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An outbreak of atypical hand, foot and mouth disease associated Coxsackievirus A6 in children from Cape Verde, 2023

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Abstract

Background Rash is a common childhood infection, mainly caused by viruses. Hand, foot, and mouth disease (HFMD), a common viral rash infection, has become one of the most common infectious diseases in Asian countries and caused outbreaks in children and adults worldwide. Following the introduction of enterovirus A71 (EVA71) vaccines, Coxsackievirus A6 (CVA6) has recently emerged. However, the disease is not commonly reported in Africa, where studies are scarce.

Methods In the current study, we focused on the HFMD outbreak that occurred in Cape Verde in July 2023 during field investigations around a cluster of patients with rash and fever. Samples collected from patients were tested using Measles and Rubella-specific immunoglobulin M and quantitative reverse transcription PCR (qRT-PCR) of a panel of viruses causing rashes and subjected to genome sequencing followed by phylogenetic analysis.

Results Eighteen out of the 22 samples were tested positive for CVA6 RNA by real-time RT-PCR, of which two tested also positive for EVA71 and Coxsackievirus A16 (CVA16). Subsequent sequencing revealed that all CVA6 sequences belonged to the D genotype, particularly the D3 sub-genotype recently described in China.

Conclusion Our study uncovers the first-ever reported outbreak of CVA6 associated with atypical HFMD in children from Cape Verde and highlights thus the need to implement an active hospital-based HFMD surveillance in Africa.

Keywords Rash, Enterovirus, Coxsackievirus A6, Children, Cape Verde, Africa, 2023

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Introduction

Viruses are the most common cause of fever and rash [1]. Measles, especially caused by the Morbillivirus, is one of the most devastating infectious diseases in humans, and it was annually responsible for over 6 million deaths during the pre-vaccination period [2]. Furthermore, Rubella that can mimic Measles syndrome can result in death or congenital Rubella syndrome (CRS) in the fetus during pregnancy [3]. Since the introduction of Measles and Rubella vaccination in routine immunization programs, there has been remarkable progress in reducing mortality and morbidity [3]. However, other viruses, including human herpesviruses, parvovirus, enteroviruses (EV), adenoviruses, and SARS-CoV-2 are also pathogens commonly associated with rash [1]. The *enterovirus* (EV), genus of the Picornaviridae family are the most common viral causes of morbilliform eruptions associated with aseptic meningitis, accounting for about 70% of cases [4]. They have also been identified as the causative agent of maculopapular rash and Hand, Foot and Mouth Disease (HFMD) [4]. Although very contagious, HFMD is usually mild and characterized by fever, mouth sores and rashes with papules and blisters on the palms, soles, or buttocks [5]. Generally, the disease is resolved within 7–10 days, but in rare cases, neurological disorders such as encephalitis, aseptic meningitis, acute flaccid paralysis, as well as cardiovascular and respiratory complications can occur [5]. The most common causative agents of HFMD are Coxsackievirus A16 (CVA16) and the enterovirus A71 (EVA71), more often associated with neurological syndromes and sometimes death, while CVA16-associated HFMD is usually mild [6].

The clinical features of CVA6-associated HFMD are slightly different from those caused by other EV. Indeed, CVA6 infections often present with atypical clinical symptoms including more severe and extensive dermatologic manifestations on the trunk, neck, legs, and perioral area, and onychomadesis which causes nail loss [5]. CVA6 strains can be classified into 4 genotypes designated as A, B, C, and D according to previous data and can be further subdivided into B1–B2, C1–C2, and D1–D3 sub-genotypes [7]. While sub-genotype D3 can be further subdivided into two evolutionary branches, which were designated as D3a and D3b [7]. Although outbreaks of HFMD have been reported in many parts of the world, knowledge remains limited on the epidemiological profile of HFMD in Africa, where no data on the incidence, morbidity and mortality of HFMD is recorded in integrated disease surveillance systems or in notifiable diseases to date.

