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ORIGINAL ARTICLE

Epidemiologic and clinical features of non-polio enteroviral infections in northern Taiwan in 2008

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Background: Non-polio enteroviruses may cause different diseases, including herpangina, hand–foot–mouth disease (HFMD), meningitis, and nonspecific febrile illness; and cause epidemic outbreak annually. This study delineates the diversity of clinical presentations based on different serotypes and different groups [human enterovirus (HEV)-A and HEV-B] of enteroviruses (EVs) during the 2008 epidemic in National Taiwan University Hospital (NTUH).
Methods: We retrospectively identified patients younger than 18 years who had positive isolates of non-polio EV in throat swabs, rectal swabs, or cerebrospinal fluid, in NTUH from January 1 to December 31, 2008. For serotyping, immunofluorescence assay and polymerase chain reaction followed by viral structure protein-1 sequencing were applied. We analyzed and compared their clinical features among different serotypes and different groups of EVs.
Results: Among 172 patients who were enrolled, 16 serotypes were identified. The major serotype in NTUH was EV71 (25.6%) followed by coxsackievirus A (CA)16 and coxsackievirus B (CB)4. EV71 manifested mostly as HFMD (89%) and was complicated with encephalomyelitis in three patients. Serotypes of HFMD included EV71 (70%), CA16 (27%), CA4, and CA6. Serotypes of herpangina were heterogeneous, and the major serotype was CA2 (35.7%) followed by CB4 (23.8%). Aseptic meningitis was entirely caused by HEV-B and mostly infected by echovirus 30 (50%). Among children with EV-related respiratory tract infection, CB4 (32%) was dominant in upper respiratory tract infection, whereas echovirus 4 (71%) was the major

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cause of lower respiratory tract infection. Cases of HEV-A were significantly younger than the cases of HEV-B ($p = 0.04$). Multivariate analysis revealed that the most significant factor associated with hospitalization is HEV-B (odds ratio, 2.2; 95% confidence interval, 1.1—4.2; $p = 0.02$).

Conclusions: At least 16 serotypes circulated in northern Taiwan in 2008. EV71 is the predominant strain in this outbreak. All patients with HFMD were infected by HEV-A, but HEV-B was associated with a higher rate of hospitalization and aseptic meningitis, which should be a cause of alert regarding public health.

Introduction

Human enteroviruses (HEVs) are RNA viruses that belong to the family Picornaviridae. HEVs were originally classified as poliovirus (P), coxsackievirus A (CA), coxsackievirus B (CB), or echovirus (Echo), based on their associated pathogenicities. Since 1990s, a new classification of HEVs has been developed based on their molecular characterization, according to which HEVs are subgrouped into poliovirus, HEV-A, HEV-B, HEV-C, and HEV-D, based on the similarities in their viral structure protein (VP) genes.[1]

Those non-polio enteroviruses (NPEVs) may cause different clinical manifestations, including herpangina, hand—foot—mouth disease (HFMD), meningitis, and nonspecific febrile illness, and they often cause epidemic outbreaks annually in certain countries, such as Taiwan. During 1998—2005, CA16 and EV71 were the predominant serotypes in Taiwan. Each of these serotypes account for 23% of reports associated with an identified serotype followed by CB3 (13%), Echo4 (6%), CB4 (5%), and Echo6 (5%). EV71 was more frequently isolated from patients with encephalitis and pulmonary edema hemorrhage. Enteroviruses (EVs) other than EV71 were more frequently isolated when the complication was aseptic meningitis.[2] In early 2008, an epidemic alert was declared by the Center for Disease Control (CDC), Taiwan because of an increasing number of cases. The final number of confirmed severe cases were 373 subjects in 2008, which is much higher than the case number in 2006 (11 subjects) and 2007 (12 subjects).[3] The purpose of this study was to try to assess the diversity of clinical presentations based on different serotypes and different groups of EVs during the 2008 epidemic in National Taiwan University Hospital, a medical center in northern Taiwan.

Methods

Patients

We retrospectively identified patients younger than 18 years who had positive isolates of NPEVs from throat swabs, rectal swabs, or cerebrospinal fluid (CSF), in National Taiwan University Hospital (NTUH) from January 1 to December 31, 2008. NTUH is a medical center serving approximately 2,700 inpatients and 7,500 outpatients daily in northern Taiwan.

