Title

Molecular epidemiology of Enterovirus D68 from 2013-2014 in the Philippines

Byline

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Running Title

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Abstract

Enterovirus D68 (EV-D68) has been recognized as an important cause of acute respiratory infections. Here we report a molecular epidemiology of EV-D68 in the Philippines from 2013 to 2014; we found cases in areas affected by the Typhoon Haiyan, and new strains in the country.
Enterovirus D68 (EV-D68) is a member of the Picornaviridae family and is primarily associated with respiratory illness (1). After its discovery in 1962 (2), EV-D68 has been detected only sporadically among patients with respiratory illness until recently (3). Since 2004, the report of EV-D68 has increased worldwide: the Philippines, Japan, Thailand, the Netherlands, the United States, New Zealand and Kenya (4-8). Besides, the United States and Canada have continued to experience a significant EV-D68 outbreak since August 2014, which has resulted in many severe infections, particularly among children (9). However, there have been few reports of the virus in other countries in 2013 and 2014.

Nasopharyngeal swab samples were collected from pediatric patients hospitalized with severe acute respiratory infection (sARI) at three hospitals in the Philippines: Eastern Visayas Regional Medical Center (EVRMC; Tacloban City, Leyte); Biliran Provincial Hospital (BPH; Naval, Biliran); and Ospital ng Palawan (ONP; Puerto Princesa City, Palawan) between September 2012 and February 2014. Nucleic acids were extracted from all samples and tested for respiratory viruses: EV-D68, adenovirus, cytomegalovirus, human metapneumovirus, influenza virus, parainfluenza virus, respiratory syncytial virus, rhinovirus, and other enteroviruses (including coxsackievirus, echovirus and polio virus), by (reverse transcription) polymerase chain reaction using previously described methods with slight modifications (10). We targeted the 5′ untranslated region of the EV-D68 genome for detection and the VP1 gene of the virus for sequencing analysis. The phylogenetic tree based on the genetic sequence of a partial VP1 region (542 nucleotides) was
We identified 20 EV-D68-positive cases among 1,854 hospitalized patients with sARI. The most common viral pathogens identified among sARI cases were respiratory syncytial virus (29.3%) followed by rhinovirus (14.0%) and influenza virus (5.1%). The detection rate of EV-D68 in this study was 1.0% (20/1,854). Age of EV-D68-positive patients range from 1 month-old to 4 year-old (median 14 month-old). During the study period, the Typhoon Haiyan (Yolanda) hit the Philippines on November 8, 2013 (12), which caused extensive damage to large areas of the country, including some of our study sites. Our study sites included EVRMC and TCHCC in Tacloban City, which is located on Leyte Island, one of the most severely damaged municipalities by the typhoon. It should be noted that our research projects were temporarily discontinued after the typhoon; thus, we were unable to collect continuous samples in this region (Fig. 1). Interestingly, we found 13 positive cases after the typhoon hit. It is, however, unclear whether the typhoon had any impact on EV-D68 circulation.

In terms of co-infection, we found 3 cases positive for both EV-D68 and respiratory syncytial virus. One patient co-infected with the viruses (2 month-old) died during the study period. All the other patients who were infected with EV-D68 recovered. Moreover, patients with asthma tend to develop severe symptoms from EV-D68 infection (9, 13). During this study period, 35% (7/20) of EV-D68-positive sARI patients exhibited wheezing.
Next, we constructed a phylogenetic tree to compare the VP1 gene sequences among the EV-D68, which showed that the samples were divided into two distinct sublineages: A11 (in lineage 3) and PL13 (in lineage 2) [Fig. 2, numbering of lineages was described in (14)]. All EV-D68-positive samples collected before May 2013 were classified into the A11-sublineage, which also consisted of strains from Thailand and China in 2011 and 2012. However, EV-D68-positive samples after October 2013 formed a distinct sublineage, PL13. Although the PL13-sublineage was closely related to the strains in Thailand and China in 2011 and 2012, this sublineage was clearly distinct. Among our samples, we found no obvious clustering by study site. As mentioned above, the PL13-sublineage emerged in October 2013 and all strains detected thereafter were classified as PL13. Notably, we found amino acid substitutions in the predicted antigenic sites of the VP1 gene in the PL13-sublineage; alanine at position 98 (98A) in the BC-loop and valine at position 148 (148V) in the DE-loop. Furthermore, sequence data of seven strains from the current 2014 outbreak in North America became available as of October 2014 (15). One of them was classified into the A11-sublineage (in lineage 3), whereas the 6 others were in lineage 2 (Fig. 2). Four of the 6 strains in lineage 2 clustered near PL13-sublineage and 2 formed a distinct cluster. Any of strains from the United States does not seem like a direct descendant of PL13-sublineage. Therefore, the phylogenetic analysis did not support the idea that PL13-sublineage viruses, from the Philippines in 2013 and 2014, were transmitted to the United States leading to the ongoing wide-spread outbreak. Interestingly, strains in the PL13-sublineage and some American strains in 2014 share common mutations in the antigenic sites mentioned above (Fig. 2).
In this study, we reported the detection and molecular analysis of EV-D68 in the Philippines from 2013 to 2014. Our analyses revealed current situation of EV-D68 infection in the country. EV-D68 has been circulating in the population and we found a large number of infections in post-disaster setting. We also detected emergence of new strains (PL13-sublineage) in the latter part of 2013. To better understand infectious disease outbreaks in post-disaster settings and associations between local and global circulation of viral pathogens, continuous global surveillance is necessary.
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Figure legends

Figure 1. Monthly distribution of EV-D68-positive cases at each study site. The total number and number of EV-D68-positive cases are shown. Arrows indicate the occurrence of the Typhoon Haiyan. The genetic sublineages of EV-D68 are shown in Figure 2.

Figure 2. A phylogenetic tree of EV-D68 strains in the Philippines and reference strains. Significant mutations (98A and 148V) mentioned in the body are shown next to nodes. Bootstrap values >70% (in 1000 tests) are shown next to the branches. Black circle, square, and rhombus indicate strains included in this study collected from Leyte, Biliran, and Palawan, respectively. Sequence data have been deposited to the GenBank database under the accession numbers AB992413–AB992443. The white triangles indicate strains previously reported from the Philippines in 2001 and 2008. The gray triangles indicate strains in the United States in 2014.