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An Investigation of a Cluster of Echovirus 6 Infection with an Encephalitis Death in Samut Prakan Province, Thailand, 2015

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Abstract

On 16 Dec 2015, the Bureau of Epidemiology in the Ministry of Public Health, Thailand, was notified of a suspected echovirus 6 encephalitis death in Samut Prakan Province. An investigation was launched for active case detection and confirmation of causative agent. Out of 32 people identified, two (6.3%) were confirmed for echovirus 6, including the index case, and there were 30 suspected cases. The index case was a 5-year-old Thai boy and kindergarten student. On 8 Dec 2015, he developed encephalitis. Echovirus 6 was detected in his cerebrospinal fluid, nasal and rectal swab samples. Chest x-ray revealed pulmonary edema and elevated cardiac enzyme as signs of rhombencephalitis. There were also five (2.9%) out of 173 asymptomatic contacts tested positive for echovirus 6 infection. Potential risks for infection were sharing of utensils and toys, and playing together. Lack of intensive health screening in the school and inadequate hand washing facilities were also observed. We recommended households and the school to improve sanitation, and health education was provided in the communities and schools. Physician's knowledge and awareness of echovirus infection among children should be raised to provide proper treatment and early referral if needed.

Keywords: Human, echovirus 6, enterovirus, encephalitis, investigation, Thailand

Introduction

There have been outbreaks of echovirus infection reported in many countries such as Brazil, China, France, Greece, Japan and Korea, where the majority of the cases had resulted with severe neurological diseases.[1-6] The data from the national event-based surveillance system in the Bureau of Epidemiology revealed that about 3% of unspecified encephalitis reported in Thailand during 2005-2015 were caused by echovirus and the case fatality rate for echovirus encephalitis was 100%. However, the echovirus encephalitis cluster had never been reported to date in Thailand.[7]

Echovirus belongs to the species *Enterovirus B*, genus *Enterovirus* of the *Picornaviridae* family. Echoviruses and other enteroviruses are found in human gastrointestinal tract, and can cause opportunistic infections and diseases. While it can be transmitted from person to person, the fecal-oral route is the predominant mode and sometimes occurs via droplets from respiration or oral secretion. Contaminated swimming and wading pools can also transmit the virus. The common incubation period ranges from 3-6 days.[8,9]

Although about 50-80% of enteroviruses infections are asymptomatic, the infections may result in a wide variety of presentations, including rash, herpangina, respiratory illness (cough, coryza, croup) or hand, foot and mouth disease (HFMD). Severe myocardial, pericardial or neurological complications can also occur with acute aseptic meningitis or encephalitis such as rhombencephalitis.[10] It can involve autonomic nervous system, resulting in autonomic dysfunction, and particularly, sympathetic overstimulation that causes high blood pressure, tachycardia, sweating, hyperglycemia as well as pulmonary edema.[11]

On 16 Dec 2015, the Bureau of Epidemiology received a notification from the Office of Disease Prevention and Control 13 of a suspected death from echovirus

encephalitis in Samut Prakan Province. The surveillance and rapid response teams (SRRT) from Bureau of Epidemiology, and local provincial health office, district health office and health promoting hospital jointly conducted a field investigation from 17 to 18 Dec 2015 to determine the cause of death, confirm the diagnosis, describe the extent of the outbreak, explore risk behaviors and provide preventive measures to the local authorities.

Methods

A descriptive study was performed to outline the index case and an investigation was carried out in the area where the case lived (Wat Si Wari Noi Sub-district, Bang Sao Thong District, Samut Prakan Province) from 29 Nov to 22 Dec 2015.

Description of the Index Case

The available clinical information and laboratory investigation of the index case died from suspected echovirus encephalitis were collected from medical records of all hospitals where the case visited. The doctors and parents were interviewed about demographic factors, clinical manifestations and management, risk behavior and activities. In the school where the case attended, the teachers and caretakers were questioned about risk behaviors and activities as well as number of students with HFMD and encephalitis cases identified in the school during the previous five years.