Data collection

The medical records of these patients were reviewed for data, including age, sex, diagnosis, laboratory results, EV serotype, hospitalization, complications, and neurologic sequelae. Questionnaires were mailed to the patients who visited the emergency department only. The questionnaires included questions regarding the patient's hospitalization to other hospitals after visiting NTUH emergency department. We arranged phone contact 2 weeks later if those patients did not reply the mail. Patients with incomplete clinical or virological data were excluded. We analyzed and compared age, sex, diagnosis, and hospitalization, among different serotypes and different groups of EVs.

Clinical definitions

The diagnosis was categorized as HFMD, herpangina, aseptic meningitis, febrile illness, viral exanthema, upper airway infection, and lower airway infection. Nonspecific illnesses included febrile illness, viral exanthem, upper respiratory tract infection (URI), and lower respiratory tract infection (LRI). HFMD was defined as a typical rash over palms, soles, buttocks, knees, or elbows. Herpangina was defined as the presentation of pharyngeal ulcers without gingival swelling or typical rashes from HFMD. Aseptic meningitis should present meningism and pleocytosis (> 5 leukocyte/mm^3 in patients older than 1 month or > 25 leukocytes/mm^3 in neonates) in CSF and negative bacterial cultures. Encephalitis was diagnosed by disturbance of the consciousness, such as lethargy, drowsiness, or coma, with characteristic slow wave on electroencephalography. Poliomyelitis-like syndrome had the characteristic of acute limb weakness with diminished reflexes and muscular strength. A diagnosis of encephalomyelitis was made when there was evidence of encephalitis and poliomyelitis-like syndrome or an evident white-matter change on magnetic resonance imaging. Viral exanthem was defined as non-itching maculopapular rash. URI included nasopharyngitis, laryngotrachitis, and tonsillitis. LRI presented as acute bronchitis, bronchiolitis, bronchopneumonia, or pneumonia.

Virus isolation and serotyping

Clinical samples were inoculated into human embryonic fibroblast (MRC-5), rhesus monkey kidney (LLC-MK2), laryngeal carcinoma (HEp-2), and RD cell cultures. When

the EV cytopathic effect was more than 50% of the cell monolayer, the cell was scraped. Indirect immunofluorescent antibody staining with pan-enteroviral antibody (Chemicon International, Inc., Temecula, CA, USA) and the serotype-specific antibody for EV71; CA Types 9, 16, 24; CB Types 1–6; and Echo Types 4, 6, 9, 11, 30, were used for serotyping. If the serotype of the samples were not identified with indirect immunofluorescent antibody, polymerase chain reaction (PCR) followed by direct sequencing was performed. Three genogroup-specific degenerate oligonucleotide primers flanking the VP1 region were made, including EntAF TNCARGCWGCNGARACNGG, EntAR outer ANGGRTTNGTNGMWGTYTGCCA, EntAR inner GGNGGNA CRWACATRTAYTG, EntBF GCNGYNGARACNGGNCACAC, EntBR outer CTNGGRTTNGTNGANGWYTGCC, EntBR inner CCNCCNGGBGGNAYRTACAT, EntCF TNACNGCNGTNGANA CHGG, EntCR outer TGCCANGTRTANTCRTCCC, and EntCR inner GCNCCWGGDGGNAYRTACAT. Amplicons of PCR with three sets of VP1-specific primers were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid, Sijhih City,Taipei County, Taiwan) before sequencing, and direct sequencing was performed with the previous genogroup-specific PCRs. All methods were carried out following the manufacturers' instructions. A specific serotype was defined when the VP1 amino acid sequence showed ≥88% homology to the VP1 amino acid sequence of the specific type of the HEV prototype strains.[4]

EVs were divided into four groups: CA Types 2–8, 10, 12, 14, 16, and EV71 belonged to HEV-A; all CB types (1–6); all Echos (Types 1–9, 11–21, 24–27, and 29–33); CA9; and EV69 were grouped as HEV-B; HEV-C comprised CA Types 1, 11, 13, 15, 17, 18, 20–22, 24, and polioviruses 1–3; and HEV-D comprised EV68 and EV70.[1]