As of 22 July 2023, an alert was received from the Integrated Disease Surveillance and Response Service (SVIR) in Cape Verde, an island nation located at the coast of West Africa, notifying an increasing number of cases with fever and cutaneous rash, mostly in children under 2 years admitted at the pediatrics department of the Dr Agostinho Neto University Hospital (HUAN), municipality of Praia. Suspected for Measles and Rubella, sera and nasopharyngeal swabs from patients with this atypical HFMD syndrome were sent to the Institut Pasteur de Dakar (IPD, Senegal) for diagnostic and sequencing.

Materials and methods

Ethical consideration

The Cabo Verdien National Ethical Committee of the Ministry of Health approved the surveillance protocol that led to the obtention of human sera and nasopharyngeal swabs as less than minimal risk research, and written consent was not required. All human samples were de-identified before any sample characterization and data analysis.

Samples collection

Most of the cases originated from the municipality of Praia and Santiago Island. After an active search, it was confirmed that cases have been firstly identified in the health center in the municipality of Praia and the neighboring municipality of Ribeira Grande at the Santiago Island and later, in the municipalities of Santa Cruz and Tarrafal, two municipalities in the center of the Santiago Island (Fig. 1). A total of 22 samples, collected from 12 patients admitted on 22 July 2023, were sent to the Institut Pasteur de Dakar (IPD). Out of the 12 patients, sera and nasopharyngeal swabs samples were collected from 10 patients. However, one sera and one nasopharyngeal swabs sample were collected from 2 patients.

Laboratory investigations

Measles and Rubella virus screening

Sera and nasopharyngeal swabs were tested for Measles and Rubella-specific immunoglobulin M (IgM) antibodies using WHO-recommended ELISA kits (Enzygnost Anti-Measles-Virus/IgM from Dade Behring or Siemens, Erlangen, Germany) according to the manufacturer's recommendations. Given the similar clinical presentations of Measles and Rubella and the WHO/AFRO Measles surveillance guidance to exclude Measles cases from Rubella screening, sera were first tested for Measles IgM antibody, with Measles-negative and equivocal samples subsequently tested for Rubella-specific IgM antibody.

In addition, all available nasopharyngeal swabs were tested by RT-PCR for the presence of Measles and then for Rubella RNA only if negative for Measles, independent of any serological results. Viral RNA was extracted from 200 µL of sample using the QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the