Active Case Finding

The situation of HFMD and encephalitis in Wat Si Wari Noi Health Promoting Hospital from 29 Nov to 22 Dec 2015 was reviewed. In addition, the laboratory findings in the National Institute of Health (NIH) for enterovirus infection during 2014-2015 were retrieved as well.

Target population of the active case finding in the communities included people in the index case's house, the neighboring houses located within 500 meter-radius, and the case's school. The active case finding was carried out to identify cases and contacts during 29 Nov to 22 Dec 2015 (two times of maximum incubation period of echovirus infection before and after the onset of the index case) using the door-to-door survey method.

A contact was a person or neighbor who lived, played or studied together with the index case. A suspected case was a contact with any symptoms of: vesicles or ulcers in oral cavity; vesicular or maculopapular rash on palm, foot or buttock; upper respiratory infection (URI); and severe manifestations (neurological deficit, cardiovascular symptoms) from 29 Nov to 22 Dec 2015. A confirmed case was a suspected case who was tested positive for echovirus 6 from fresh stool or throat swab specimen by polymerase chain reaction (PCR) or viral isolation. Asymptomatic infection included contacts without any symptoms, yet found to have echovirus 6 by laboratory testing of fresh stool or throat swab by PCR or viral isolation.

Clinical specimens from contacts and symptomatic cases were collected during 17-18 Dec 2015 for testing echovirus 6 and other enterovirus infections. All specimens in viral transport media were kept at 4°C and transported with ice packs to the NIH, the Neuroscience Centre for Research and Development under Faculty of Medicine in Chulalongkorn University, and the Faculty of Medicine in Ramathibodi Hospital. The specimens were tested for 12 species of enterovirus, including *Enterovirus A*, *B* (including echovirus), *C*, *D*, *E*, *F*, *G*, *H* and *J*, and *Rhinovirus A*, *B*, *C* by PCR and viral isolation.

Contact Tracing

Fresh stool specimens were collected from the index case's household members, neighborhood contacts and all students studying in the same class with the index case. Throat swabs were obtained from the symptomatic contacts, including household members, neighborhood contacts and students in the same school, with URI symptoms, or vesicles/ulcers in oral cavity, or vesicular/maculopapular rash at palm, sole or buttock within seven days before specimen collection.

Phylogenetic Analysis

Furthermore, a phylogenic study was also conducted using 500 bootstrap method/maximum composite likelihood approach to find the epidemiological linkage of the virus.

Environmental Study

An environmental study was also performed at the index case's house, the neighborhood area and the school via a walk-through survey, and findings were recorded using an environmental checklist. General sanitation, drinking water, water for washing, toilet and waste container were observed. The residual chlorine level in the water was tested as well. Moreover, personal behaviors such as daily activity, eating, playing and hand washing were inspected.

Results

Description of Index Case

The index case was a Thai boy aged four years and 11 months, without any underlying disease. He studied in the kindergarten and lived in Wat Si Wari Noi

Sub-district, Bang Sao Thong District, Samut Prakan Province. On 8 Dec 2015, he had high fever, poor appetite and occasional myoclonic jerk. However, he had no URI symptoms, nausea, vomiting, skin lesion or oral ulcer. Although he was treated at home with symptomatic treatment, his illness did not improve. Hence, his mother brought him to a private hospital (Hospital A) where he received out-patient treatments on 10 Dec 2015. Despite that, he continued to have fever, had poor oral intake, went to another private hospital (Hospital B) on 11 Dec 2015 and was admitted.

At Hospital B, he had fever, tachycardia and mild tachypnea. No abnormal neurological finding was detected at the time of admission. He received intravenous antibiotic as well as intravenous acyclovir, yet the fever was still persistent and later, he developed nausea and vomiting. At 4 am on 12 Dec 2015, as he developed high grade fever and seizures, he was referred to another private hospital (Hospital C) and admitted in the intensive care unit. Due to seizures and cardiac arrest, endotracheal intubation and cardiopulmonary resuscitation were performed. Despite the efforts, he died at 9:15 am on 13 Dec 2015.

