Enterovirus D68 (EV-D68) is a subtype of Enterovirus enterovirus D within the family Picornaviridae. It was first isolated in the United States in 1962 from respiratory specimens obtained from 4 pediatric patients with pneumonia and bronchiolitis (1). Since then, EV-D68 infections have occurred in small numbers and accounted for only 26 reports among enteroviruses detected in the United States during the 36-year surveillance period from 1970–2005 (2). Between 2008–2010, clusters of EV-D68 emerged in the Philippines, Japan, the Netherlands, and the United States (3). EV-D68 infections have also appeared in Italy (2010–2012), France (2009–2010), China (2009–2012), New Zealand (2010), Great Britain (2009–2010), Kenya (2008–2011), and Thailand (2009–2011) (4–12). A large outbreak in the United States and Canada in 2014 affected many more than the 1,100 laboratory-confirmed cases (13). EV-D68 strains found in the United States since 2009, when it was first identified in Thailand, and compiled records of clinical manifestations in children with confirmed EV-D68 infection. From 837 samples, 5 samples (0.6%) tested positive for EV-D68. All patients presented with viral pneumonia and required hospitalization. Phylogenetic analysis of the VP4/VP2 regions revealed that EV-D68 strains circulating in Thailand between 2012 and 2014 were closely related to strains reported in Japan, United Kingdom, China, and France. Continued surveillance of probable EV-D68-associated severe respiratory tract infection and the development of a rapid diagnostic test for EV-D68 are essential in supporting awareness and facilitating disease prevention and control.

SUMMARY: Enterovirus D68 (EV-D68) is associated with severe lower respiratory tract infection and neurological abnormalities including acute myelitis and cranial nerve dysfunction. To determine whether an increased incidence of EV-D68 occurs in Southeast Asia, we retrospectively tested specimens collected from Thai pediatric patients who were less than 5 years of age and presented with acute respiratory tract infections between 2012 and 2014. Reverse transcription-polymerase chain reaction and nucleotide sequencing of the 5'UTR/VP2 region were used to identify EV-D68. We also examined the epidemiological pattern of EV-D68 since 2009, when it was first identified in Thailand, and compiled records of clinical manifestations in children with confirmed EV-D68 infection. From 837 samples, 5 samples (0.6%) tested positive for EV-D68. All patients presented with viral pneumonia and required hospitalization. Phylogenetic analysis of the VP4/VP2 regions revealed that EV-D68 strains circulating in Thailand between 2012 and 2014 were closely related to strains reported in Japan, United Kingdom, China, and France. Continued surveillance of probable EV-D68-associated severe respiratory tract infection and the development of a rapid diagnostic test for EV-D68 are essential in supporting awareness and facilitating disease prevention and control.
commercial Viral Nucleic Acid Extraction Kit (RBC Bioscience, Taipei, Taiwan) and cDNA synthesis using random hexamers was performed. Semi-nested PCR using a primer set specific for enterovirus 5’UTR/VP2 region was performed as previously described (6,22). After nucleotide sequencing, fragments were assembled using SeqMan DNASTAR software (v5.0) and aligned with reference strains from GenBank using CLUSTAL W with BioEdit software (v7.0.9). The phylogenetic tree of the VP4/VP2 regions was generated using the neighbor-joining method implemented in MEGA software (v6) with 1,000 bootstrap replicates. Epidemiological data of EV-D68 since 2009 were compiled, and clinical manifestations of children with confirmed EV-D68 infection during 2012–2014 were evaluated. Five out of 837 samples (0.6%) tested positive for EV-D68. The review of clinical charts showed that the patients from whom the EV-D68-positive samples were obtained were hospitalized for viral pneumonia (Table 1). One patient (TH-CB103) required mechanical ventilation and 1 patient (TH-TU45) was afebrile. Three patients had no prior episodes of wheezing, while 2 had histories of recurrent wheezing. None had neurological symptoms, and no co-infections with other respiratory viruses were detected. All patients recovered from their illness. To examine the epidemiological trend of EV-D68 in Thailand since 2009, we compiled the available data (Fig. 1). The observed prevalence of EV-D68 was 0.9% in 2009 (5/584), 1.6% in 2010 (10/611), 4.3% in 2011 (10/232), 0% in 2012 (0/238), 0.9% in 2013 (2/232), and 0.8% in 2014 (3/367). The highest prevalence was in 2011. The seasonal pattern of EV-D68 in-

