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LETTER TO THE EDITOR

Enterovirus D68 detected in children with severe acute respiratory illness in Brazil

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Dear Editor,

In recent years, enterovirus D68 (EV-D68) infection has been increasingly associated with clusters of respiratory illness worldwide[1]. Since its first isolation from hospitalized children with lower respiratory infection in California in 1962[2], relativity small numbers of EV-D68 cases have been reported. In August 2014, outbreaks of respiratory illnesses of varying severity occurring among young children in the USA were confirmed to be due to EV-D68[3]. Infection clinically presented as an influenza-like illness, and severe symptoms including wheezing and breathing difficulties were reported. Children with asthma have been identified as a risk group for severe illness[4,5]. Additionally, concerns have been raised regarding the etiological role of EV-D68 in neurological complications observed in some cases[6]. Here, we report two cases of EV-D68 infection in children with severe acute respiratory illness in 2009; to the best of our knowledge, this is the first report of the virus circulating in Brazil.

A total of 594 nasopharyngeal specimens collected from children (0–12 years of age) presenting with respiratory illness during the 2009–2010 pandemic year and referred to the Laboratory of Respiratory Viruses (Oswaldo Cruz Institute/Fiocruz, Rio de Janeiro, Brazil) as part of the national influenza surveillance program were selected for further investigation. Samples were tested by FTD Respiratory Pathogens 21 PLUS (Fast Track Diagnostics, Luxembourg, Belgium) a multiplex real-time reverse transcription-polymerase chain reaction (PCR) assay for the detection of 21 respiratory pathogens. Samples that were positive for enterovirus by the multiplex assay were submitted for PCR amplification and sequence analysis of the viral protein (VP1) gene using primer pairs 222 and 229[7]. Sequences were assembled and edited using the Sequencher software, a BLAST query was performed, and a phylogenetic tree was constructed through MEGA 6.

Partial gene sequences of the VP1 gene were deposited in the GenBank database under accession numbers KT023106 and KT023107.

Of the 594 samples tested, 18 (3%) samples were enterovirus positive. Two samples were identified as EV-D68 positive based on VP1 gene target sequencing. Both samples were negative for influenza A, influenza A(H1N1)pdm09, and other respiratory viruses by the multiplex assay. Samples had 99% similarity to EV-D68 sequences available in GenBank, and phylogenetic analysis of the Brazilian strains compared to strains circulating worldwide placed them within major group 3.

EV-D68-positive patient 1 was a four-year-old boy admitted to Hospital Aliança, Salvador, Bahia on the 21st October 2009 with a three-day history of an afebrile cough and runny nose that evolved into respiratory distress. The child attended the emergency department of the referral hospital with respiratory failure, a low-grade fever (37.9 ℃) and diarrhea. The child had contracted meningitis in the first year of life and had suffered from neurologic sequelae with late neuropsychomotor development. He was transferred to the pediatric intensive care unit on 22nd October 2009 with psychomotor agitation, respiratory symptoms with wheezing, rawls in both lungs, intercostal and sub-diaphragmatic retractions, and hepatomegaly. His leukocyte count was 16 000 without a left shift (aspartate aminotransferase = 110, alanine transaminase = 144, glucose = 183 mg/dL and urea = 22 mg/dL). The chest radiograph showed bilateral interstitial infiltrates. The patient received oseltamivir and clarithromycin for five days. The respiratory symptoms gradually improved, and the patient was transferred to the pediatric ward on the 25th October 2009 and discharged in good medical health on 27th October 2009.

Patient 2 was a boy, 2 months old, admitted to the intensive care unit of Ernesto Simões Filho Hospital, Salvador, Bahia on 22nd October 2009 in a debilitated state with a five-day history of fever, acute influenza-like symptoms, and tachycardia. The child was treated with oseltamivir and vasoreactive drugs, and he received packed red blood cells. He was intubated after a cardiopulmonary arrest, which was reversed after resuscitation. The respiratory symptoms gradually improved, and the patient was transferred to the pediatric ward on 9th November 2009, where he remained in good general health until 10th December 2009, when he was medically discharged.

EV-D68 has been recently reported in other South American countries[8,9] and worldwide[1]. The impact of EV-D68 as an agent of infection in small outbreaks of respiratory illness may be underestimated in South American countries, both in hospitalized patients and in the community. Although the clinical spectrum of illness remains unclear, seasonal surveillance of EV-D68 has also associated infection with mild illness[10]. The cases of EV-D68 infection described here both occurred in 2009 and were due to viruses placed phylogenetically within major group 3, which contains strains recently reported from around the world[11]. Both cases described here had severe acute respiratory illness; however, a full clinical investigation was not within the

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scope of this study. Due to the emergence of the virus in the 2014 outbreaks in the USA and the reported severity of the illness, more systematic testing should be conducted on recent specimens to identify possible outbreak clusters in Brazil, and future works will aim to establish a more comprehensive clinical picture of the illness. Rapid detection strategies can aid the surveillance of emerging EV-D68 strains, contributing to and strengthening existing respiratory virus surveillance programs, as well as guiding the early mobilization of resources and control measures when necessary.

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