The laboratory investigation revealed leucocytosis, hyperglycemia and elevated cardiac enzymes, with creatinine phosphokinase (CPK) of 462 U/L (normal range = 22-198 U/L) and the cardiac marker (CK-MB) of 56 IU/L (normal range = 5-25 IU/L). Peribronchial thickening was found in the chest X-ray.

No influenza and respiratory syncytial virus was identified from his nasal swabs. Samples of cerebrospinal fluid (CSF) were tested negative for herpes and rabies, yet positive for Japanese encephalitis (JE) immunoglobulin G. Echovirus type 6 (E6) was identified from throat and rectal swabs, and CSF samples by PCR only after he died.

The index case had received complete vaccination as recommended in the immunization schedule, including JE vaccine. He had no travel history two weeks prior to his illness. Furthermore, he did not go to school or go to play outside after he developed the symptoms. There were other nine persons in the same household and 16 persons in the neighboring houses within 500 meter radius. For the exposure history, his cousin had herpangina about two weeks before he developed symptoms. However, his cousin was not included in this study as the onset of illness was beyond the study period.

Active Case Finding

For active case finding in the hospitals, none were diagnosed with HFMD or encephalitis in Wat Si Wari Noi Health Promoting Hospital. In addition, there was no previous outbreak of echovirus infection or HFMD in Wat Si Wari Noi Sub-district during the study period.

As there were two houses within 500 meter-radius from the index case's house, active case finding was conducted in these two houses and the index case's school. Among total 211 persons identified, 204 (96.7%) persons were interviewed. Total 31 people were detected from the active case finding, including 30 suspected cases and one confirmed case who was the index case's sister confirmed as echovirus 6 infection by viral isolation. Hence, total two (6.3%) cases were confirmed, including the index case. The overall attack rate was 15.6% (Table 1).

The most common symptoms were nasal discharge (81.3%), cough (75.0%) and sore throat (15.6%). The first suspected case had onset on 1 Dec 2015 and the peak of an epidemic occurred during 10-17 Dec 2015, followed by a downward trend (Figure 1).

There were two suspected cases reported clinical URI symptoms earlier than the index case. The first suspected case (suspected case A) who developed URI symptoms on 1 Dec 2015 was a 6-year-old Thai female. She studied in the kindergarten together with the index case. She still went to school even after she got sick and shared some activities with the index

Table 1. Number of villagers and echovirus cases identified in Wat Si Wari Noi Sub-district, Bang Sao Thong District, Samut Prakan Province, Thailand, 29 Nov - 30 Dec 2015

Finding		Household & neighborhood	School		Total
			Kindergarten	Others	
Found/Total		25/25	45/45	135/142	205/212
Case	Suspected	3	6	21	30
	Confirmed	2	0	0	2
Attack rate (%)		20.0	13.3	15.6	15.6
Asymptomatic		1	4	0	5