A phylogenetic tree was constructed for 18 studied strains of CA2. The tree was outgroup rooted using CA16 prototype G10 strain (U05876); EV71 strains (98-984-S3, U22622, and U22521); and nine CA2 reference strains (AY919539, AB162722, AB188506, AY421760, AB162720, AB162721, AB119642, AB119643, and AB188507); it was built using the neighbor-joining method and bootstrap analysis with 1,000 bootstrap replications, and p-distance substitution model was used to evaluate the strength of the

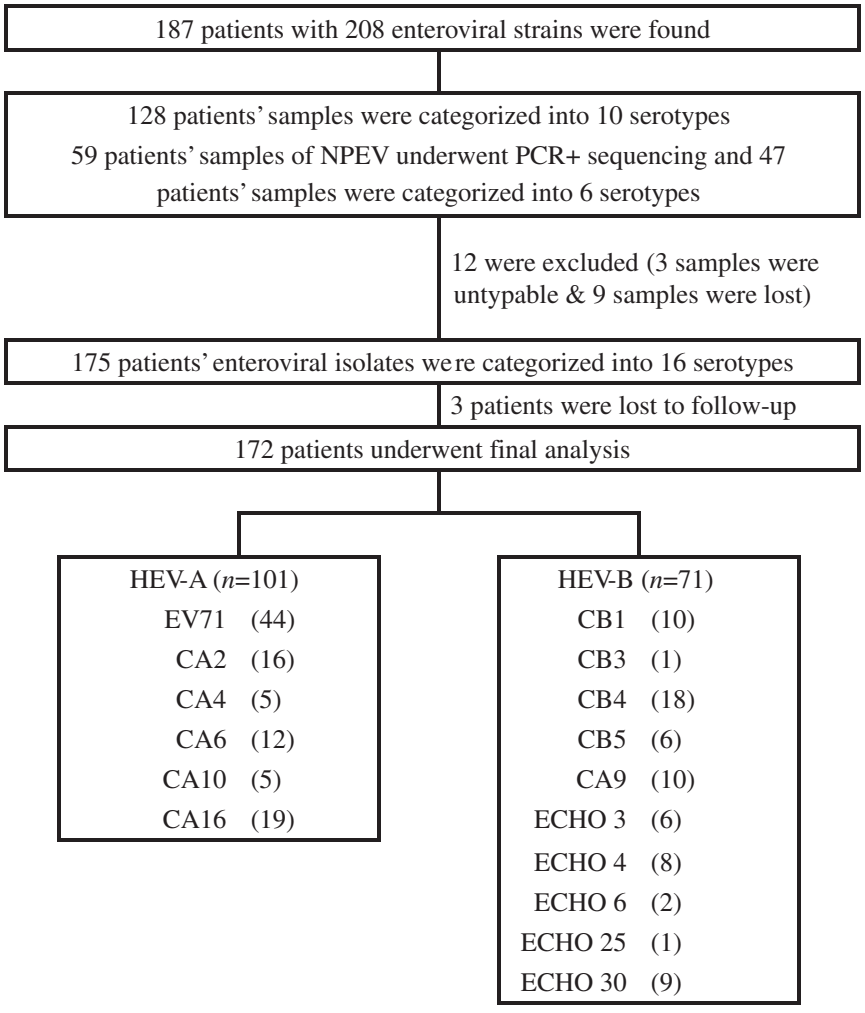

Figure 1. Flowchart of the enrolled patients and their serotype distribution. CA = coxsackievirus A; Echo = echovirus; EV = enterovirus; HEV = human enterovirus; NPEV = non-polio enterovirus; PCR = polymerase chain reaction.

topologies by MEGA Version 4.0.[5] The genetic distance was calculated by a pairwise estimation of percent divergence among the sequences.

Statistical analysis

Continuous variables were analyzed by Student *t* test, and categorical data were compared with χ^2 tests. Significance was defined as *p* value less than 0.05. Data were collected in a Microsoft Excel database and analyzed with SPSS software for Windows (Release 15.0; SPSS, Inc., Chicago, IL, USA).

Results

Serotypes of EVs

We found a total of 187 children with 208 EV isolates from throat, anus, or CSF. The EV strains of 128 patients were categorized into 10 serotypes initially after viral culture and indirect immunofluorescent antibody staining, and the other EV isolates from 47 patients underwent PCR and VP1 sequencing, but nine samples were lost before this investigation and three strains were untypable. Therefore, a total of 16 serotypes, including CA2, CA4, CA6, CA10, Echo3, and Echo25 were detected. Subsequently, we analyzed the clinical and epidemiological features of 175 patients infected by 16 serotypes, and 172 patients with complete profile entered final analysis. There were 101 patients infected by HEV-A and 71 patients infected by HEV-B. Figure 1 shows the flowchart of the enrolled patients and their serotype distribution. EV71, CA16, and CB4 were the major three isolated strains in NTUH laboratory in 2008.