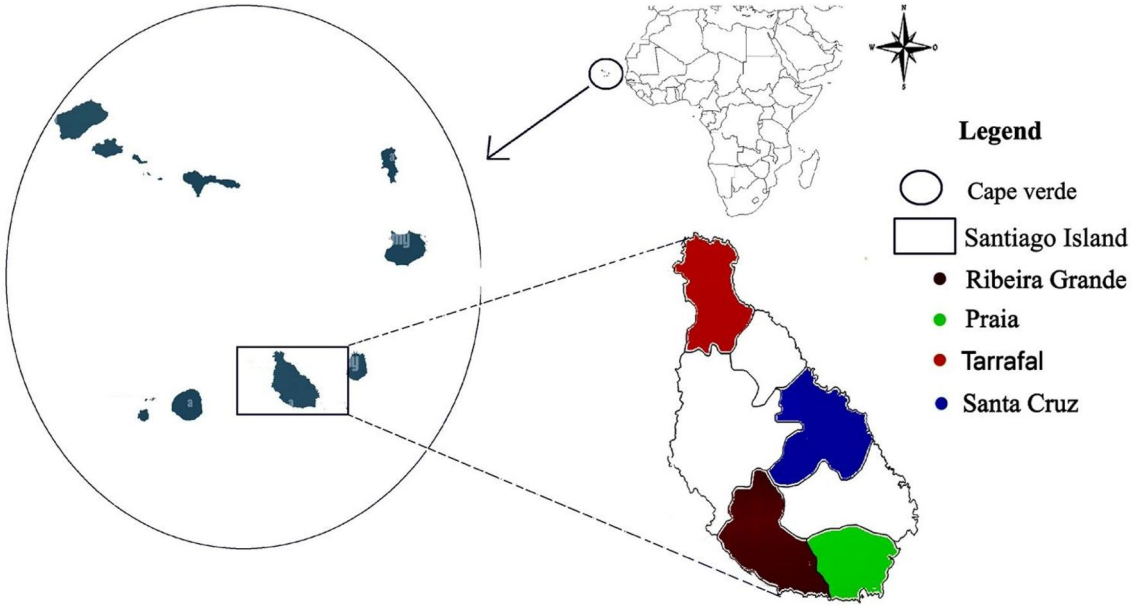

Fig. 1 Map showing Cabo Verde, Santiago Island

manufacturer's instructions. Extracted RNA is eluted in 60 µL of the elution buffer and tested for the presence of Measles or Rubella RNA by real-time RT-PCR as per World Health Organization (WHO) standard protocols [8].

Screening for arboviruses

With the clinical hallmark of fever, both samples were then tested for classical arboviruses, including Chikungunya, Yellow Fever, West Nile, Zika and Dengue, hemorrhagic fever viruses (Ebola, Marburg, Lassa, CCHF and RVF) using specific real-time reverse-transcription polymerase chain reaction (RT-PCR) assays [9], while only sera were screened for anti-IgM antibodies against Chikungunya, Yellow Fever, West Nile, Zika, Dengue, CCHF and RVF by Enzyme-Linked ImmunoAssay (ELISA) testing [10].

Bacterial screening by isolation and qPCR

Sera were analyzed for bacteria using isolation on selective and non-selective media and qPCR targeting *Shigella spp*, invasive *Escherichia coli*, *Yersinia enterocolitica, Vibrio cholerae, Clostridium difficile, Salmonella spp, Shigatoxin-producing E. coli (STEC), Enteropathogenic E. coli enteropathogenic (EPEC), E. coli enterotoxinogenic (ETEC), E. coli enteroaggregative (EAEC.)* [11].

Screening for enteroviruses

The extracted RNA from all samples were tested for EV by real-time RT-PCR using a pan-enterovirus (panEV) assay with the LightMix® Modular Enterovirus 500 kit (Roche-Ref 50-0656-96, TibMolBiol, Berlin, Germany) [13] with the qScript™ XLT One-Step RT-PCR (Quanta Bio, Beverly, MA, USA) according to the manufacturer's instructions. In addition, differential singleplex RT qPCR assays for specific detection of EVA71, CVA6 and CVA16 [14] were performed using specific primers and probes for each virus genus rigorous validate based on their high accuracy (EV-A71, 90.6%; CV-A6, 92.0%; CV-A16, 100%), sensitivity (EV-A71, 96.5%; CV-A6, 95.8%; CV-A16, 99.0%), and specificity (EV-A71, 100%; CV-A6, 99.9%; CV-A16, 99.9%) in testing [14].

In addition, to assess any case of coinfection all samples were screened for Human Herpesviruses (HHV) including Herpes simplex virus 1 (HSV1), Herpes simplex virus 2 (HSV2), Varicella zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human herpesvirus 6 (HHV6) and Human herpesvirus 7 (HHV7) by RT-PCR using the Allplex Meningitis-V1 kit, according to the fabricant's instructions [12].

PCR amplification of Enterovirus Capsid protein-encoding region and sequencing using the Oxford nanopore technology

For viral amplification, cDNA was synthesized from enterovirus-positive RNA using the Protoscript II First Strand cDNA Synthesis Kit 350 (New England Biolabs Inc.) according to the manufacturer's instructions. Amplicons of the entire coding-region of the Capsid protein were then generated using the One*Taq*® DNA

Polymerase kit (New England Biolabs Inc.) with a PCR method targeting this region, as previously described [15].

To determine EV's genotype sequences, the entire coding-region of the Capsid was purified using AMPure XP magnetic beads and quantified using the dsDNA High Sensitivity Kit on a Qubit 3.0 fluorometer (Thermo Fisher). The purified entire-coding-regions of the Capsid were barcoded using the Rapid Barcoding Kit (SQK.110.96) with the MRT001 expansion module (Oxford nanopore technology) and pooled in a single tube. The libraries were then purified and sequenced on a GridIon instrument (Oxford nanopore technology).

