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Molecular diagnosis of enterovirus infections in Chungnam Korea in 2010

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The epidemiological data of enteroviruses isolated in Chungnam, Korea since 2005 has been reported, and this current study extends these observations in 2010. During this period, 178 of the total 920 samples (19.3%) were positive for enterovirus, and the types were identified using nucleotide sequence analysis in VP1. The temporal distribution was similar to previously reported data, however, coxsackievirus A6 and coxsackievirus B5 showed different patterns in detail. Children aged < 1 year accounted for 89 (50%) of the total 178 isolates. Most coxsackievirus A6, coxsackievirus A9 and enterovirus 71 isolates were identified in children < 5, < 6 and < 4 years old, respectively. CVB5 was distributed evenly in children < 7 years old. Enterovirus serotypes were identified by sequence determination of the VP1 region, which resolved the isolated enteroviruses into 16 types in 2010; coxsackievirus A4, A5, A6, A9, A10, A12, A16, B2, B3, B4, B5, echovirus 17, 18, 25, 30 and enterovirus 71. The prevalent enterovirus types were coxsackievirus A6 (14.6%), B5 (15.7%), and enterovirus 71 (15.7%). This is the first time E17 has been identified in Korea since surveillance began in 1993. The presentations of enterovirus-infected patients were respiratory illness (30.3%), septicemia (19.7%), central nervous system infections (16.3%), gastrointestinal infections (11.2%), herpangina or hand-foot-mouth disease (10.7%), and genitourinary (5.6%).

Key words: Enterovirus, reverse transcriptase-polymerase chain reaction (RT-PCR), epidemiology, molecular type.

INTRODUCTION

The human enteroviruses (HEV) comprise a large genus belonging to the *picornaviridae* family. HEV types are distinguished by antigenic and genetic sequence differences, and newer HEVs are classified by numbering.

On the basis of nucleotide and amino acid sequences of the viral protein genes, HEVs have been subgrouped into four species named HEV-A to HEV-D by the International Committee for taxonomy of Viruses (http://talk.ictvonline.org and http://www.picornaviridae.com). Although 110 additional types have been described, several types account for the majority of disease. No HEV diseases are uniquely associated with any specific type, although certain manifestations are preferentially associated with specific types (Kim, 2010). Most HEV infections are asymptomatic or result in only mild symptoms, for example, nonspecific febrile illness or mild upper respiratory symptoms. In addition, HEV infections may cause a wide variety of other clinical illness, including aseptic meningitis, hand-foot-mouth disease (HFMD), acute hemorrhagic conjunctivitis, myocarditis, encephalitis, undifferentiated rashes, neonatal sepsis-like disease, poliomyelitis, and so on (Chang et al., 1998; Thoelen et al., 2003; Saitoh et al., 2004; Cha, 2008). The most commonly encountered illness associated with HEV infections occurs in young children, and often appears in the form of outbreaks (Lee and Davies, 2007). In Korea, HEV epidemics typically peak during the summer and early fall, and various serotypes are often associated with a single outbreak. The predominant HEV types vary from year to year, with echovirus (E) 6, 9, 13, 18, 30, coxsackievirus A (CVA) 16, coxsackievirus B (CVB) 1, 5, and enterovirus A71 (EV-A71) being the most frequently isolated in Europe and the United States over the past few years (Chomel et al., 2003; Khetsuriani et al., 2006; Jacques et al., 2008; CDC, 2010; Khanh et al., 2012; Qiaoyun et al., 2013; Smura et al., 2013). Since 1993, when nationwide surveillance began in Korea, there had been reports of summer outbreaks of enteroviruses, involving E5, 6, 7, 9, 13, 18, and 30, CVA 24, CVB 3 and 5, and EV-A71 (Jee et al., 2004; Baek et al., 2009, 2011; Kim and Baek, 2011). Laboratory detection of HEVs was performed by the gold standard method of real-time reverse transcription- polymerase chain reaction (RT-PCR) in the 5' non-coding region (NCR) (Watkins-Riedel et al., 2004). In addition, conventional RT-PCR based on amplification of the VP1 region was developed to identify the HEV serotype (Oberste et al., 1999; Nix et al., 2006). The partial VP1 nucleotide sequences were compared with a database of complete HEV VP1 sequences of all serotypes to determine whether the isolates were related genetically to any known HEV serotype. In this study, HEVs from hospitalized patients with HEV-related diseases were detected and identified using RT-PCR and sequence analysis, the epidemiological features by HEV types were determined in Chungnam Korea in 2010.