Table 1 Demography, clinical entities, and medical care of HEV-A and HEV-B

Demographic characteristics	HEV-A ($n = 101$)	HEV-B ($n = 71$)	*p*
Age (yr)			
Mean ± SD	3.4 ± 2.8	4.8 ± 4.2	0.04
Median (range)	2.6 (0.5−17.4)	4.4 (0.01−17.7)	
Sex			
Male	59	49	
Female	42	22	0.21
Clinical manifestations			
HFMD	56	0	<0.01
Herpangina	41	18	0.05
Aseptic meningitis	0	19	<0.01
Nonspecific illness	4	34	<0.01
Medical care			
No hospitalization	72	38	
Hospitalization	29	33	0.03
ICU care	3	0	

HEV-A includes EV71, CA2, CA4, CA6, CA10, and CA16, and HEV-B includes CB1, CB3, CB4, CB5, CA9, Echo3, Echo4, Echo6, Echo25, and Echo30.
CA = coxsackievirus A; CB = coxsackievirus B; EV = enterovirus; HEV = human enterovirus; HFMD = hand−foot−mouth disease; ICU = intensive care unit; SD = standard deviation.

Demography

Table 1 shows that the mean [standard deviation (SD)] age is 3.4 (2.8) years and the median age is 2.6 years, ranging from 0.5 years to 17.4 years in HEV-A group. In the HEV-B group, the mean (SD) and median (range) ages are 4.8 (4.2) years and 4.4 (0.01−17.7) years. The age of the HEV-A group was significantly lesser than that of the HEV-B group ($p = 0.04$). The age distribution of both groups (HEV-A and HEV-B) was mostly less than 7 years (83%), and 47% of the cases were found in children younger than 3 years. No significant gender difference was found between these two groups. Among cases of HFMD, the mean age between EV71 and non-EV71 groups is similar (3.2 ± 2.6 years *vs.* 3.9 ± 2.2 years, $p = 0.15$). In the group of meningitis, the mean (SD) age was slightly greater in patients of Echos than that in patients of coxsackieviruses (8.0 ± 5.1 years *vs.* 4.6 ± 4.5 years, $p = 0.13$). The mean age and gender of individuals who were excluded from final analysis showed no difference in comparison with those of the enrolled patients. Sixty percent of the EV cases were identified between April and July, which was the same period of the major peak of EV activity in Taiwan.

Clinical entities among different serotypes

All patients with HFMD were infected by HEV-A. The leading cause of HFMD was EV71 (70%) followed by CA16 (27%). The mean age and gender between EV71 and non-EV71 patients with HFMD were of similar distribution. Fifteen percent of patients with EV71-related HFMD were afebrile at the time of diagnosis. Encephalomyelitis presented in three patients with EV71. All of them were diagnosed with febrile HFMD and needed intensive care. Elective intubation, intravenous immunoglobulin (1 g/kg) and milrinone were administered. The first patient entered the stage of cardiopulmonary failure, progressed to irreversible neurological damage, and passed away. The second patient experienced cardiopulmonary failure 3 days after disease onset, regaining consciousness with sequelae of psychomotor retardation, and needed tube feeding because of dysphagia and a tracheostomy because of failure from weaning ventilator. The last patient presented conscious disturbance with poliomyelitis-like syndrome and progressed to the stage of cardiopulmonary failure 2 days later, but fully recovered without subsequent limb weakness.

Herpangina was mostly caused by HEV-A (69.5%, $p = 0.05$), and the leading strains were CA2 (25%) and CA6 (17%). About 11% patients with EV71 manifested herpangina clinically. Figure 2 shows that the phylogenetic tree of the 18 CA2 strains, drawn on the basis of the alignment of the VP1 gene sequences, is considered homologous. These isolates were close to the reference strain AY919539 from America in 2005.