Table 1. Clinical characteristics of the 5 hospitalized cases due to EV-D68 infection in Thailand during 2012–2014

| Patient | Strain | Age | Sex | Sample | Date of collection | Symptom | Diagnosis | Severity | Accession number (5’UTR/VP2) |
|---------|--------|-----|-----|--------|-------------------|---------|-----------|----------|-------------------------------|
| 1       | TH-CB103 | 4   | M   | Tracheal suction | 27-Sep-13 | Fever, Cough, Runny nose, Dyspnea, Wheezing, Respiratory failure | Viral pneumonia | Required intubation and mechanical ventilation | KR080363 |
| 2       | TH-CB108 | 4   | M   | NP suction | 27-Sep-13 | Fever, Cough, Runny nose, Dyspnea, Wheezing | Viral pneumonia | Required hospitalization | KR080364 |
| 3       | TH-TU44  | 3   | M   | NP suction | 11-Dec-14 | Fever, Cough, Vomiting, Wheezing, Chest retractions | Viral pneumonia | Required hospitalization | KR080360 |
| 4       | TH-TU45  | 2   | F   | NP suction | 13-Nov-14 | Cough, Runny nose, Wheezing (third episode) | Viral pneumonia | Required hospitalization | KR080361 |
| 5       | TH-TU48  | 1   | F   | NP suction | 13-Dec-14 | Low grade fever, Cough, Runny nose, Vomiting, Wheezing (fourth episode), Dyspnea, Chest retraction | Viral pneumonia | Required hospitalization | KR080362 |

![Fig. 1. (Color online) Seasonal distribution of EV-D68 in Thailand between 2009 and 2014. Bar graphs showed the number of cases with confirmed EV-D68 infection. The annual rainy season is generally between June and October.](image-url)
Fig. 2. Phylogenetic relationship of EV-D68 detected among Thai pediatric patients during 2009–2014. The trees were constructed from nucleotide alignments of VP4/VP2 using neighbor-joining method and MEGA program. The genetic distances were calculated according to the Kimura-parameter model. Bootstrap support values of 1,000 pseudo-replicates are indicated at the branch nodes. Squares denote samples from this study, while circles indicate strains previously reported from Thailand between 2009 and 2011. Triangles denote strains from the 2014 outbreaks in the United States, Canada, and China.
Infection was similar to that of the influenza virus as it generally peaked during the rainy season (between May and October). Interestingly, several cases also appeared in the drier months (between November and April). Phylogenetic analysis of the VP4/VP2 region showed that most EV-D68 TH-strains clustered into group 1, along with the EV-D68 strains found in 2014 in the United States, Canada, and China (Fig. 2). Furthermore, EV-D68 TH-strains identified more recently in 2013 and 2014 were very closely related to the EV-D68 reported in China in 2014 (GenBank accession number KP240936). Whole genome sequences of EV-D68 identified in Thailand in 2011 confirmed that it closely resembled strains in the 2014 US outbreak (23). In addition, alignment of the nucleotide sequence of the 5′-UTR region among the 5 TH-strains shown in Table 1 revealed a 23-nucleotide deletion at positions 682–704 and a 12-nucleotide deletion at positions 721–732 (data not shown).

In recent years, the circulation of EV-D68 has risen worldwide as exemplified by the outbreak in the United States. Our study examined the prevalence of EV-D68 in Thailand in children of less than 5 years of age, as they appeared to be the most vulnerable to infection (13). The EV-D68 prevalence of 0.6% found in Thailand between 2012 and 2014 (and 0.8% overall) is lower than the rates reported in Europe in recent years: 7.7% in Germany (24), 2% in the Netherlands (16), 17% in Norway (14), and 11% in Denmark (15). Our data suggest that the prevalence of EV-D68 in Thailand varies annually between 0.4–4.3% (mean = 1.5%) and slightly above the 0.45% found in China (7) and 0.87% in Japan (11).

EV-D68 infection has consistently been associated with severe respiratory infection and occasionally severe bronchospasm (13,25). Several Thai children with EV-D68 had wheezing and all presented with severe bronchospasm at the time of their first hospital visit. These children, however, had no neurological symptoms, unlike those in reports from Colorado and California (20,21). Additionally, EV-D68 infection in Thailand paralleled peak influenza outbreak during the rainy season, which differs from the EV-D68 pattern observed in the summer and autumn elsewhere (26,27).

Although phylogenetic analysis of the VP4/VP2 region showed that EV-D68 TH-strains in 2009–2011 clustered with those circulating in the United States in 2014 (28), recent Thai strains from 2013–2014 were more closely related to strains isolated in Japan, the United Kingdom, China, and France. The viral genome also revealed 2 deletions at the 5′-UTR spacer region between the end of the internal ribosome entry site and the beginning of the polyprotein open reading frame. These same deletions have been found in EV-D68 isolated in South Africa (29), China (30), and the United States (28). Whether these deletions affect the initiation of translation and virulence will require further investigation. Continued monitoring for severe respiratory tract infection especially in children may help control EV-D68 and other emerging enteroviruses.

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Conflict of interest None to declare.

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