MATERIALS AND METHODS

A total of 920 clinical samples were obtained from pediatric patients (children aged under 16) whose symptoms such as aseptic meningitis, respiratory illness, acute gastroenteritis, herpangina, HFMD were consistent with HEV infections admitted to pediatric hospitals in Chungnam, Korea in 2010. Samples of central nervous system (CNS) infections such as aseptic meningitis were derived from cerebrospinal fluid (CSF), and samples of respiratory illness were taken by throat swabs. Samples of other symptoms were taken from stools.

The CSF and throat swab samples were used directly, and fecal specimens were diluted to 10% suspensions with phosphate-buffered saline and clarified by centrifugation at 8,000 X g for 15 min. Viral RNA was extracted from the specimens using a viral nucleic acid prep kit (Greenmate Biotech, Seoul, Korea), according to the manufacturer's instructions. The extracted RNA was dissolved in 50 µL of nuclease-free water and stored at -80°C until used for RT-PCR. It was assayed for HEVs in clinical samples using an *AccuPower*® Enterovirus Real-Time RT-PCR Kit (Bioneer, Daejeon, Korea) based on 5' NCR of a highly conserved region in the HEV genome, according to the manufacturer's instructions. Positive samples were subjected to semi-nested RT-PCR in the VP1 coding region for molecular typing as described previously (Nix et al., 2006). Next, the VP1 amplicons generated by semi-nested RT-PCR were sequenced by using internal primer sets.

The molecular type of each isolate was determined by the serotype of the highest scoring strain in GenBank using the Basic Local Alignment Search Tool (BLAST); that is, the sequence of the HEV strain that gave the highest nucleotide similarity value with the query sequence (Oberste et al., 2000).

RESULTS

In 2010, a total of 920 samples obtained from patients with various HEV-related diseases were subjected to a diagnostic real-time RT-PCR corresponding to a highly conserved domain in the 5' NCR. A total of 178 HEV isolates were detected from 920 cases (19.3%) in 2010. For molecular typing, the VP1 amplicons generated in the semi-nested PCR were sequenced and were determined to correspond with a 372-bp VP1 region. Gapped Basic Alignment Search Tool (BLAST) analyses were carried out, and each virus was assigned to the type that gave the highest VP1 identity score. The four most common HEV types (CVB5, EV71, CVA6 and 9) accounted for 100 (56.1%) of the total isolates and HEV detections had prominent summer to fall seasonality, with June to September accounting for 137 (76.9%) of the total 178 isolates in 2010 (Table 1). In detail, CVA 6 was detected in May and June; CVB 5 was mainly detected in July and September. The CVA 9, 10 and EV-A71 were isolated

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Abbreviations: HEV, Human enteroviruses; **HFMD,** hand-foot-mouth disease; **E,** echovirus; **CVA,** coxsackievirus A; **CVB,** coxsackievirus B; **EV-A71,** enterovirus A71; **RT-PCR,** reverse transcription-polymerase chain reaction; **NCR,** non-coding region; **CNS,** central nervous system; **CSF,** cerebrospinal fluid; **BLAST,** basic alignment search tool.

Table 1. The number of identified enteroviruses and temporal distribution of each enterovirus detected in Chungnam Korea in 2010.