Diagnosis of aseptic meningitis was totally related to HEV-B ($p < 0.01$), and most of it was caused by Echo30 (42%). Table 2 shows that CB1, CB4, CB5, CA9, and Echo6 were also isolated from patients with aseptic meningitis in 2008. All patients with aseptic meningitis recovered well

from the acute illness and had no need of intensive care and no long-term sequelae.

Nonspecific illnesses, including fever, viral exanthem, URI, and LRI, were significantly associated with HEV-B ($p < 0.01$). Echo4 (21%), CB4 (18%), and CA9 (18%) are the most common strains. Two siblings who were first diagnosed of flu-like illness were infected by Echo3. Respiratory tract infection is the major presentation—around 76% of all nonspecific illnesses. All patients with EV-associated respiratory tract infection are listed in Table 3. The circulation strains causing respiratory tract infection included CA2, CA6, CA10, CA16, CB1, CB4, CB5, CA9, Echo3, and Echo4. Among children with airway infection, 22 (76%) had URI and 7 (24%) had LRI. CB4 (32%) was dominant in URI, whereas Echo4 (71%) was dominant in LRI. Patients with URI manifested frequently with tonsillitis and rhinopharyngitis (common cold), whereas bronchiolitis and bronchopneumonia were the most common diagnoses in LRI. Five patients developed mixed lower airway infection by respiratory syncytial virus (RSV), proven by a rapid antigen test.

