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Research Article

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First Identification of Member of a New Coxsackievirus A16 Genetic Group in Senegal

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Abstract

Five enterovirus-positive cases have been identified in Southern Senegal in December 2022, during field investigations around a cluster of patients with acute gastroenteritis syndrome including 4 deaths. Two enterovirus species were identified using genomic sequencing, including Coxsackievirus A4 and Coxsackievirus A16 strains with sequence length of 306 and 5788 nucleotides, respectively. The generated sequences were screened for recombination. Similarity plot and Bootscan analyses of the newly characterized Coxsackievirus A16 sequence revealed a recombination with Coxsackievirus A4 at the VP3-VP1 Capsid region with a breakpoint at the nucleotide position 2212. These results were confirmed by using phylogenetic inferences. Our data are noteworthy by exhibiting the first recombinant Coxsackievirus A16-Coxsackievirus A4 strain in Africa which belongs to a distinct phylogenetic group. This new sequence expands the number of Coxsackievirus A16 genome sequences and could be useful in future studies focused on the molecular epidemiology of Coxsackievirus A16 in Africa. It is important to continue using next-generation sequencing in surveillance of enteroviruses in Senegal not only for monitoring the circulation dynamic of this new recombinant enterovirus phylogenetic group, but also for rapid characterization of emerging enteroviruses that could be probably associated to severe diseases such as acute gastroenteritis. In addition, more studies focusing on the impact of recombination on enterovirus fitness could be promoted for better understanding of their pathogenesis.

Keywords: Acute gastroenteritis; Coxsackievirus A16; Recombination; Southern Senegal; 2022

Introduction

Gastroenteritis is one of the most typical causes of morbidity and mortality in humans of all age groups particularly, in children [1,2,3,4] and low-income and middle-income countries [5,6]. It is also a common reason for admission to hospital in developed countries.

Viruses that are the most common cause of acute gastroenteritis include rotavirus, calicivirus, adenovirus, and astrovirus [6,7,8,9]. However, enteroviruses have also been recently associated with acute gastroenteritis [10,11].

Members of the Picornaviridae family, the Human enteroviruses (EV) include enteroviruses A to D species such as coxsackieviruses, rhinoviruses, polioviruses, and echoviruses. They are often associated with clinical manifestations ranging from mild infection to severe diseases including myopericarditis, pneumonia, aseptic meningitis, herpangina, enteroviral vesicular stomatitis, encephalitis and acute flaccid paralysis [12,13,14]. In humans, enterovirus (EVs) are among the most common infectious agents worldwide responsible for major public health concerns [15,16]. The association of EVs infection with diarrheal diseases is not well confirmed. However, during the last decade, EV have been added to the list of causative agents of gastroenteritis [10] and diarrheal disease in several countries, including Thailand, Italy, Japan, Vietnam, Djibouti, and India [11,17,18,19,20]. However, human EV species A (EV-A) such as Coxsackievirus (CV)-A2, CVA4, CVA5, CVA6, CVA10, CVA12, CVA16 and Enterovirus A71 (EV-A71) are known as etiologic agents of hand-foot-and-mouth disease (HFMD) [21,22,23]. Since 2004, several HFMD outbreaks associated with co-circulation of EV-A71 and CVA16 have been reported in the Asia-Pacific region [24,25].

First isolated in South Africa in 1951 [26,27] and subsequently sequenced in 1994 [28], the CVA16 has been reported as a major pathogen responsible for self-limited and mild HFMD worldwide. However, occasionally, it can cause serious infections with complications including encephalitis, myocarditis and acute flaccid paralysis (AFP) [29,30]. As for other EV, the CVA16 genome is a single-stranded positive-sense RNA molecule of approximately 7500 nucleotides (nt), encompassed by a 5‘untranslated region (UTR) of approximately 750 nt and a short 3’UTR of approximately 70–100 (nt). The large polyprotein encoding for a single open reading frame (ORF), is divided into three regions (P1–P3). The P1 region contains four structural proteins (VP1–VP4) whereas the P2 and P3 regions together contain seven non-structural proteins (2A–2C and 3A–3D) [31,32]. Based on the VP1 protein, which is the most diversified region between EVs, the CVA16 species is divided into three genotypes (A, B and D). Additionally, the genotype B can be further divided into B1a, B1b, B1c, B2a, B2b, and B2c sub-genotypes [32,33,34].

In Africa, only limited data regarding the epidemiology and genetic diversity of CVA16 exists due to the lack of active surveillance programs or diagnostic resources for detection of EV. To date, only few sequences of CVA16 are available from Africa and most of them are associated with cases of AFP [35,36].