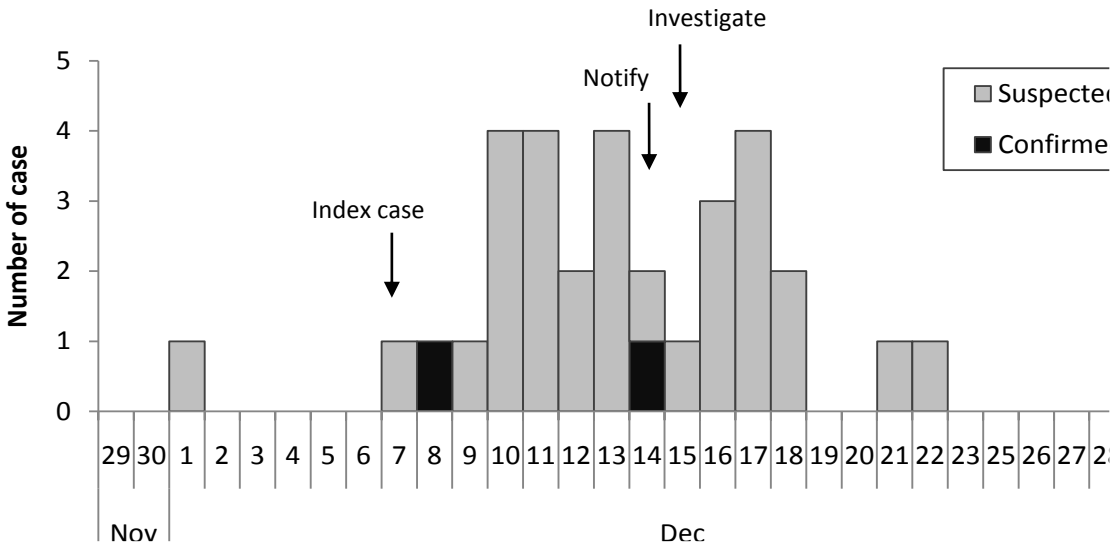

Figure 1. Number of echovirus cases in Wat Si Wari Noi Sub-district, Bang Sao Thong District, Samut Prakan Province, Thailand, 29 Nov - 30 Dec 2015 (n= 32)

case, including having lunch. Her symptoms lasted for three days and however, laboratory testing did not find enterovirus from her throat swab and fresh stool specimens.

Another suspected case (suspected case B) was an 8-year-old Thai female and reported clinical URI symptoms on 7 Dec 2015. She studied at the primary level in the same school. However, she did not go to school after she got sick or share any activities with the index case. Her symptoms lasted for four days. Her throat swab specimen also showed negative results for enterovirus.

Contact Tracing

Regarding to asymptomatic household and neighbor contacts, one household contact out of 21 fresh stool specimens collected was positive for echovirus 6. In addition, five out of 22 specimens from asymptomatic contacts in the kindergarten were tested to have enterovirus, including four for echovirus 6 and one for coxsackie virus A16. In addition, one out of 21 throat swab specimens from symptomatic contacts in the primary level was positive for coxsackie A16 (Table 2).

Phylogenetic Analysis

Six positive specimens for echovirus 6 (2 confirmed and 4 asymptomatic cases) were undergone the phylogenetic analysis and found that all had the same nucleic base sequencing. The VP1 gene 635 bp from 719 bp were sequenced and the viral isolate showed 96% identity to the E6 strain prevalent in Shandong, China during 2011-2012 (E6SD11CHN HFMD, accession number: JX976771.1)[12] (Figure 2).

Information from National Institute of Health

The data obtained from NIH between 2014 and 2015

Table 2. Laboratory results for enterovirus infection in Wat Si Wari Noi Sub-district, Bang Sao Thong District, Samut Prakan Province, Thailand, 29 Nov-30 Dec 2015

Specimen	Contact	Specimen collected			Positive for enterovirus		Type
		Total	Symptomatic	Asymptomatic	Symptomatic	Asymptomatic	
Throat swab (n=31)	Household & neighborhood	4	4	0	1 (index case)	0	Echovirus 6
	School						
	- Kindergarten	6	6	0	0	0	-
	- Others	21	21	0	1	0	Coxsackie A16
Fresh stool (n=52)	Household & neighborhood	25	4	21	1 (index case's sister)	1	Echovirus 6 (2)
	School						
	- Kindergarten	27	5	22	0	5	Echovirus 6 (4) Coxsackie A16 (1)