Enterovirus		Monthly distribution								Total
		Apr	May	Jun	July	Aug	Sep	Oct	Nov	
	Coxsackievirus A4				3	4	1	1		9 (5.1%)
	Coxsackievirus A5								1	1 (0.6%)
	Coxsackievirus A6	3	10	10	1	2				26 (14.6%)
HEV-A	Coxsackievirus A10		1	3	6	2		4		16 (9.0%)
	Coxsackievirus A12			1		1				2 (1.1%)
	Coxsackievirus A16		1	2						3 (1.7%)
	Enterovirus A71	3	1	4	12	6	1	1		28 (15.7%)
	Coxsackievirus A9		3	3	6	5	1			18 (10.1%)
	Coxsackievirus B2				1	2	4	1	1	9 (5.1%)
	Coxsackievirus B3		2							2 (1.1%)
	Coxsackievirus B4					2	4	1		7 (3.9%)
HEV-B	Coxsackievirus B5	1	1		10	2	10	3	1	28 (15.7%)
	Echovirus 17		1	1						2 (1.1%)
	Echovirus 18				1	6	3		1	11 (6.2%)
	Echovirus 25			2	4	7	2			15 (8.4%)
	Echovirus 30							1		1 (0.6%)
Total		7 (3.9%)	18 (10.1%)	26 (14.6%)	46 (25.8%)	39 (21.9%)	26 (14.6%)	12 (6.7%)	4 (2.2%)	178 (100%)

in July, and E18 and 25 were detected in August most frequently. Children of less than 1 year old accounted for 89 (50%) of the total 178 isolates. Most CVA6, CVA9 and 6 and < 4 years old, respectively. CVB5 wasdistributed evenly in children < 7 years old relatively (Figure 1). The major clinical presentations of each HEV-infected patient are summarized in Table 2. Among 178 HEV–positive cases, 30.3% (n = 54) had respiratory infections such as bronchiolitis, pneumonia and asthma, 19.7% (n = 35) had septicemia that was sepsis-like and 16.3% (n = 29) had CNS infections such as aseptic meningitis. The cases of gastrointestinal infection, such as diarrhea, were 11.2% (n = 20), herpangina or HFMD cases were 10.7% (n = 19), cases of genitourinary infection, such as nephritis and pyelonephritis, were 5.6% (n = 10). The major clinical presentations of CVA6-infected patients were respiratory illness and herpangina-HFMD, and the clinical symptoms of CVA9-infected cases were CNS and respiratory disease. The main clinical presentation of patients infected with CVA10 and E18 was septicemia, and the most common clinical symptom of CVA10 and E25 infections was respiratory illness. The major clinical presentations of CVB5-infected patients were diseases associated with the CNS and respiratory tract, and all patients with CVA16 infections had HFMD. In EV-A71, the clinical presentations of infected patients were variously distributed (Table 2). Of the total 178 isolates, 111 were from males and 67 were from females, giving a male-to-female ratio of approximately 1.66:1.

DISCUSSION

The epidemic occurrence of HEVs isolated in Chungnam, Korea has been reported since 2005 (Baek et al., 2009, 2011). The present study extended these findings by investigating the epidemic pattern in 2010. The predominant serotypes vary from year to year, and HEV epidemics in Korea may be affected by environmental shifts induced by climate change due to global warming (Baek et al., 2009, 2011). Therefore, systemic, long-term epidemiological studies should be conducted to prevent the spread of HEVs and to identify proper anti-viral

