted from the blood sample using the High Pure Viral RNA Kit (Roche, Indianapolis, Ind., USA), and was suspended in DNase/RNase-free water. Thereafter, reverse transcriptase-polymerase chain reaction (RT-PCR) and nested PCR were performed as previously described (2–4). Amplicons were purified using the High Pure PCR Product Purification Kit (Roche), and the nucleotide sequence was determined using direct se-

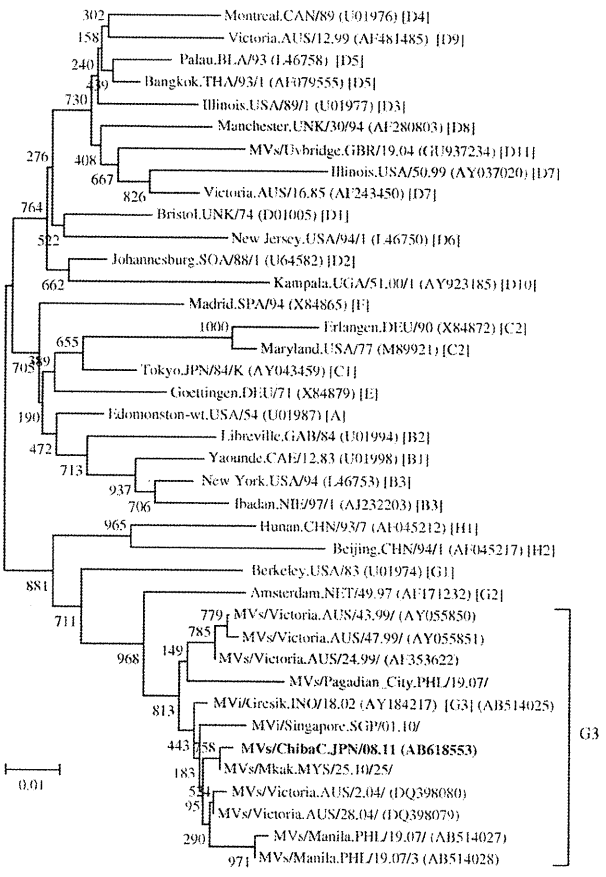

Fig. 1. Phylogenetic tree based on the nucleotide protein (*N*) gene sequences of various strains of the measles virus. The evolutionary distance was calculated using Kimura's two-parameter method, and the tree was plotted using the neighbor-joining method. Numbers at each branch indicate the bootstrap values of the clusters supported by that branch. Genbank accession numbers are given in parentheses. The present strain is represented in bold type.

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quencing method (4). The nucleotide sequence of the partial *N* gene (450 bp) of MVs was analyzed phylogenetically using Molecular Evolutionary Genetics Analysis (MEGA) software (version 4) (2–4). Evolutionary distances were estimated using Kimura's two-parameter method and the phylogenetic tree was constructed using the neighbor-joining (NJ) method (2–4). Reliability of the phylogenetic tree was estimated by 1,000 bootstrap replications.

We constructed a phylogenetic tree based on the *N* gene of the detected MV strain and the reference strains (Fig. 1). The strain was genotyped as MV G3 in the phylogenetic tree. The homology between the reference strain (MVi/Gresik.INO/18.02 [G3], GenBank accession no. AY184217) and the present strain was 99.1% at the nucleotide level and 98.7% at the amino acid level. Epidemiological investigations have not reported occurrence of measles among the patient's family and colleagues.

To the best of our knowledge, this is the first report on MV G3 detection in Japan. The genotype G3 was first detected in Australia and East Timor in 1999 (5). Infection with G3 has not been frequently reported in these countries after 1999. However, this may be attributed to the lack of aggressive MV surveillance in these countries. At present, a small number of the population in Chiba Prefecture may be susceptible to measles because of lack of immunization against the disease. However, as measles is highly contagious in humans (6), and spreads rapidly from one area to another, up-to-date information on the epidemiological status of this disease in our country is needed.

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Conflict of interest None to declare.