Whole genome sequencing

Furthermore, Next-Generation Sequencing with Illumina was applied to all samples for whole genome sequencing. Briefly, the host's background RNA was depleted as previously described [16] using mammal ribosomal RNA-specific primers and RNAseH enzyme (NEB). Purified viral RNA was reverse transcribed into first-stranded cDNA using the Invitrogen SuperScript IV Reverse Transcriptase kit (Thermo Fisher, Waltham, MA, USA). Then, amplicons were tagged using the "index tags" incorporated into the Nextera kit XT DNA Library Preparation kit (Illumina, San Diego, CA, USA) based on the manufacturer's instructions. The obtained libraries were quantified, normalized, pooled, and loaded onto an Illumina MiSeq using the Miseq reagents kit v3 (Illumina, San Diego, CA, USA), according to the manufacturer's protocol.

Passed reads were analyzed using the "Genome Detective Virus Tool" software (version 2.40) (https://www.genomedetective.com/app/typingtool/virus/) and the consensus sequences were analyzed using the online Basic Local Alignment Search (BLAST) program (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on August 25, 2023) to compare the sequence homology with previously available data. In addition, the enterovirus genotype was confirmed using the online Enterovirus Genotyping Tool (RIVM) program (enterovirus/typingtool/, accessed on August 25, 2023).

Phylogenetic and recombination bioinformatic

Multiple alignments of our dataset, including our newly characterized genome sequences and sequences previously available from Genbank (https://www.ncbi.nlm.nih.gov/), were performed using BioEdit version 7.2.5 [17]. The maximum likelihood (ML) phylogenetic trees based on VP1 and near-complete genome sequences were inferred using IQ-TREE (version 1.6.12) [18] for 1000 replications with the GTR+G found as the best substitution model. The ML tree topology was visualized with (FigTree version 1.4.4) [19]. Nodes were supported by Bootstrap values.

To confirm the occurrence of recombination events, similarity plots and bootscan analyses were conducted against sequences closely related by using the SimPlot program, version 3.5.1 [20], with a 400-nt window moving in 20-nt steps and using a Kimura two-parameter method with a transition-transversion ratio of 2 with 1000 resampling. In addition, the RDP4 (Recombination Detection Program version 4) program [21] was also used to detect the potential recombinants confirmed by at least 6 of the 7 selected methods using default settings, including RDP, GENECONV, Maxchi, Siscan, Chimaera, 3Seq and LARD. Full genome sequences were used as queries for BLASTn and sequences with the highest homologies and complete genomes were used in the recombination analysis.

Results

Preliminary clinic and epidemiological data

The initial investigations carried out with the support of the Praia Health Precinct revealed that Symptoms included cutaneous eruptions as erythematous macules and papules, with limited margins, sometimes grouped together, which evolved into crusts, sometimes preceded by vesicles. Skin rashes of varying intensity usually affected large areas and were most often localized on the trunk, upper and lower limbs. Lesions were identified less frequently in the palmar region of the hands, the plantar region of the feet, and the mouth region, affecting mucous membranes and the skin around the mouth. Lesions of the oral cavity appear, usually 2–3 days after the onset of fever (in up to 90% of cases), in the form of erythematous macules that evolve into vesicular lesions and, when they rupture, lead to ulcerated lesions generally located on the tongue, palate and oral mucosa. Sixty percent of the cases had concomitant respiratory symptoms such as cough and/or rhinorrhea.

The disease mainly affects children under 5 years, with a particular incidence in the under 2 age group, affecting both sexes proportionally. Patients were treated in an outpatient clinic. The disease had a benign evolution and the clinical symptoms resolved in 6–10 days with a compensatory treatment. Patients do not usually return for a follow-up visit and no confirmed death was reported.

Clinical and demographic characteristics of confirmed cases

Based on the case-based form, the main clinical signs and symptoms included mainly cutaneous rash (100%; 10/10) and fever (70%; 7/10).

In addition, among the 12 patients's data on gender, 2 patients were missing. Thus, the male to female sex-ratio

was 0.43 (3/7), with a median age of 6 years (ranging from 9 months to 12 years). Eighty percent (80%; 8/10) were under 5 years.