As of December 19, 2022, an alert was received by the Senegalese Ministry of Health and Social actions (MoHSA) from the team of the Bounkiling health district, in the Sédhiou medical region in Southern Senegal, signalling a cluster of human cases with acute gastroenteritis. The first case was recorded on November 23, in a house-hold of 320 members including a koranic school. A total of 4 deaths were reported, all including men aged between 10 and 37 years-old. From December 25 to December 30, 2022, a jointed national multidisciplinary and multi-sector mission (epidemiologists, clinicians, toxicologists, socio-anthropologists, biologists, environmentalists,..) supported by partners (OMS, CDC, IPD, PATH) and including teams from the Senegalese MoHSA, the Institut Pasteur de Dakar (IPD), the Mobile Advanced Coordination Unit (UMCA) based in the Kolda region, the Sédhiou medical region and the Bounkiling health district, was deployed for investigation around the reported deaths, contacts tracing, rapid identification of suspected cases and evaluation of risk factors in order to formulate necessary public health recommendations for timely management of patients and outbreak control.

In this study, we reported the identification and analysis of a recombinant CVA16-CVA4 strain detected during investigation of an epidemic of acute gastroenteritis in the Bounkiling health district, Sédhiou medical region, Southern Senegal.

Materials and Methods

Ethical Consideration

The Senegalese National Ethical Committee of the MoHSA approved the surveillance protocol as a piece of research with less than minimal risk, and written consent forms were not required. Oral consent to participate was obtained from all patients or parents/guardians of minors included in this study, as required by the Senegalese National Ethical Committee of the MoHSA. All samples were de-identified before we performed virus characterization and analysis.

Specimen Collection and Study Location

Clinical samples used in this study were obtained from 32 patients admitted at hospital for acute gastroenteritis in the Bounkiling health district during the field investigations in December 2022. For each suspect or contact case, blood, nasopharyngeal swab, urine and stool samples were collected. Bounkiling is a city of

196,874 Inhabitants for a surface of 3,005 Km² (a population density of 66 people/Km²), located in the Sédhiou region, in Southern Senegal (13° 8' 13" north and 15° 36' 42 west) and share borders with The Gambia in the Northern part and Guinea in the Southern part (Figure 1).

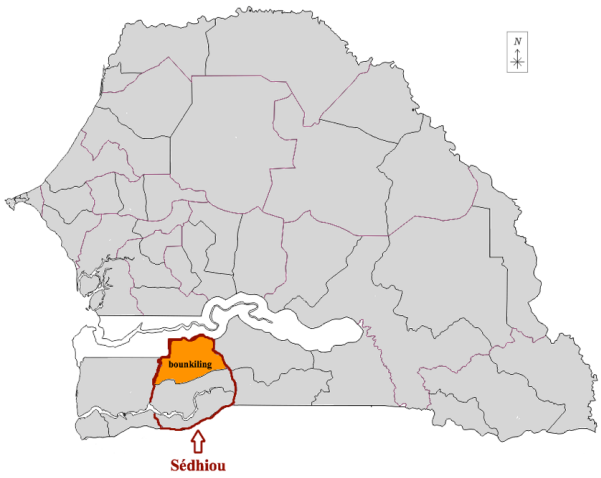

Figure 1: Map showing Bounkiling city (orange) and the Sédhiou region (red circle).

First-Line Testing

Blood and urine samples were tested for classical arboviruses including Chikungunya, Yellow Fever, West Nile, Zika and Dengue) and hemorrhagic fever viruses (Ebola, Marburg, Lassa, CCHF, RVF) by real-time reverse-transcription polymerase chain reaction (RT-PCR) and anti-IgM antibodies by Enzyme-Linked Immunoassay (ELISA) testing. In addition, stool samples were analysed for bacterials 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 coli enteropathogenic (EPEC), E. coli enterotoxinogenic (ETEC), E. coli* enteroaggregative (EAEC.).

Treatment of stool samples and RNA extraction

A total of 32 stool samples were collected with their case-based form containing data on patient, demographics and clinical symptoms. Samples were sent to the World Health Organization's Regional Polio Laboratory hosted at IPD in Senegal for further investigation and identification of the potential associated pathogen and were stored at approximately 4 °C during transport.

At the IPD, stool samples were treated with 1 mL Chloroform, 5 mL Phosphate Buffer Solution and 1 g of glass beads. The mixture was shaken for 20 minutes and centrifuged at 1500× g for 20 minutes to obtain clarified stool suspensions. Viral RNA was extracted from 200 µL of clarified stool suspensions using the QIAmp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Extracted RNA was eluted in 60 µL of nuclease-free water and stored at -20 °C until use.