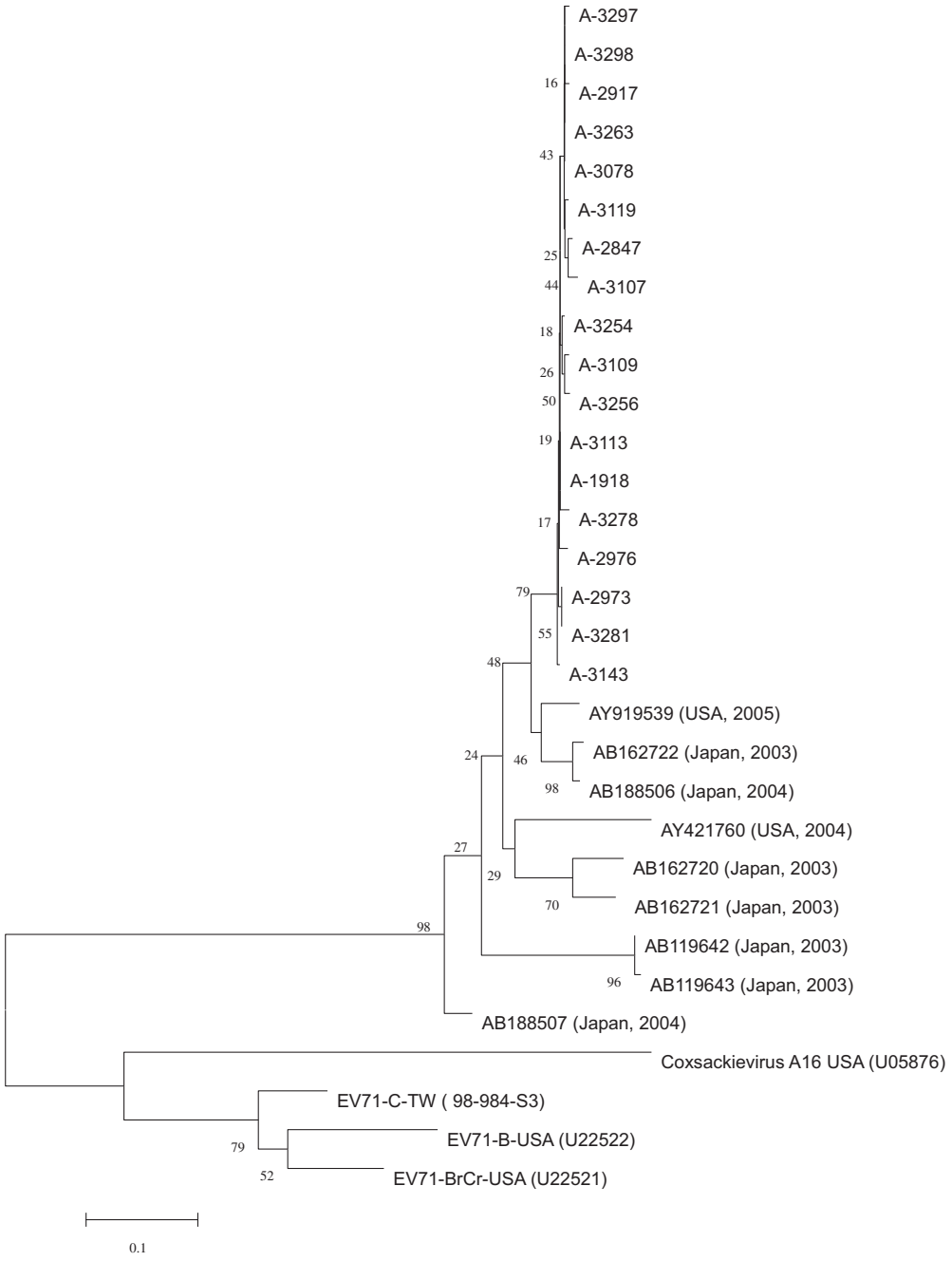

Figure 2. Phylogenetic tree constructed using viral structure protein-1 gene sequences of coxsackievirus A2 (CA2). The strains labeled A-1918 to A-3298 are CA2 strains isolated in National Taiwan University Hospital in 2008. The outgroup was using CA16 prototype G10 strain (U05876); enterovirus 71 strains (98-984-S3, U22622, and U22521); and nine CA2 reference strains (AY919539, AB162722, AB188506, AY421760, AB162720, AB162721, AB119642, AB119643, and AB188507).

Table 2 Clinical entities among different serotypes and groups of enteroviruses

Clinical manifestations	HEV-A						HEV-B										Total
	EV71	CA2	CA4	CA6	CA10	CA16	CB1	CB3	CB4	CB5	CA9	Echo3	Echo4	Echo6	Echo25	Echo30	
HFMD	39		1	1		15											56
Herpangina	5	15	4	10	4	3	4	1	9	2		1			1		59
Meningitis							2		2	2	3			2		8	19
Nonspecific illness		1		1	1	1	4		7	2	7	5	8			1	38

CA = coxsackievirus A; CB = coxsackievirus B; Echo = echovirus; EV = enterovirus; HEV = human enterovirus.

Other bacterial or atypical pathogens were not detected with EV infection.

Factors associated with hospitalization

The hospitalization rate by individual diagnosis was as follows: 29% in HFMD; 27% in herpangina; 100% in aseptic meningitis; and 29% in nonspecific illnesses, including 100% of LRI.

The overall hospitalization rates of HEV-A and HEV-B groups were 31% and 46%, respectively ($p = 0.03$). Patients with HFMD who were infected by EV71 had more demand for hospitalization than those who were infected by other strains in HEV-A, but the hospitalization rate was not statistically significant (33% *vs.* 18%, $p = 0.38$).

Figure 3 shows the age distribution and individual hospitalization numbers of HEV-A and HEV-B groups. The peak age of inpatients in the HEV-A group was between 1 year and 2 years, and 76% of the inpatients were younger than 6 years. In the hospitalized patients of HEV-B group, the major peak was less than 1 year (36%) and the minor peak was 6—8 years (24%).

The multivariate analysis in Table 4 shows the association between serogroup and hospitalization. HEV-B was a significant risk factor for hospitalization after age was adjusted (odds ratio, 2.2; 95% confidence interval, 1.1—4.2, $p = 0.02$).

Discussion

This study presents NPEV infections in one medical center in northern Taiwan in 2008. During this same epidemic period, at least 16 serotypes of EVs circulated in the community of northern Taiwan, and EV71 and CA16 ranked as the Top 2. The most common clinical presentations are still HFMD and herpangina. We found that all patients with HFMD were infected by HEV-A, but HEV-B was associated with a higher rate of hospitalization and aseptic meningitis.

The EV surveillance result between 2000 and 2008 from CDC, Taiwan shows that the ranking of EV71 had slipped down after 2005 until the epidemic recurred in 2008.[3,6] It is supposed that the seropositivity rate was decreasing with years in the population, especially among the infants and toddlers who are most susceptible to EV infections.[6,7] We speculate that the accumulation of the susceptible hosts during the past few years may explain the occurrence of EV71 outbreak in 2008.