Overall, all sera and nasopharyngeal swabs were tested negative for Measles and Rubella by both specific anti-IgM ELISA and RT-PCR. In addition, no targeted arbovirus, hemorrhagic fever virus, Varicella zoster virus, Epstein-Barr virus or bacteria were detected from the screened samples.

EV was detected in 91.6% (11/12) of suspected cases, corresponding to 18 samples with Ct values ranging from 22 to 27 and 30 to 39 respectively for nasopharyngeal swabs and serums. As suspected cases, the median age among positive cases was 6 years (ranging from 9 months to 12 years) with a male–female sex-ratio of 0.43 (3/7).

HHV7 and CMV were detected in 33.3% (4/12) and 8.3% (1/12) patients respectively, including only nasopharyngeal swabs. In addition, coinfection of EV and HHV7 was noted with 18.2% (n = 4), followed by EV and CMV with 4.5% (n = 1) of samples. Among EV genotypes, the coinfection of CVA6 and CVA16 with 16.6% (n = 2) and the coinfection of CVA6 and EVA71 with 4.5% (n = 1) was also observed. However, Ct values for HHV7, CMV, CVA16 and EVA71 ranged from 32 to 39 (Table 1).

As investigated, the case-based form indicates that the confirmed case had cutaneous rash (100%; 10/10) followed by fever (70%; 7/10). Non-dermatological clinical signs were also found in some patients, including cough (20%; 2/10) one of which is co-infected CVA6, HHV7 and Rhinitis (10%; 1/10) in CVA6 infection (Table 1).

Enterovirus sequencing

Overall, 9 entire coding-regions of the Capsid protein (≈ 4000 pb) and 2 sequences of the VP1-VP3 region (≈ 800 pb) were generated out of the 18 EV-positive samples, including only nasopharyngeal swabs, using the Oxford nanopore technology. The generated EV sequences correspond to EVA species and CVA6 genotype based on BLAST and RIVM analysis. However, any whole genome sequences were generated with Illumina.

The obtained sequences were deposited in the GenBank database under the accession numbers (Table 1).

Table 1 Laboratory results of all suspected cases by age, gender, occurrence of symptoms and GenBank accession numbers of the newly characterized sequences of CVA6

Sample ID	Specimen type	Pathogens detected	Age (month)	Sex	Clinical characteristics	GenBank accession no
CAV-2023-004	N.Swab	CVA6, CVA16	10	M	Cutaneous rash, fever, rhinitis, cough	OR778598
	Serum	CVA6				
CAV-2023-005	N.Swab	CVA6, HHV7	9	F	Cutaneous rash, cough	OR778599
CAV-2023-006	N.Swab	CVA6, CMV	36	F	Cutaneous rash, fever	OR778600
	Serum	CVA6				
CAV-2013-007	N.Swab	CVA6	12	M	Cutaneous rash, fever	OR778601
	Serum	CVA6				
CAV-2023-008	N.Swab	CVA6	48	F	Cutaneous rash, fever	OR778602
	Serum	CVA6				
CAV-2023-009	N.Swab	CVA6, HHV7	144	F	Cutaneous rash, fever	OR778603
	Serum	CVA6				
CAV-2023-010	N.Swab	CVA6	132	F	Cutaneous rash, fever	OR778604
	Serum					
CAV-2023-011	N.Swab	CVA6 HHV7	NA	NA	NA	OR778605
	Serum	CVA6				
CAV-2023-012	N.Swab	CVA6	18	F	Cutaneous rash	OR778606
	Serum	CVA6				
CAV-2023-013	N.Swab	CVA6, HHV7, EV-A71	24	M	Cutaneous rash, fever	OR778607
	Serum					
CAV-2023-014	N.Swab	CVA6, CVA16	24	F	Cutaneous rash	OR778608
	Serum					
CAV-2023-015	Serum		NA	NA	NA	

Phylogenetic analysis

Classification of the genotypes and their genetic relationships were also assessed by the construction of subgenomic ML phylogenetic trees using aligned sequences based on the entire coding-region of the Capsid protein (Fig. 2A) and the VP1-VP3 region (Fig. 2B). Further, the ML tree showed that the Cape Verde CVA6 sequences clustered with several strains that circulated in the Asia countries in recent years (2018–2023), particularly with Thailand isolates in 2019 with 98% of nucleotide homology and seemed to all belonged to the D3a sub-genotypes recently described (Fig. 2B).

