

Detection of Novel Respiratory Viruses From Influenza-Like Illness in the Philippines

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Several novel viruses have been recently identified in respiratory samples. However, the epidemiology of these viruses in tropical countries remains unclear. The aim of the present study was to provide an overview of the epidemiology of novel respiratory viruses, including human metapneumovirus, human bocavirus, new subtypes of human coronavirus (NL63 and HKU1), KI virus, WU virus, and Melaka virus in the Philippines, a tropical country. Nasopharyngeal aspirates from 465 patients with influenza-like illness were collected in 2006 and 2007. Reverse transcription polymerase chain reaction (RT-PCR) and PCR were performed to detect viruses from culture-negative specimens. Human metapneumovirus, human coronavirus HKU1, KI virus, and WU virus were detected for the first time in the Philippines; Melaka virus was not found. **J. Med. Virol.** 82:1071–1074, 2010. © 2010 Wiley-Liss, Inc.

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were identified in respiratory samples. However, it is difficult to assess the pathogenic role of these viruses [Sloots et al., 2008].

Furthermore, a reovirus unknown previously, named Melaka virus (MelV), was identified from a patient in Melaka, Malaysia, who had been suffering from high fever and acute respiratory disease in 2007 [Chua et al., 2007]. The detection of this virus has only been reported in Malaysia and Indonesia [Chua et al., 2007; Iwakiri et al., 2008], although the global epidemiology of MelV is still unclear.

A number of studies have shown the prevalence, seasonal distribution, and/or significance of novel respiratory viruses in different communities, mostly in developed countries in the temperate zones [Chano et al., 2005; Sasaki et al., 2005; Choi et al., 2006; Kaida et al., 2006; Kesebir et al., 2006; Weissbrich et al., 2006; Abed et al., 2007; Han et al., 2007; van der Zalm et al., 2008]. However, the epidemiology of the infections caused by respiratory viruses identified recently in tropical countries is still unclear. Their seasonal distribution might differ between temperate and tropical zones [Shek and Lee, 2003].

The aim of the present study was to provide an overview of the epidemiology of novel respiratory viruses in the Philippines.

INTRODUCTION

Respiratory infections are one of the most common causes of death in children under 5 years of age [Lopez et al., 2006]. Influenza virus, respiratory syncytial virus (RSV), and rhinovirus are known to be common pathogens of acute respiratory infection. Recently, novel viruses such as human metapneumovirus (hMPV) [van den Hoogen et al., 2001] and new subtypes of human coronavirus [HCoV NL63, van der Hoek et al., 2004 and HCoV HKU1, Woo et al., 2005] were identified by molecular techniques and are considered to be the causative agents of acute respiratory infection. Similarly, human bocavirus (HBoV) [Allander et al., 2005] and polyomaviruses unknown previously and named provisionally KI polyomavirus (KIV) and WU polyomavirus (WUV) [Allander et al., 2007; Gaynor et al., 2007]

Experimental ethics: The study was approved by the Institutional Review Boards in Tohoku University, Japan and Research Institute for Tropical Medicine, the Philippines.

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MATERIALS AND METHODS

Specimens

The Research Institute for Tropical Medicine in Alabang, Muntinlupa, the Philippines, has been implementing influenza surveillance and collecting nasopharyngeal aspirates from patients exhibiting influenza-like illnesses at sentinel health care facilities. Demographic patient information was also collected. Among these sentinel sites, the Tunasan Healthcare Center in Muntinlupa was selected as a study site for the years 2006 and 2007.

Nasopharyngeal aspirates were collected and transported to the Research Institute for Tropical Medicine. They were inoculated into MDCK and HEp-2 cells. Viral cultures were performed to detect influenza A and B viruses, adenovirus, enterovirus, herpes simplex virus type 1 (HSV-1), parainfluenza viruses 1 and 3, rhinovirus, and RSV. The viral cultures were then confirmed by hemagglutination inhibition tests, neutralization tests, and/or immunofluorescence staining.

