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Short communication

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

Rita C.C. Carmona [a,*], Bráulio C. Machado [a], Fabricio C. Reis [a], Adriana M.V. Jorge [a], Audrey Cilli [a], Amanda M.N. Dias [a], Daniele R. Morais [a], Lucas Leme [a], Ana L.F. Yu [c], Marcela R. Silva [c], Telma R.M.P. Carvalhanas [c], Maria C.S.T. Timenetsky [b]

[a] Enteric Diseases Laboratory, Virology Center, Adolfo Lutz Institute, São Paulo, Brazil
[b] Virology Center, Adolfo Lutz Institute, São Paulo, Brazil
[c] Divisão de Doenças de Transmissão Respiratoria, Centro de Vigilancia Epidemiológica "Prof. Alexandre Vranjac", Secretaria de Estado da Saúde de 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 investigated 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 Sao Paulo State, during the COVID-19 pandemic.

* Corresponding author at: Instituto Adolfo Lutz, Centro de Virologia, Núcleo de Doenças Entéricas. *Av.* Doutor Arnaldo, 355 | CEP 01246-902| Pacaembu, SP, Brazil
E-mail address: rita.carmona@ial.sp.gov.br (R.C.C. Carmona).

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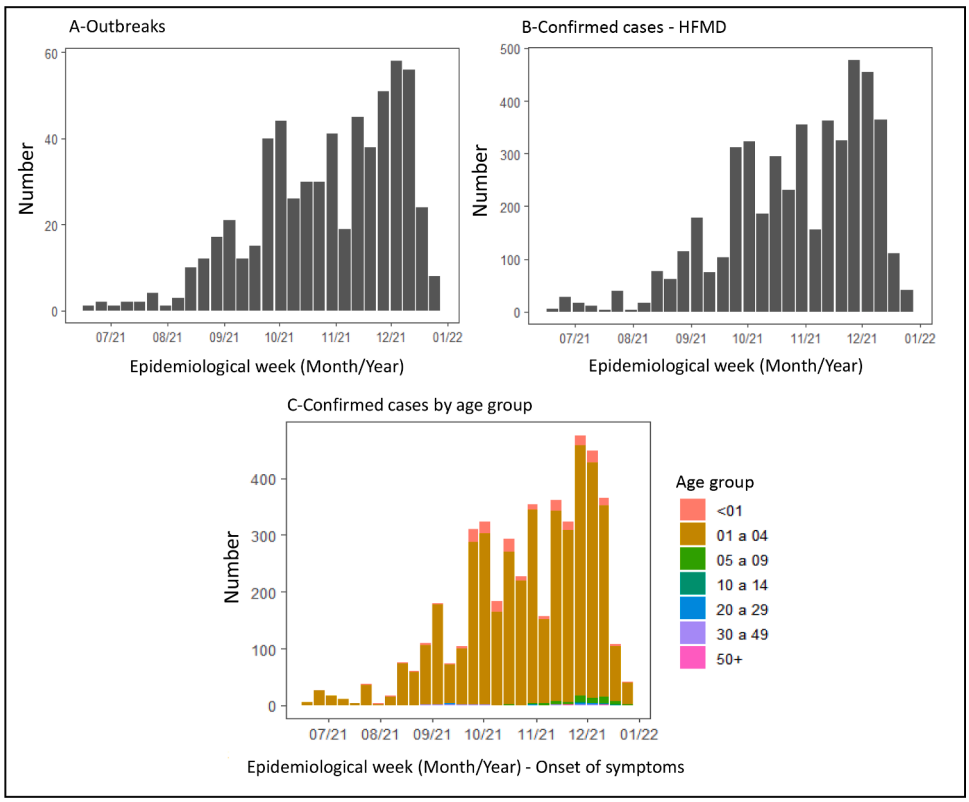

Fig. 1. Distribution of HFMD outbreaks (A), case-patients (B) and case-patients by age (C) in the State of São Paulo, between 01-01–2021 and 12–31–2021.

Table 1
Total number of HFMD case-patients and hospitalization notified by age group.

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.