Furthermore, no recombination event was identified in the capsid-encoding region.

Discussion

Fever with rash caused by viral infections can occur in both children and adults, sometimes associated with other symptoms such as sore throat, vomit, diarrhea, fatigue, irritability, anorexia, conjunctivitis, cough, and insomnia [1]. The generalized EV infection-associated rash can resemble that of Measles, Rubella or roseola. Nevertheless, Coxsackieviruses cause diffuse maculopapular or vesicular exanthems associated with fever and rarely with more severe manifestations (meningitis, pneumonia, hepatitis) [4].

Beyond, atypical HFMD has a high rate of misdiagnosis and is more likely to present as bullous lesions and diverse rashes [4]. In rare instances, disease involves the central nervous system and in severe cases it can cause death [22]. Major causes of HFMD include CVA16 and EVA71, which is the main pathogens responsible for severe cases [22]. Though CVA6 was previously detected in sporadic cases, it was largely neglected earlier and subsequently became the third predominant viral pathogen to cause HFMD outbreaks worldwide [4]. Since 2008, CVA6 has caused several HFMD outbreaks in Europe and Asia and clinical symptoms mainly included fever, skin rash, desquamation, and onychomadesis [22].

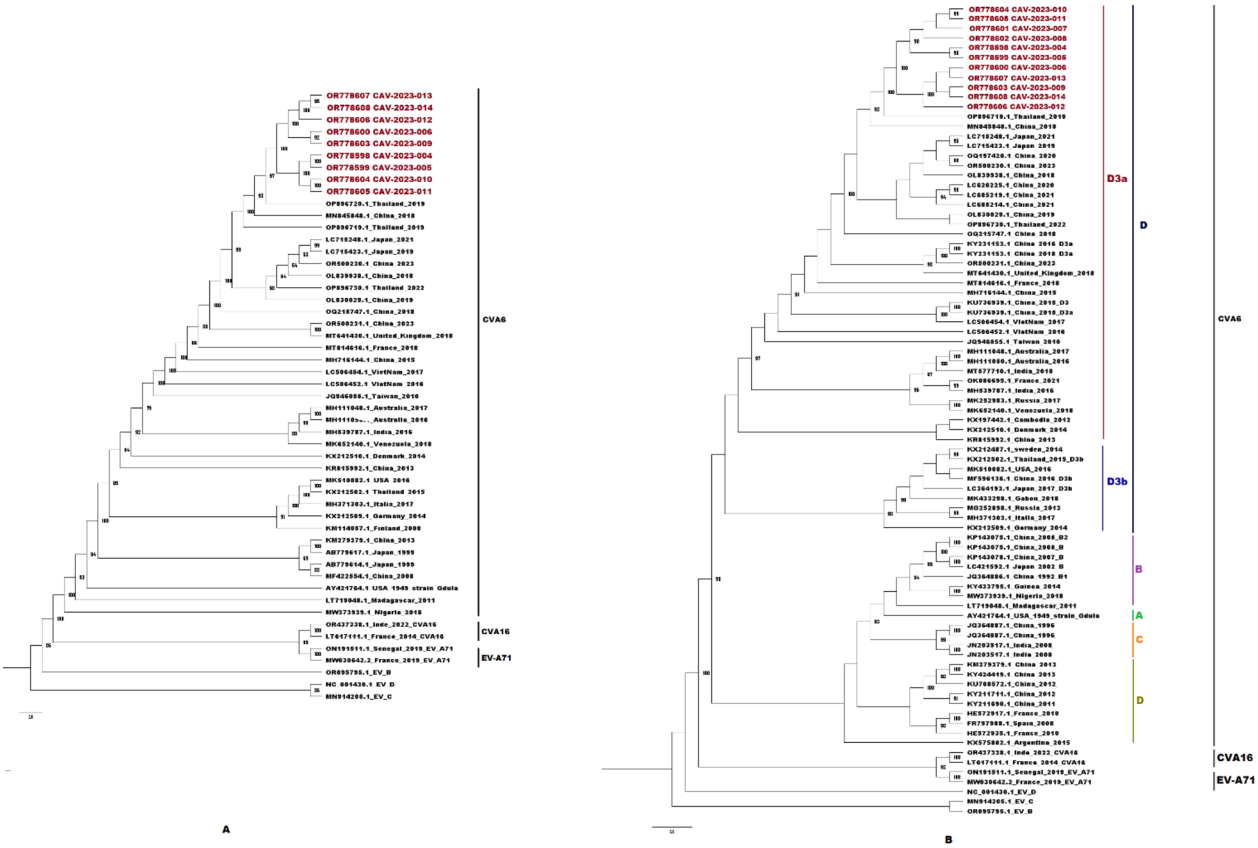

Fig. 2 Phylogenetic comparisons trees based on **A** 51 sequences of the entire coding-region of the Capsid (≈ 4000 pb) and **B** 84 VP1–VP3 sequences (≈ 800 pb) from the newly characterized CVA6 and previously available sequences obtained from GenBank. The newly characterized sequences are highlighted in red. Bootstrap values ≥ 90 are shown on the tree. The scale bar indicates the distances of the branches. GenBank accession numbers for published sequences are shown in the tree. Sequences obtained from GenBank included CVA16 and EVA71. Sequences of EV-B, EV-C and EV-D species were used as outgroups

Despite some studies that reported the circulation of EVA71, CVA16 and CVA6 in Africa [23], data from Sub-Saharan Africa on the incidence of HFMD and its probable causative agent are not available so far. In this study, 12 patients with rash tested positive for CVA6, CVA16 and EVA71 RNA, confirming an outbreak of atypical HFMD in Cape Verde. Our data are then noteworthy by describing the first-ever reported outbreak of CVA6, CVA16 and EVA71 associated with HFMD in the Cape Verde islands, which was consistent with previous reports [22]. Moreover, coinfection of CVA6 with EVA71 and CVA16 