RT-PCR/PCR

Specimens of nasopharyngeal aspirates from which virus was not isolated by culture were tested by reverse transcription polymerase chain reaction (RT-PCR) and PCR. RT-PCR/PCR assays were done to detect hMPV, HCoV, HBoV, WUV, KIV, and MeIV. Briefly, RNA was extracted from stored samples using the PureLink Viral RNA/DNA MiniKit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Viral RNA was reverse transcribed to complementary DNA by Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase with random hexamer primers. A standardized PCR protocol was followed to amplify the hMPV matrix protein genes [Bellau-Pujol et al., 2005], the NP1 genes of HBoV [Allander et al., 2005], the ORF 1b of the polymerase genes of all known coronaviruses (including those newly discovered) [Moes et al., 2005], and the VP2 gene of WUV and KIV [Norja et al., 2007]. Information on the primer for MeIV was kindly provided by Dr. Linfa Wang (CSIRO's Australian Animal Health Laboratory). All amplified products were then separated on agarose gels. PCR-amplified fragments were sequenced using BigDye Terminator version 1.1 (Applied Biosystems, Foster City, CA) and analyzed using Applied Biosystems

3130 Genetic Analyzer (Applied Biosystems) to confirm the results. Viruses were analyzed phylogenetically using Molecular Evolutionary Genetics Analysis (MEGA) software version 4 [Kumar et al., 2008]. Phylogenetic trees were constructed using the neighbor-joining method.

RESULTS

Clinical specimens were collected between January 2006 and December 2007 (Table I). Four hundred sixty-five specimens were tested and viruses were isolated by culture from 54 (11.6%) samples. Utilizing the viral culture technique, influenza virus (n = 24), adenovirus (n = 13), enterovirus (n = 5), HSV-1 (n = 4), parainfluenza virus (n = 5), rhinovirus (n = 1), and RSV (n = 2) were isolated.

Four hundred eleven samples were subjected to the RT-PCR and PCR. Two hundred two of those samples (49.1%) were collected from males and 209 (50.9%) from females. The age of the patients ranged from 1 month to 54 years, with a median of 4.5 years.

Table II shows the results of virus detection by RT-PCR and PCR. Samples positive for hMPV (n = 2) were collected from 1-year-old patients in October. By phylogenetic analysis, they were categorized into group A2 described by a published study [van den Hoogen et al., 2004a] (data not shown). Samples positive for HBoV (n = 6) were collected from patients between 4 months and 6 years of age. These specimens were collected between January and July. Phylogenetic analysis showed that the strains in the Philippines are genetically related to each other (data not shown).

Positive samples (n = 3) for HCoVs (2 for HCoV HKU1 and 1 for HCoV OC43) were collected in May and June. Samples positive for KIV (n = 2) and WUV (n = 6) were detected in patients from 9 months to 29 years of age. They were collected between January and July. None of the samples were found to be positive for MeIV. Samples coinfecting with two or more novel viruses were not detected by RT-PCR/PCR.

DISCUSSION

In this study, hMPV, HBoV, HCoV HKU1, KIV, and WUV were found in patients with influenza-like illness

TABLE I. Distribution of Viruses (Isolated by Viral Culture)

	January	February	March	April	May	June	July	August	September	October	November	December	Total
Influenza virus (A/H1N1)	0	0	0	0	0	6	5	2	0	0	0	0	13
Influenza virus (A/H3N2)	0	0	0	0	0	0	0	0	1	1	1	2	5
Influenza virus (B)	0	0	0	0	0	0	0	0	5	0	1	0	6
Adenovirus	1	1	2	1	4	2	1	0	1	0	0	0	13
Enterovirus	0	1	0	0	0	1	2	0	0	0	1	0	5
HSV-1	0	0	0	0	0	2	1	0	0	0	0	1	4
Parainfluenza virus 1	0	0	0	0	0	1	2	1	0	0	0	0	4
Parainfluenza virus 3	0	1	0	0	0	0	0	0	0	0	0	0	1
Rhinovirus	0	1	0	0	0	0	0	0	0	0	0	0	1
RSV	0	0	0	0	0	0	0	0	0	0	0	2	2
Negative	56	49	24	14	28	30	48	34	40	25	34	29	411
Total	57	53	26	15	32	42	59	37	47	26	37	34	465