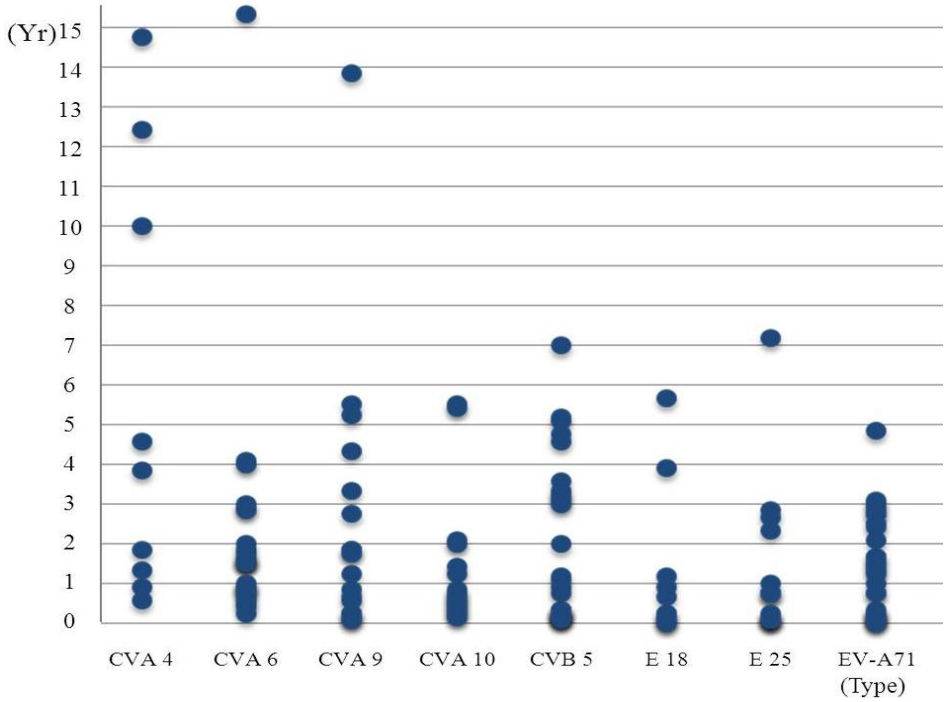

Figure 1. Distribution of patient age of enterovirus detection in Chungnam Korea in 2010.

therapies (Park et al., 2012). The prevalence types among HEV epidemic were different each year in Chungnam, Korea; CBV 5 and E18 in 2005, E5 in 2006, E6 and 30 in 2008, CVB1 and 5 in 2009, and CVA6, CVB5, and EV-A71 in 2010. This is the first time E17 has been identified in Korea since surveillance began in 1993 (Jee et al., 2004; Park et al., 2012). In particular, EV-A71 was the major prevalence type in Chungnam, Korea in 2010. Although recent reports of the outbreak of EV-A71 infection in several Asian countries, including Taiwan, China, and Malaysia, indicate that some EV-A71-infected patients have severe neurological complications (Cardosa et al., 2003; Yang et al., 2009; Ooi et al., 2007), there were no severe sequelae (neurologic dysfunction requiring assistance) cases among EV-A71-infected patients in this period of this study. Since 2005, the predominant CVB5 was detected most frequently in these periods (n = 70, 13.5%). Epidemics of HEV have been noted to peak in summer until early autumn in Korea (Jee et al., 2004; Baek et al., 2009, 2011). Totally, the results of the present study revealed a similar temporal distribution as found from 2005 to 2009; however, CVA6 and CVB5 showed a different pattern in detail. Generally, the peak age for children with HEV infection was < 1 year old, the results of the present study were in accordance with the general appearance. Long-term circulation patterns for individual serotypes varied but were consistent with the epidemic (for example, E9, E13, E30, and CVB5) or endemic patterns (for example, CVA9, CVB2, 4 and HEV-A71) (Khetsuriani et al., 2006). The age distribution by type was not significantly different, but the age of CAV4- and CVB5-infected patients was widely distributed. In the same area, because outbreaks of E18 in 2005 and E25 in 2006 occurred, the low infected age distributions of these viruses can be presumed. In this investigation, the major clinical presentations of HEV-infected patients were respiratory illness, septicemia, CNS infections, gastrointestinal disease, herpangina or HFMD, and genitourinary. In a previous study, HEVs were most often detected from patients with aseptic meningitis. However, it was many detected from patients with respiratory symptoms in this study. Since the symptoms of HEV infection are quite variable, surveillance for other presentations of HEV infection including aseptic meningitis may be required. For example, a case report of an acute nephritis patient with CBV infections and a report of HEV RNA shedding in the genital tract of childbearing-aged women were described (Papachristou and Printza, 2005; Jacques et al., 2006). Although, HEVs were isolated from the stools of patients with major clinical presentation of genitourinary and gastrointestinal infections, it was not possible to establish a causal link between the clinical symptoms and the presence of an HEV strain (Bessaud et al., 2012). Generally, EV-A71 infection tended to present with HFMD and meningoencephalitis (Ryu et al., 2010), however, EV-A71 was detected in patients with various clinical presentations in this study. No disease is uniquely associated with any specific type, although certain manifestations are preferentially associated with specific

Table 2. Distribution of clinical presentation of each enterovirus detected in Chungnam Korea in 2010.