was also observed. This finding agrees with studies from Spain and China [24]. Indeed, in most years, one of the three serotypes has dominated the epidemic activity, representing about 50% of enterovirus-positive cases. Thereby, EVA71 was the main causative agent of HFMD in 2009, 2011, 2013, and 2015 but in 2010, 2012 and 2014, CVA16 was the main causative agent [22]. Furthermore, in 2018, CVA6 and CVA16 were the main causative agents [25]. This reduction in the incidence of EVA71 may be due to the successful use of the EVA71 vaccine since 2016. Thus, data monitoring in many Chinese cities has shown that the pathogenic composition of HFMD has changed immensely, and there has been a sharp rise in the detection rate of CVA6 [25]. Nevertheless, coinfection between EVA71 and CVA6 has been observed and associated with serious central nervous system complications and exacerbated disease [25]. Therefore, the pattern of infection might contribute significantly to the clinicopathological, thereby the symptoms of the disease.

Mixed infections by HHV, CMV and EV, were often detected in a case of meningitis [26]. In the same way, herpesviruses were only isolated in a small proportion of HFMD cases [27]. However, the identification of HHV7 from 4 samples could have reflected silent excretion of this virus or the patients may actually be suffering from acute gingivostomatitis due to this virus in addition to HFMD.

Fever accounted for 70%, nearly similar to previous data reported in the Chinese studies in 2015, where the proportion of fever in the CVA6 group was 78.69% [25]. Measles-like rash was reported in these cases even though they were not well detailed in the case-based form. However, a prospective study in France showed that CVA6-associated rashes could spread to the limbs and face [25]. In addition, children from Spain developed papulovesicular rash on the palms, soles, buttocks, and mouth [24], while skin rash, fever, desquamation, onychomadesis and loss of all their fingernails were recorded for some CVA6-associated HFMD cases in children from Beijing [5]. In our study, we recorded 2 cases of cough and 1 case of rhinitis. This observation is consistent with previous data described from EV infections and HFMD cases in India [28]. Further retrospective investigations of mothers of these two children will be interesting to determine possible transmission routes, as a case of congenital CVA6 pneumonia/sepsis caused by maternal infection after HFMD in an older sibling has been recently reported in Japan [25].