TABLE II. Distribution of Novel Viruses (Detected by RT-PCR and PCR)

Number of cases, n (%)	Type	Age ^a	Sex	Collected month	Year
hMPV 2 (0.5%)		1Y	M	October	2006
		1Y	M	October	2006
HBoV 6 (1.5%)		1Y	M	January	2006
		6Y	M	January	2007
		4M	M	February	2007
		7M	F	May	2006
		9M	M	July	2006
HCoV _s 3 (0.7%)	HKU1	8M	F	May	2007
	OC43	9M	M	May	2007
	HKU1	2Y	F	June	2007
KIV 2 (0.5%)		9M	M	July	2007
		2Y	F	July	2007
WUV 6 (1.5%)		5Y	M	January	2007
		1Y	F	February	2006
		7Y	F	February	2007
		29Y	M	May	2007
		10Y	M	June	2007
MelV 0 (0%)		—	—	—	—
		3Y	M	July	2007

^aAge is described in months-old (M) or years-old (Y).

symptoms in the Philippines. Notably, this is the first reported detection of these viruses in the Philippines.

The demographic pattern of hMPV- and HBoV-positive cases in this study is similar to that reported in several published reports [van den Hoogen et al., 2004b; Arnold et al., 2006; Kesebir et al., 2006; Heikkinen et al., 2008], which showed that most of these infections are seen in infants and children. Positive samples for hMPV and HBoV were collected from patients less than 2 years of age, except for one 6-year-old. This finding suggests that hMPV and HBoV are prevalent in children under 2 years of age, as in temperate zones.

In temperate countries, specific seasonal distribution of hMPV and HBoV has been reported: hMPV and HBoV infection peaks are reported from late winter to spring [Kaida et al., 2006; Kesebir et al., 2006; Weissbrich et al., 2006]. In the present study, two hMPV-positive specimens were collected in October. HBoV-positive specimens were collected from January to July. HCoV_s, including OC43 and HKU1, circulate during winter in the temperate zone [Vabret et al., 2003; Gerna et al., 2007] but OC43- and HKU1-positive samples were detected in May and June. Therefore, the current study is not in complete agreement with previous reports. These findings suggest that seasonal distribution of these viruses in tropical countries might differ from the seasonal distribution in temperate zones. However, it is not possible to determine the seasonal distribution of detected viruses by observing the situation for only two consecutive years.

In most reported cases, WUV and KIV infections were detected in children under 3 years of age and were rarely seen in immunocompetent adults [Gaynor et al., 2007; Bialasiewicz et al., 2008; Ren et al., 2008]. But, in eight WUV and KIV positive samples in the study, four belonged to patients over 3 years of age (5, 7, 10, and 29 years old). Unfortunately, the detailed clinical data of these cases were missing. WUV was found from January to July and KIV was found in July. The seasonal distribution of WUV and KIV has not yet been determined in temperate countries [Han et al., 2007; Norja et al., 2007; Ren et al., 2008].

Disparity exists in the regional distribution of MeIV. For example, it has been found in clinical specimens in Malaysia and Indonesia [Chua et al., 2007; Iwakiri et al., 2008], but it has also been reported as negative in a group of European children with respiratory diseases [Schildgen et al., 2008]. MeIV is likely to spread only amongst countries in the same geographical neighborhood. In the present study, MeIV was not found in the Philippines, although the Philippines is situated in close proximity to Malaysia and Indonesia. This suggests that MeIV infection might occur endemically in a very narrow region.

Samples coinfecting with two or three viruses were not detected in this study. However, several studies have reported this common phenomenon [Han et al., 2007; Le et al., 2007; Bialasiewicz et al., 2008]. This study did not test for coinfection by novel viruses and commonly found viruses. Also, the sample size in this study might not have been sufficient to observe such a phenomenon.

In conclusion, novel viruses, including hMPV, HBoV, HCoV HKU1, KIV, and WUV, exist in the Philippines. Additionally, their seasonal distribution may differ from that observed in temperate zones. Furthermore, the findings lay the groundwork for large-scale, multicenter surveillance in tropical countries to obtain a broader picture of the viral epidemiology of acute respiratory infection.

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