Enterovirus		Clinical presentation							Total
		CNS infection	Respiratory infection	Genitourinary	Herpangina-HFMD	Gastrointestinal infection	Septicemia	Others	
HEV-A	Coxsackievirus A4		3	1	1	1		3	9
	Coxsackievirus A5						1		1
	Coxsackievirus A6		13	1	7	2	3		26
	Coxsackievirus A10	1	7	1	1	1	2	3	16
	Coxsackievirus A12				1	1			2
	Coxsackievirus A16				3				3
	Enterovirus A71	6	5	1	5	5	5	1	28
HEV-B	Coxsackievirus A9	5	8			2	2	1	18
	Coxsackievirus B2	1				2	6		9
	Coxsackievirus B3				1	1			2
	Coxsackievirus B4	1	3			1	2		7
	Coxsackievirus B5	9	6	4		2	4	3	28
	Echovirus 17	1	1						2
	Echovirus 18		2	1		1	7		11
	Echovirus 25	4	6	1		1	3		15
	Echovirus 30	1							1
Total		29(16.3%)	54(30.3%)	10(5.6%)	19(10.7%)	20(11.2%)	35(19.7%)	11(6.2%)	178

types. Generally, herpangina or HFMD were mainly caused by CVA16 and EV-A71, and all CVA16 was detected in HFMD in this study. CVA 6, 9, 10 and E25 were detected from patients associated with respiratory infection, CVB 5 and EV-A71 were detected from patients associated with CNS infection, and CVB2 and E18 were detected in septicemia patients. But, the association analysis of specific serotype and clinical presentations is very difficult, and further study is required to address this issue.

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REFERENCES

Baek K, Park K, Jung E, Chung E, Park J, Choi H, Baek S, Jee Y, Cheon D, Ahn G (2009). Molecular and epidemiological characterization of enteroviruses isolated in Chungnam, Korea from 2005 to 2006. J. Microbiol. Biotechnol. 19:1055-1064.

Baek K, Yeo S, Lee B, Park K, Song J, Yu J, Rheem I, Kim J, Hwang S, Choi Y, Cheon D, Park J (2011). Epidemics of enterovirus infection in Chungnam Korea, 2008 and 2009. Virol. J. 13(8):297.

Bessaud M, Pillet S, Ibrahim W, Joffret ML, Pozzetto B, Delpeyroux F, Gouandjika-Vasilache L (2012). Molecular characterization of human enteroviruses in the Central African Republic: Uncovering wide diversity and identification of a new human Enterovirus A71 genogroup. J. Clin. Microbiol. 50(5):1650-1658.

Cardosa MJ, Perera D, Brown BA, Cheon D, Chan HM, Chan KP, Cho H, McMinn P (2003). Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 and VP4 genes. Emerg. Infect. Dis. 9:461-468.

Centers for Disease Control and Prevention (CDC) (2010). Nonpolio enterovirus and human parechovirus surveillance - United States, 2006-2008. Morb. Mortal. Wkly. Rep. 59(48):1577-1580.

Cha SH (2008). Recently prevalent infectious diseases among children: Meningitis due to enteroviral infection. J. Korean Med. Assoc. 51:935-941.

Chang LY, Huang YC, Lin TY (1998). Fulminant neurogenic pulmonary oedema with hand, foot, and mouth disease. Lancet 352:367-368.

Chomel JJ, Antona D, Thouvenot D, Lina B (2003). Three ECHOvirus serotypes responsible for outbreak of aseptic. Meningitis in Rhone-Alpes region, France. Eur. J. Clin.

