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Hand, foot, and mouth disease outbreak by Coxsackievirus A6 during COVID-19 pandemic in 2021, São Paulo, Brazil.

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ABSTRACT

Introduction: Hand, foot, and mouth disease (HFMD) is an acute febrile illness characterized by fever; sore throat; and vesicular eruptions on the hands, feet, and oral mucosa. Outbreaks of HFMD in children aged <5 years have been reported worldwide and the major causative agents are Coxsackievirus (CV)A16, enterovirus (EV)-A71 and recently CVA6.

Aim and methods: The aim of this study was to investigate a large outbreak of Hand, foot, and mouth disease during COVID-19 pandemic in 2021 from clinical samples of 315 suspected cases, in São Paulo State, Brazil. Diagnostic evaluation was performed by RT-qPCR, culture cell isolation and serological neutralization assay. EV-positive were genotyped by partial VP1 genome sequencing.

Results: One hundred and forty-nine cases analyzed were positive for enterovirus (47.3%; n = 149/315) by neutralizing test (n = 10 patients) and RT-qPCR (n = 139 patients), and identified as CVA6 sub-lineage D3 by analysis of VP1 partial sequences.

Conclusions: This finding indicated the reemergence of CVA6 in HFMD, soon after the gradual easing of non-pharmaceutical interventions during-pandemic COVID-19 and the relevance of continued surveillance of circulating enterovirus types in the post-COVID pandemic era.

1. Introduction

Non-Polio Enterovirus infections (NPEV) are associated with a wide spectrum of illnesses, including mild diseases like a febrile illness until severe neurological disorders [1,2]. Hand, foot and mouth disease (HFMD) is a common infectious disease that occurs most often in children aged <5 years [3]. In most cases, the disease is mild and self-limiting, with common symptoms including fever, sore throat, and vesicular eruptions on the hands, feet, and oral mucosa. However, more severe symptoms such as meningitis, encephalitis, polio-like paralysis and myocarditis may occur [3]. Enterovirus (EV)-A71 and coxsackievirus (CV)A16 were the most frequent serotypes involved in HFMD outbreaks throughout the world [4]. However, CVA6 has emerged as a new important pathogen worldwide and more severe and extensive dermatologic presentations has been reported [4]. In early 2020, after the declaration of the COVID-19 pandemic by the World Health Organization [5], non-pharmaceutical interventions (NPI) were implemented in Brazil to reduce and contain the disease [6]. These measures adopted, in addition to reducing the prevalence of COVID-19 also reduced reported cases of diverse infections caused by virus, included HFMD. However, in 2021 through the gradual return of Child Daycare Centers and Preschool activities, HFMD outbreaks have been reported in the São Paulo State, Brazil. HFMD is not a reportable disease in Brazil, but notification of outbreaks is mandatory. The aim of this study was to investigate the outbreaks of HFMD that occurred in São Paulo State, during the COVID-19 pandemic.

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2. Methods

During January and December 2021, 613 HFMD outbreaks were reported in the São Paulo State in the Notifiable Diseases Information System (SINAN), Brazilian Ministry of Health, with 4,718 related cases (Fig. 1). Clinical samples from 315 patients with suspected HFMD were sent to the Enteric Diseases Laboratory, Adolfo Lutz Institute, Reference São Paulo State for Enterovirus Laboratory Surveillance (see Supplementary material, Table 1).

We prospectively analyzed 313 clinical samples (163 stool samples and 150 nasopharyngeal/oropharyngeal swabs) by Reverse transcription-quantitative Polymerase Chain Reaction (RT-qPCR) (see Supplementary material, Table 1). Samples were also inoculated in culture cell line human rhabdomyosarcoma (RD, CCIAL-039, World Federation for Culture Collection) and RT-PCR was applied to amplify a partial region of viral protein 1 (VP1) and subsequently identify EV types by partial VP1 sequencing [7]. In addition, paired serum samples

Table 1

| HFMD | Age group (year) | Case-Patients n | Hospitalization n |
|------|------------------|-----------------|------------------|
|      | <01              | 217             | 0                |
|      | 01 to 04         | 4387            | 12               |
|      | 05 to 09         | 57              | 0                |
|      | 10 to 14         | 4               | 0                |
|      | 20 to 29         | 9               | 0                |
|      | 30 to 49         | 13              | 0                |
|      | 50+              | 3               | 0                |
|      | NI               | 28              | 0                |
|      | Total            | 4,718           | 12               |

NI: No information.

Source: Notifiable Diseases Information System (SINAN), outbreak module. Data update 03-24-2022.

Fig. 1. Distribution of HFMD outbreaks (A), case-patients (B) and case-patients by age (C) in the State of São Paulo, between 01-01–2021 and 12–31–2021.

Fig. 2. The Maximum Likelihood tree based on partial VP1 nucleotide sequences of CVA6 (nt 2569 to 2892 according to prototype Gdula strain AY421764). The numbers at nodes (>70% were indicated) represent the percentage of 1000 bootstrap replicates that supported the distal cluster. GenBank accession numbers and location as well as year of isolation are included. Scale bar indicates branch distances per 100 nucleotides. A difference of at least 15% in the partial VP1 region was used to distinguish sub-lineage. Representative strains isolated in this study are marked with black circles indicate strains from São José do Rio Preto region; gray circles – São Paulo region; white circle Ourinhos region and withe square other Brazilian isolates.
had an important effect on other communicable diseases such as HFMD outbreaks began soon after the gradual easing of NPIs by Brazilian government authorities.

The widespread circulation of NPEV, the epidemic pattern of EV infections and the recent types EV involved in severe neurological conditions warrant reinforcement of the surveillance of EV infections [10]. This study shows that a syndromic and laboratory surveillance network are of value for timely detect outbreaks HFMD, monitor circulating emergent types EV, and recommend control measures that prevent and contain the spread of disease.

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Ethical statement

The planning, conduct, and reporting of human research reported in this manuscript are in accordance with the Helsinki Declaration as revised in 2013. Approval to conduct research was obtained from Adolfo Lutz Institute Human Research Ethics Committee, São Paulo, Brazil.

Declaration of Competing Interest

The authors declare do not have any competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.jcv.2022.105245.

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