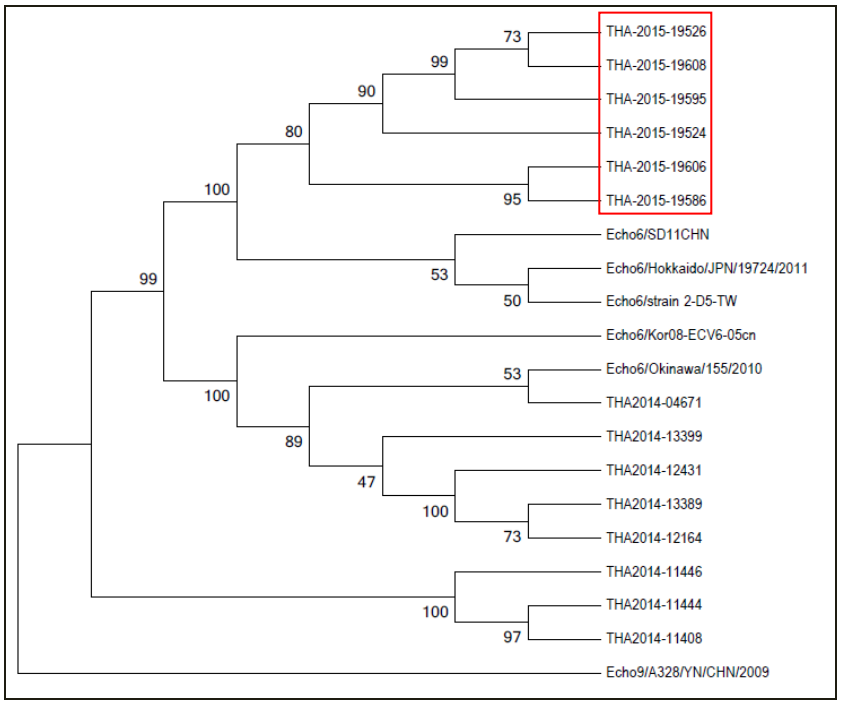

Figure 2. Phylogram of partial VP1 sequences of human echovirus 6 isolates from Wat Si Wari Noi Sub-district, Bang Sao Thong District, Samut Prakan Province, Thailand, 29 Nov - 30 Dec 2015

showed that 1,909 samples were sent for enterovirus detection, 349 samples (18.3%) were tested positive. The most common serotype was enterovirus 71 (39.0%), followed by coxsackie A16 (25.2%). During 2014 to 2015, echovirus was identified from 4% of the total samples (Table 3).

Table 3. Distribution of enterovirus serotypes isolated in the National Institute of Health, Thailand, 2014-2015

Serotype	Number	Percent
Enterovirus 71	136	39.0
Coxsackie A16	88	25.2
Unidentified	47	13.5
Coxsackie A6	35	10.0
Echovirus 6	14	4.0
Coxsackie A10	6	1.7
Coxsackie A2	5	1.4
Echovirus 3	4	1.2
Poliovirus	3	0.9
Coxsackie A24	2	0.6
Echovirus 11	2	0.6
Human Rhinovirus	2	0.6
Coxsackie A8	1	0.3
Coxsackie A14	1	0.3
Echovirus 9	1	0.3
Echovirus 14	1	0.3
Coxsackie B4	1	0.3

Environmental Study

The index case lived in a 3-generation extended family, with total 26 people. The index case and his sister were confirmed to have echovirus 6 infection, and were the grandchildren in the family.

Two (50.0%) out of four symptomatic and one (4.5%) out of 21 asymptomatic family members and neighbors were tested to have positive for echovirus 6 infection. They occasionally ate dinner together, shared glasses, dining utensils and toys, watched TV, and played together. They shared toilets and kitchen, and usually washed their hands with soap before meal and after using the toilet. The index case slept in the same bedroom with his younger sister and mother.

They consumed bottled water as drinking water and used tap water for washing. The residual chlorine level was adequate (⩾0.5 ppm) on the investigation day, 17 Dec 2015. They used water privy toilets with good sanitation. There were adequate trash bins with cover as waste containers around their houses.

In the index case's school, there were kindergarten and primary levels 1-4. The index case studied in the kindergarten in a single-story building while the primary students studied in another building. One playground was shared for all students. There were total 45 students in the kindergarten and had lunch together in their classroom. They did not usually wash their hands before meal and after using the toilet. Daily health screening was done roughly by

teachers in the mornings. There was no isolation room for sick students.

The school provided bottled drinking water for students and tap water for washing. The residual chlorine level was also adequate (≥0.5 ppm). The toilets for the kindergarten students and primary students were separated. Two water toilets with good sanitation were provided for the kindergarten students. There were only two washing sinks, yet one was broken. There were waste containers with cover in the school.

Discussion

A cluster of echovirus 6 infection with an encephalitis death was confirmed. The cause of death was likely to be brainstem infection. Although the source of the outbreak was inconclusive, it was most probably from a school contact.