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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-

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Table 1. Patient and human metapneumovirus data

Patient	Age (y)	Sex	Diagnosis	Onset date	Sampling date	Strain	Subgroup	GenBank accession no.
1	65	F	Pneumonia	14 Oct. 2009	23 Oct. 2009	Shimane 09-17	B2	AB594742
2	55	M	Pneumonia	16 Oct. 2009	23 Oct. 2009	Shimane 09-18	B2	AB594743
3	69	M	Fever, Cough	17 Oct. 2009	23 Oct. 2009	Shimane 09-16	B2	AB594741
4	56	F	Fever, Cough	20 Oct. 2009	23 Oct. 2009	Shimane 09-15	B2	AB594740
5	65	F	Pneumonia	21 Oct. 2009	23 Oct. 2009	Shimane 09-19	B2	AB594744
6	56	F	Fever, Cough	22 Oct. 2009	23 Oct. 2009	Shimane 09-14	B2	AB594739
7	61	M	Fever, Cough	29 Oct. 2009	30 Oct. 2009	Shimane 09-20	B2	AB594745

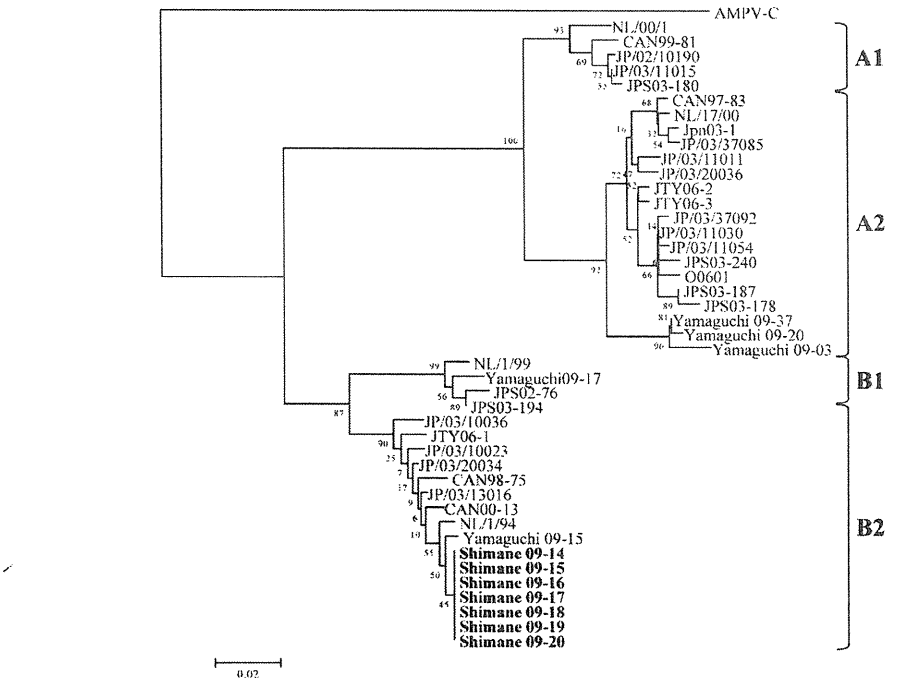

Fig. 1. Phylogenetic tree constructed on the basis of partial sequences of the human metapneumovirus *F* gene. Distance was calculated using Kimura's two-parameter method, and the tree was plotted using the neighbor-joining method. Numbers above the branches are bootstrap probabilities (%). Reference 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), Jpn03-1 (AB503857), JP/03/37085 (AB119485), JP/03/11011 (AB113371), JP/03/20036 (AB126612), JTY06-2 (EU127918), JTY06-3 (EU127919), JP/03/37092 (AB119486), JP/03/11030 (AB119489), JP/03/11054 (AB119491), JPS03-240 (AY530095), O0601 (EF589610), JPS03-187 (AY530093), JPS03-178 (AY530091), Yamaguchi 09-37 (AB533251), Yamaguchi 09-20 (AB533245), Yamaguchi 09-03 (AB533239), NL/1/99 (AY304361), Yamaguchi09-17 (AB533244), JPS02-76 (AY530089), JPS03-194 (AY530094), JP/03/10036 (AB126611), JTY06-1 (EU127917), JP/03/10023 (AB126608), JP/03/20034 (AB119493), CAN98-75 (AY297748), JP/03/13016 (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.

ple with severe ARIs.

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Conflict of interest None to declare.

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