The case numbers of EV71 are far more than those of CA2 strains in NTUH, and this result is different from the ranking data of EVs from CDC-TW in the same period, which showed that CA2 was the top one. This might be related to different patient characteristics in NTUH and in local hospitals or clinics because more severe cases or HFMD cases tended to seek medical care in medical centers rather than local hospitals or clinics. In this study, most of the patients presented with HFMD, which was known as the most common presentation of EV71 and the common preceding syndrome of severe EV cases with central nervous system (CNS) involvement in Taiwan.[8]

We select CA2 for phylogenic analysis because it takes the first place in whole-country surveillance of NPEV infection in 2008. CA2 is known as a less important strain for HEV outbreaks in Taiwan after 1998.[6] In addition to the

Table 3 Clinical diagnosis and virological features of 29 pediatric patients with an enterovirus-related respiratory infection

Diagnosis	No. of patients	Mean age (yr)	Male/female	Multiple infections	Admission, *n*(%)	Serotypes (*n*)
Tonsillitis	12	5.4 ± 3.0	10/2	—	1 (8)	CB4 (5), Echo3 (2), Echo4 (1), CB1 (1), CA9 (1), CA2 (1), CA6 (1)
Rhinopharyngitis	10	5.1 ± 3.9	5/5	—	0 (0)	CB4 (2), CB5 (2), CB1 (2), CA9 (3), Echo4 (1)
Bronchiolitis	4	0.8 ± 0.5	2/2	RSV (3)[a]	4 (100)	Echo4 (2), CA10 (1), CA16 (1)
Bronchopneumonia	3	4.6 ± 4.9	3/0	RSV (2)[a]	3 (100)	Echo4 (3)
Total	29	4.6 ± 3.7	20/9		8 (28)	

[a] RSV infection was diagnosed by sputum antigen test.

Data are presented as *n*, *n*(%) or mean ± standard deviation. RSV = respiratory syncytial virus; SD = standard deviation.

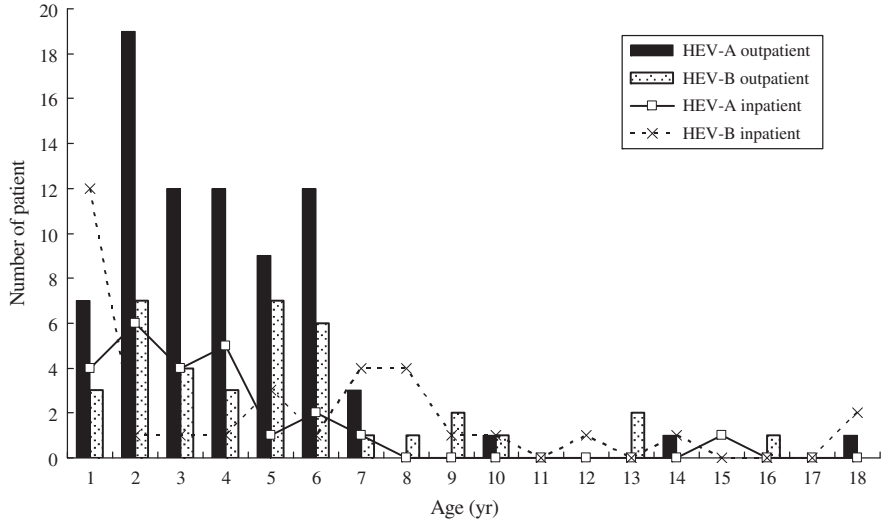

Figure 3. Age distribution and medical care of HEV-A and HEV-B. HEV = human enterovirus.

theory of susceptibility in general pediatric group, viral circulation in environment may represent a problem of public health. The recent survey of EVs in environmental water and hot spring water showed CA2 to be a dominant serotype in Taiwan.[9,10] We suspect that CA2 may circulate in the environment and, thus, play some role in the outbreak of 2008.

Some symptoms are particularly characteristic of certain serotypes, such as HFMD of EV71 and CA16, and aseptic meningitis of Echos.[11] Herpangina is usually related to HEV-A rather than HEV-B. In a recent report, all the serotypes accounting for herpangina in the annual epidemic data of Japan show CA.[12] The serotype distribution of herpangina in our result is compatible with that in Taiwan during 2000—2005.[6] Echos and coxsackieviruses are known as the dominant pathogens of pediatric aseptic meningitis, and outbreaks of Echo30 infection are frequently discussed in Europe, South America, and Asia.[6,13] Although coxsackieviruses caused fewer outbreaks in history, the clinical entities are not significantly different from those of echoviruses.