Enterovirus Diagnosis and Molecular Genotyping

The extracted RNA was tested for EV by real-time RT-PCR using a pan-enterovirus (panEV) assay which targets highly conserved sites in the 5'-nontranslated region (5'-NTR) of all enteroviruses, with the LightMix® Modular Enterovirus 500 kit (Roche-Ref 50-0656-96, TibMolBiol, Berlin, Germany) [37] and the qScript™ XLT One-Step RT-PCR (QuantaBio, Beverly, MA, USA).

The viral RNA detection was performed using the CFX96TM Real-Time PCR system (Bio-Rad, Singapore) and the following conditions: reverse transcription at 50 °C for 10 minutes, initial denaturation at 95 °C for 1 minute, followed by 45 cycles of denaturation at 95 °C for 10 seconds, and 60 °C for 1 minute. Samples with Ct values <35 were considered as EV-positive and RNA was stored at -80 °C until further testing.

Enterovirus VP1 Gene Amplification

For viral amplification, RNA was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific™ Vilnius, Lithuania) with random-hexamers primers according to the manufacturer's instructions. Amplicons of the VP1 region were then generated using The GoTaq® DNA Polymerase kit (Promega, Madison, WI, USA) with a PCR method targeting the VP1 region as previously described [38].

The PCR was carried out in a final volume of 50 µL containing 5 µL of the cDNA product, 10 µL of the PCR buffer (Promega), 4 µL of MgCl2, 1,2 µL of dNTP (10 mM), 1 µL of forward primer (20 µM), 1 µL of reverse primer (20 µM) and 5 µL of GoTaq® DNA Polymerase (5 u/µL). This reaction mixture was amplified using the following conditions: 3 minutes at 95 °C followed by 40 cycles of 30 seconds at 95 °C, 30 seconds at 45 °C and 1 minute at 72 °C, plus a final extension step at 72 °C for 10 min. The PCR product was verified using electrophoresis in a 1% Agarose gel with Bromide Ethidium staining under UV visualization.

Enterovirus Sequencing and Genome Analysis

To determine EV's genotypes, cDNA and amplicons of the VP1 gene were purified using AMPure XP magnetic beads and quantified using the dsDNA High Sensitivity Kit on a Qubit 3.0 fluorometer (Thermo Fisher). The purified cDNA and VP1 amplicons were barcoded using the Rapid Barcoding Kit 110.96 with MRT001 expansion (Oxford nanopore technology) and pooled in a single tube. The libraries were then purified and sequenced on a GridIon instrument (Oxford nanopore technology).

Passed reads were analyzed using the "Genome detective virus

tool" software (version 2.40) 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 January 2, 2022) to compare the sequence homology with previously available data. In addition, the genotype was confirmed using the online Enterovirus Genotyping Tool (RIVM) program which reliably assign the genotype based on Sequences of all regions of the enterovirus genome (https://www.rivm.nl/mpf/typingtool/enterovirus/, accessed on January 2, 2022) [39].

Phylogenetic Analysis

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 [40]. The maximum likelihood (ML) phylogenetic trees based on VP1 and near-complete genome sequences were inferred using IQ-TREE (version 1.6.12) [41] for 1,000 replications. The ML tree topology was visualized with (FigTree version 1.4.4) [42]. Nodes were supported by Bootstrap values.

Recombination Analysis

To confirm the occurrence of recombination events, similarity plot and bootscan analysis were conducted against sequences closely related by using the SimPlot program, version 3.5.1 [43], 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 [44] 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, Bootscan, Siscan, Chimaera, 3Seq and LARD. Full genome sequences were used as queries for BLASTn and sequences with highest homologies and complete genomes were used in the recombination analysis.

Maximum likelihood (ML) phylogenetic trees based on sequences of the recombinant region and concatenate sequences of the non-recombinant regions were inferred using the FastTree program [45] for 1,000 replications. The ML tree topology was visualized with (FigTree version 1.4.4) [42]. Nodes were labelled by local support values computed using the Shimodaira-Hasegawa test.

Results

Demographic and clinical characteristics of investigated cases

A total of 32 patients including 18 males and 14 females, was identified with a sex ratio of 1.28 and a median age of 26 years (ranging from 14-38 years). The 15–29-years-old group had the highest proportion of cases (34.4%). The majority of cases were found among housewives and students with 25.7 % and 23.3 %, respectively. The signs and symptoms frequently reported were fever & headache (68.8%), vomiting (35.5%), abdominal pain (35.5%) and asthenia (35.5%). Diarrhea was reported in 19 % of the patients investigated. One person reported urinary signs such as polyuria/polydipsia. However, no haemorrhagic or neurological signs were reported.