Children under 5 years were the major age group of infected patients, similar to data reported in previous studies focusing on HFMD in China [6]. In fact, children are highly susceptible to the EVs due to an immature immune system and clustering at the pre-kindergarten stage. In addition, the notion that males are more likely to contract CVA6 infection, as previously reported [6], was not observed in our study, but this could be due to the low number of patients included.

Our data showed that all patients were admitted in July. Interestingly, this time span is encompassed by a period of high number of HFMD cases in some parts of China [6], generally ranging from April to July, which corresponds to the summer period in this country. Thus, the average temperature and other prevailing weather conditions could have promoted the highest circulation rates of EV during the summer.

Sequence and phylogenetic analyses of the newly characterized CVA6 shared high nucleotide identity with recent CVA6 strains isolated in Asian countries, particularly with the strain from Thailand in 2019. These data suggest that the CVA6 strain responsible for this outbreak could have been probably imported from Asia, considering the important transboundary circulation of human populations beyond the COVID-19 pandemic. In addition, incongruent tree topologies between the VP1 protein and other genomic regions indicated that they seem to be closely related to the D genotype, particularly the D3a sub-genotype. During the last decade, the D3 and E2 sub-genotypes have become the dominant sub-genotypes circulating in Southeast Asia and Europe, causing the outbreak of HFMD in 2013 and thereafter in China [7]. Then the D3a were the predominant evolutionary branch in China from 2017 to 2019 and co-circulated with other CVA6 [7]. The main reason for the sub-genotype D3 persistent international circulation may be probably associated with its stronger transmissibility [7].

In addition, the absence of recombination in the capsid-encoding region confirms that recombination is probably constrained by genetic and/or structural requirements, unlike the non-structural region [29].

Through this emergence, the role of CVA6 in other diseases also needs to be investigated. It has been reported as a causative agent of meningitis, encephalitis, and acute flaccid paralysis [30]. Due to the limited number of CVA6 sequences generated during our study and the lack of

publicly available data from the other African countries, we were not able to determine the exact origin and spread of these viruses in the continent. Thus, regional studies targeting viruses associated with skin rash could be important to address these questions, rapidly identify emerging or re-emerging viruses of public health concern such as CVA6 and determine the public health burden of CVA6-associated diseases. The newly characterized sequences from Cape Verde could serve not only in further phylodynamic studies for assessment of the CVA6 spread worldwide and its introduction period to the African countries but also in the development of a multivalent vaccine with CVA6, EVA71 and CVA16 which is urgently needed.

Conclusion

Our study is relevant for reporting the first-ever outbreak of HFMD and guiding the diagnosis, prevention, and control of a CVA6-associated HFMD outbreak in Cape Verde. However, a more detailed case-based form could be developed by the Cape Verdean Ministry of Health for gathering all necessary data for better characterization of the type of skin rash in the future. Nevertheless, our findings underpin the crucial need for longitudinal hospital-based and multicenter surveillance of HFMD across Africa, if possible based on the Measles surveillance program, to better understand risk factors driving the severity, molecular epidemiology and public health burden of CVA6-associated diseases.

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Author contributions

Conceptualization: NN and MF; funding acquisition: OF, AAS, AS, ND and MF, field investigations: DT, CCDSL, UDF and MS; formal analysis: NN, ND, GF, YD, OK, FDT and MF; supervision: OF, BD, MF; writing: NN and MF; review and editing: NN, DT, CCDSL, OF, AS and MF; All authors have read and agreed to the final version of the manuscript.

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Availability of data and materials

All the data are available in the present manuscript.

Declarations

Competing interests

The authors declare that they have no competing interests.

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