The clinical manifestations of hyperglycemia, tachycardia, leucocytosis, pulmonary edema, and myocarditis suggested reticular formation involvement and autonomic dysfunction, which implied the potential brainstem involvement resulted from echovirus 6 infection. The brainstem infection that could cause cardiopulmonary failure was also corresponded with the previous report in Malaysia[13]. However, the pathological confirmation was not available as the brain imaging, autopsy or necropsy was not performed. WHO has recommended using intravenous immunoglobulin in enterovirus infection. One public health critique relating to the index case's death was the awareness of echovirus infection, which could led to proper diagnosis and treatment such as intravenous immunoglobulin[14,15]. Hence, this was a cluster of echovirus 6 infection that caused URI as well as severe manifestations such as encephalitis and myocarditis.

Although the index case's cousin had herpangina two weeks before his onset, the direct infection from his cousin was less likely as the incubation period for echovirus infection is commonly reported as 3-6 days[8,9]. Out of two suspected cases (suspected cases A and B) with earlier date of onset than the index case, the suspected case A might be the source of the cluster as she still went to the school during the infectious period. Nonetheless, the epidemiological linkage could not be confirmed as the laboratory results for echovirus infection revealed negative for the suspected case A. Despite that, as the specimens for this study were collected only on 17 Dec 2015, laboratory testing might fail to detect etiologic agent.

Two confirmed cases of echovirus 6 were identified in the same household, highlighting the risk behavior of sharing glasses, utensils and toys. Most enteroviruses are resistant to acid pH, ether, alcohol and deoxycholate. Appropriate means of disinfection include using sodium hypochlorite, chlorine, glutaldehyde, formaldehyde, boiling at 50-60 ^{o}C for 30 minutes, sterilization[16] and ultraviolet.[10] Therefore, the disease could spread easily in the community if control measures such as disinfection are not applied properly.

The predisposing factor of this cluster could be the overcrowding condition in the kindergarten with total 45 students, which is the common risk factor of enterovirus outbreak.[17] Furthermore, a higher rate of infection was normally found in children due to exposure, hygiene and immunity status.[18] Sharing of glasses, dinning utensils and toys, and lack of appropriate hand washing and proper health screening were also observed as the potential risks of transmitting infection in the school. Thus, provision of spacious classrooms, avoid sharing of materials, conducting intensive health screening and managing an isolation room for sick children at the school as well as frequent hand washing could help limiting the outbreaks.[19]

The phylogram for partial VP1 sequences of human echovirus 6 isolates was the first of its kind developed in NIH, which identified a viral isolate similar to the echovirus 6 strain prevalent in Shandong, China. Although the linkage to China was not revealed from this study, the phylogram would be useful as a reference for the future investigations and studies in Thailand.

Limitations

Specimens could not be collected from some close contacts, which might have failed to detect some epidemiologically linked cases or asymptomatic infections. Long working period for laboratory process might result in delayed case detection and affect the control measures.

Lack of official health care network between public and private sectors caused challenges in referral and consulting systems. Reluctance among some local staff, teachers and parents adversely affected the cooperation for disease control as well as delay in specimen collection for laboratory confirmation within the appropriated time period.

Public Health Recommendations

Communities

Regular cleaning of glasses, dining utensils and toys should be performed with prohibiting sharing of materials among students. Hand washing behavior should be promoted in the school under supervision of the teacher before meals and after using toilet.

Close monitoring of cases, especially among under five children, should be carried out in schools and day-care centers to prevent further transmission. Health education highlighting the importance of the disease should be performed among local staff, teachers and parents. The disease surveillance should be strengthened in schools, in terms of conducting daily intensive health screening, isolating sick children and giving advice to keep infected children at home.

Health Sectors

Physician's awareness should be raised for echovirus among children who come with neurological symptoms as well as URI symptoms. The guideline for clinical case management of severe enterovirus infection should be developed and distributed to all health services. Importance of echovirus infection and consultation of atypical clinical infection to infectious disease experts in specialized areas should be accessible among medical personnel in both public and private medical centers to achieve correct diagnosis. Availability of intravenous immunoglobulin treatment for echovirus infection should be informed to pediatricians.

Surveillance in the communities should be improved for disease detection, prevention and referral by establishing a network between epidemiologists and pediatricians. Public education about echovirus infection through various media should be implemented as well.

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