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

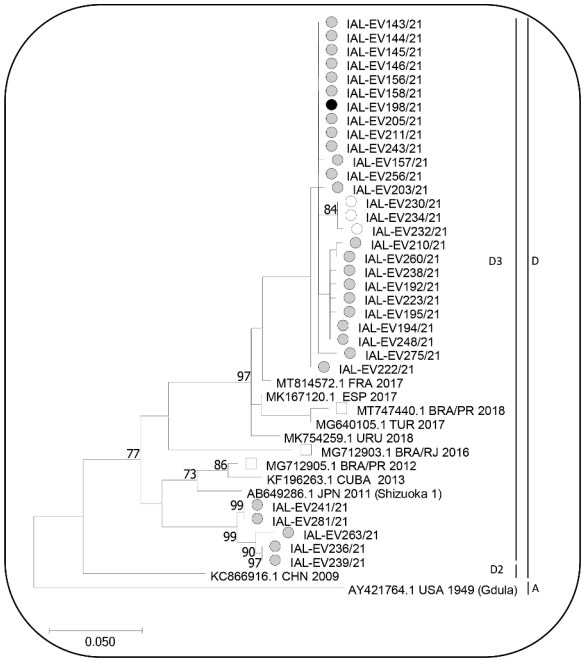

Fig. 2. The Maximum Likelihood tree based on partial VP1 nucleotide sequences of CVA6 (nt 2569 to 2892 according to prototype Gdula strain AY421764). The numbers at nodes (>70% were indicated) represent the percentage of 1000 bootstrap replicates that supported the distal cluster. GenBank accession numbers 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.

were collected from 51 patients to test for the presence specific neutralizing antibodies or seroconversion against EV-A71, CVA16 and CVA6 [8] (see Supplementary material, Table 1).

3. Results

Throughout the study period, 44.4% ($n = 139/313$) samples (stool or NPS or OPS) were positive for enterovirus by RT-qPCR and cell cultures isolation. The overall seroconversion rate for CVA6 was 27.5% (14/51) over the follow-up period. Among the 315 cases of HFMD with laboratory analysis, 47.3% ($n = 149/315$) cases were positive for enterovirus by neutralizing test ($n = 10$ patients with seroconversion) and RT-qPCR ($n = 139$ patients).

Among the reported cases of HFMD, 2,558 (54.2%) were males while 2,160 (45.8%) were females; mean and median age of 2.2, respectively. Twelve case-patients were hospitalized. There were no deaths during the study period (Table 1). EV-positive were typed as CVA6 by partial VP1 genome sequencing. The phylogenetic analysis of the CVA6 genotypes was performed by aligning the sequences obtained in this study ($n = 31$) and closely related sequences obtained through Basic Local Alignment Search Tool (BLAST) search ($n = 11$) (see Supplementary material, Table 2), being selected those with a percentage of identity $> 85\%$. According to the analysis, it was possible to observe the formation only 1 clade distinct sub-lineage (D3), including sequences isolated in this study (85–90%) to the CVA6 D3 shizuoka_1 prototype strain isolates in Japan (Fig. 2). Cluster D3 was composed of strains isolated in France, Spain, Turkey, Uruguay, Cuba and Brazil (Rio de Janeiro and Parana States). It was possible to observe the formation of a sub-cluster grouping the isolates IAL-EV241/21; EV281/21; EV263/21; EV236/21 and EV239/21, from the southern region of São Paulo city, sharing the same amino acid substitution at position S911T. In addition, the isolate IAL-EV263/21 also showed an amino acid substitution D941N. Nucleotide sequences determined in this study have been deposited in GenBank under the accession numbers ON012609 - ON012639. Some epidemiologic data from cases EV sequenced are available in Supplementary material, Table 3. This is the first report of CVA6 sub-lineage circulation in São Paulo State.

4. Discussion

Brazil's Information System for Notifiable Diseases (SINAN) revealed a large-scale outbreak of HFMD in São Paulo State, in 2021. Laboratory surveillance showed that this outbreak was exclusively associated with the CVA6 D3 sub-lineage. CVA6 strains have been categorized into 4 genotypes designated as A, B, C, and D according data genome sequencing and subdivided into seven sub-genotypes or sub-lineage (B1–B2, C1–C2, and D1–D3). D3 has been the predominant sub-genotype/sub-lineage outbreaks-HFMD in several countries from Asian and European continents [1]. The detection of a single CVA6 lineage is probably due to the health regulations in force during the COVID-19 pandemic, which must have mitigated the circulation of other types of EV. During laboratory surveillance HFMD outbreaks in 2018 and 2019, a wide diversity of EV types was observed, in São Paulo State: 12 EV types (mainly CVA6 and CVA16, followed by E11, CVA1, CVA9, CVA24, E25, EV-C99, CV-A5, E13, E14 and E18) and 7 EV types (mainly CVA6, followed by CVA16, EVA71, EVC-99, EV-C116, E9 and CVA5), respectively (Carmona RCC, unpub. data). In 2020, there were no reports of HFMD outbreaks in Brazil. NPIs helped control the source of infection, prevented transmission and protected susceptible people; they might have also had an important effect on other communicable diseases such as HFMD in addition to COVID-19, consequently leading to a greater susceptibility to infections in children [9,10]. This phenomenon has also been observed in other countries such as recently reported outbreak of HFMD in France in September 2021, respiratory syncytial virus (RSV) infections in Japan in July 2021 and Europe, which was substantially larger and earlier compared with previous years [10]. In Sao Paulo State, 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 laboratorial 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, Sao 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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