EV-related respiratory tract infection was analyzed to a lesser extent in previous reports. Our understanding of the epidemiology and clinical profile of EV-related respiratory infection is mostly from the surveillance of respiratory viral infections. The average rate of positive EV isolation for pediatric respiratory infections in Taiwan ranged from 12.7% to 22%.[14,15] A Japanese analysis of respiratory viruses from children in 2004 and 2005 displayed an approximate positive EV rate of 20% among children with acute respiratory infections. Another comprehensive retrospective study in France that reviewed a pediatric cohort from 1999 to 2005 gave the positive EV rate of 11.6%.[16] Other countries showed a lower rate of about 0.2—5.7% in Singapore, Czechoslovakia, Brazil, and United States.[17—20] The seasonal distribution in France revealed a greater case number during spring—fall season and a peak in June to July.[16]

Till date, it is unclear which are the dominant serotypes or groups in airway infections.[16,17,21,22] CB3, CB5, and Echo20 were addressed to be related to the so-called "summer grippe" or common cold.[23] CA2, CA4—6, CA10, CB1, CB2, and CB5, and Echo9, Echo18, and Echo30 had also been isolated or identified from children with acute tonsillitis in earlier studies.[24,25] There might be a trend that HEV-B is the dominant group in patients with acute respiratory infections, but there is no detailed control study to support this. Another concern is whether EVs are true pathogens or an incidental colonization in airway infections. A study of nasopharynx colonization in children showed that EV RNA may be detected among asymptomatic children, although previous respiratory symptoms or contact with symptomatic family members can be traced.[26] Another article reported that EV RNA may disappear in nasal cavity about 2—5 weeks after acute respiratory infection.[27] This might indicate that colonization is possible, and EV-related respiratory infections might be overestimated. In our study, five patients were detected with concomitant Echo4 and RSV at the same time. Rawlinson et al.[28] studied the relationship between viruses in nasopharynx and acute exacerbation of asthma and found EVs from nasopharyngeal aspiration (NPA) in 29% of children with asthma in the summer. Jartti et al.[29] described mixed viral infections, mostly RSV and EV (19%), from NPA, in a prospective clinical trial for children with acute expiratory wheezing. Because analysis of pathogens of all respiratory tract infections by throat swab or NPA might be misinterpreted by concurrent acute infection, further

Table 4 Multivariate analysis of factors associated with hospitalization

Parameter	OR (95% CI)	*p*
Age, yr $\leq$3 *vs.* >3	1.7 (0.9—3.3)	0.12
Sex male *vs.* female	1.8 (0.9—3.5)	0.11
Species HEV-B *vs.* HEV-A	2.2 (1.1—4.2)	0.02
Serotypes(non-EV71 *vs.* EV71)	1.2 (0.6—2.6)	0.57

CI = confidence interval; EV = enterovirus; HEV = human enterovirus; OR = odds ratio.

surveillance of the EV prevalence in healthy controls may be needed.

EV71 can result in disability or even death; hence, risk factors of EV71-related CNS involvement have been clinically focused after the outbreak occurred in Taiwan in 1998. Young age, especially less than 3 years, is the most important risk factor of the unfavorable outcome in children.[30] Our study chooses hospitalization as an indicator of disease severity because the clinical management cannot be interfered in the retrospective review. After adjusting for age and gender, it is interesting to note that the hospitalization rate of non-EV71 infection is not lower than that of EV71 infection. The first-line physicians enforced an effective screening by stage-based management, which can primarily differentiate patients with and without the potential risk of CNS involvement and cardiopulmonary failure.[31] Patients infected by HEV-B might be more toxic and show the need for inpatient care at the time of medical visit. The major diagnoses, aseptic meningitis and LRI, were reasonable to present toxic appearance initially. This issue proves that EVs other than EV71 may be a burden on public health, especially on the cost of health insurance. Our study is limited by the short period, single medical center, and the retrospective nature. However, it still represents a regional epidemiology in the outbreak of 2008.

In conclusion, we found at least 16 serotypes that circulated in northern Taiwan in 2008. EV71 is back to be the predominant strain in this outbreak. Clinically, HEV-B group is associated with a higher rate of hospitalization and aseptic meningitis, compared with HEV-A, which should be a cause of alertness regarding public health.

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