Microbiol. Infect. Dis. 22:191-193.
Jacques J, Bélec L, Moret H, Grésenguet G, Longo Jde D, Chomont N, Brodard V, Matta M, Graesslin O, Andréoletti L (2006). Enterovirus RNA shedding in the genital tract of childbearing-aged women living in Central Africa. J. Med. Virol. 78:591-597.
Jacques J, Moret H, Minette D, Leveque N, Jovenin N, Deslee G, Lebargy F, Motte J, Andreoletti L (2008). Epidemiological, molecular, and clinical features of enterovirus respiratory infections in French children between 1999 and 2005. J. Clin. Microbiol. 46:206-213.
Jee YM, Cheon DS, Choi WY, Ahn JB, Kim KS, Chung YS, Lee JW, Lee KB, Noh HS, Park KS, Lee SH, Kim SH, Cho KS, Kim ES, Jung JK, Yoon JD, Cho HW (2004). Updates on enterovirus surveillance in Korea. Inf. Chemother. 36:294-303.
Khanh TH, Sabanathan S, Thanh TT, Thoa le PK, Thuong TC, Hang Vt, Farrar J, Hien TT, Chau NV, van Doorn HR (2012). Enterovirus 71-associated hand, foot, and mouth disease, Southern Vietnam, 2011. Emerg. Infect. Dis. 18(12):2002-2005.
Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA (Centers for Disease Control and Prevention) (2006). Enterovirus surveillance--United States, 1970-2005. MMWR Surveill. Summ. 55(8):1-20.
Kim JI, Baek KA (2011). Sequencing and molecular characterization of the genome of echovirus 30 isolated from a Korean aseptic meningitis patient. J. Bacteriol. Virol. 41(4):301-307.
Kim KH (2010). Enterovirus 71 infection: An experience in Korea, 2009. Korean J. Pediatr. 53(5): 616-622.
Lee BE, Davies HD (2007). Aseptic meningitis. Curr. Opin. Infect. Dis. 20: 272-277.
Nix WA, Oberste MS, Pallansch MA (2006). Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J. Clin. Microbiol. 44: 2698-2704.
Oberste MS, Maher K, Flemister MR, Marchetti G, Kilpatrick DR, Pallansch MA (2000). Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. J. Clin. Microbiol. 38:1170-1174.
Oberste MS, Maher K, Kilpatrick DR, Pallansch MA (1999). Molecular evolution of the human enteroviruses: Correlation of serotype with VP1 sequence and application to picornavirus classification. J. Virol. 73:1941-1948.
Ooi MH, Wong SC, Podin Y, Akin W, del Sel S, Mohan A, Chieng CH, Perera D, Clear D, Wong D, Blake E, Cardosa J, Solomon T (2007). Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. Clin. Infect. Dis. 44:646-656.
Papachristou F, Printza N (2005). Acute nephritis complicating Coxsackie B infection. Indian Pediatr. 42: 838-839.
Park KS, Choi YJ, Park JS (2012). Enterovirus infection in Korean children and anti-enteroviral potential candidate agents. Korean J. Pediatr. 55(10):359-366.
Qiaoyun F, Xiongfei J, Lihuan L, Angao X (2013). Epidemiology and etiological characteristics of hand, foot and mouth disease in Huizhou City between 2008 and 2011. Arch. Virol. 158(4):895-899.
Ryu WS, Kang B, Hong J, Hwang S, Kim J, Cheon DS (2010). Clinical and etiological characteristics of enterovirus 71-related diseases during a recent 2-year period in Korea. J. Clin. Microbiol. 48:2490-2494.
Saitoh A, Sawyer MH, Leake JA (2004). Acute disseminated encephalomyelitis associated with enteroviral infection. Pediatr. Infect. Dis. J. 23:1174-1175.
Smura T, Kakkola L, Blomqvist S, Klemola P, Parsons A, Kallio-Kokko H, Savolainen-Kopra C, Kainov DE, Roivainen M (2013). Molecular evolution and epidemiology of echovirus 6 in Finland. Infect. Genet. Evol. 16:234-247.
Thoelen IP, Lemey, Van der Donck I, Beuselinck K, Lindberg AM, Van Ranst M (2003). Molecular typing and epidemiology of enteroviruses identified from an outbreak of aseptic meningitis in Belgium during the summer of 2000. J. Med. Virol. 70:420-429.
Watkins-Riedel T, Woegerbauer M, Hollemann D, Hufnagl P (2002). Rapid diagnosis of enterovirus infections by real-time PCR on the Light Cycler using the TaqMan format. Diagn. Microbiol. Infect. Dis. 42(2):99-105.
Yang F, Ren L, Xiong Z, Li J, Xiao Y, Zhao R, He Y, Bu G, Zhou S, Wang J, Qi J (2009). Enterovirus 71 outbreak in the People's Republic of China in 2008. J. Clin. Microbiol. 47:2351-2352.