First-line testing

Blood and urine samples tested negative for classical arboviruses and hemorrhagic fever viruses. Only a blood sample from a 26 years-old woman residing in the village of Ndiama Peulh, tested positive for Dengue's anti-IgM antibodies by serology testing. In addition, the bacteriological examination of stool samples was negative for all cases, apart from a 35 years-old woman residing in the village of Ndiama Peulh, who tested positive for *Salmonella spp*.

Clinical and Demographic Characteristics of Confirmed Cases

EV were detected in 5 stool samples from 32 (15.62%) with a sex-ratio of 1.5. The most frequent age-group was those aged between 10-20 years (60%). The most common clinical signs in EV-confirmed cases were fever & headache (80%), asthenia (80%), vomiting (60%) and abdominal pain (40%). However, these data are not enough to confirm these statements.

Enterovirus Genotyping

Overall, two out of 5 EV were successfully amplified including one with partial VP1 sequence (300 nt) from an amplicon and one with P1, P2 and partial P3 region (5637 nt) from a cDNA, corresponding to CVA4 and CVA16 genotypes, respectively, based on BLAST analysis. In addition, the RIVM confirmed the genotyping data.

The patient with CVA4 infection was a 15 years-old boy with fever, headaches, vomiting and asthenia (SEN/023.2022) while the patient infected with CVA16 was a one year-old boy with fever and diarrhea (SEN/020.2022). The obtained sequences were deposited in the GenBank database under the accession numbers (OQ607171; OQ550958 for the CVA16 and CVA4 sequences, respectively).

Phylogenetic Analysis

Classification of the genotypes and their genetic relationships were also assessed by construction of sub-genomic ML phylogenetic trees using aligned sequences based on the VP1 gene and the near-complete genome from our study and sequences of the EV-A genotype available from the GenBank database.

The phylogenetic data confirmed the RIVM's results. Interestingly, Both SEN/023.2022 and SEN/020.2022 isolates grouped with CVA4 sequences on the VP1-based tree (Figure 2A) while

considering the near-complete genome-based tree, the isolate SEN/020.2022 clustered only with CVA16 sequences (Figure 2B).

In addition, the VP1-based tree showed the phylogenetic analysis showed that the SEN/020.2022 isolate was closed to a previous CVA4 sequence from Guinea (KY433793.1- Guinea/2014) with a 90% nt homology (Figure 2A). However, its newly characterized near-complete sequence (P1, P2 and partial P3) was close to the prototype Human enterovirus A isolated from South Africa in 1951 (U05876.1-South Africa/1951) with a 100% nt homology and a CVA16 strain from China (EU812514.1 -China/2008) with a 99.8% nt homology (Figure 2B).

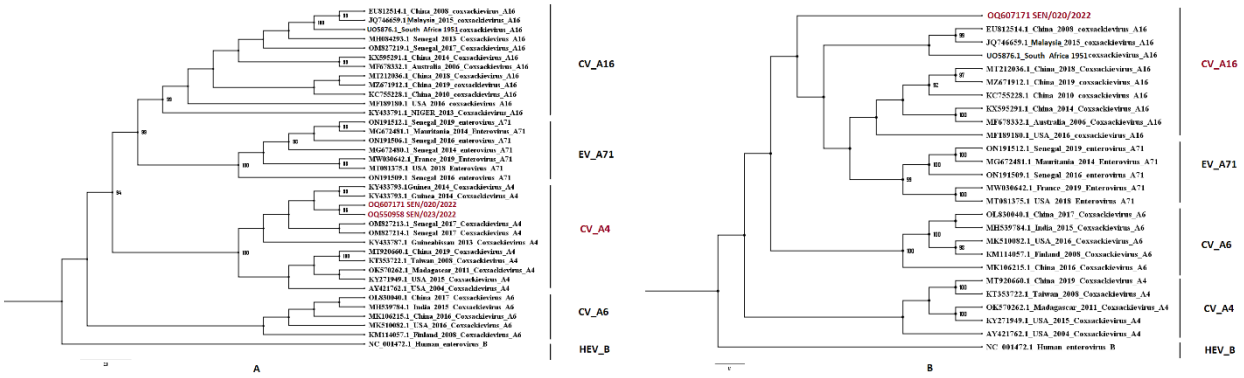

Figure 2: Maximum-Likelihood phylogenetic trees based on (A) 37 partial VP1 sequences (~306 bp) and (B) 26 sequences (~5637 bp) from the newly characterized EV-A and previously available sequences obtained from GenBank. The newly characterized sequences are highlighted in red. Bootstrap values ≥ 90 are 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, EV-A71, CVA6, CVA4 and EV-B as an outgroup.

Moreover, to better ratify the genetic relationships between the CVA16 genomes, we analyzed separately recombinant and concatenated non-recombinant regions from the SEN/020.2022 isolate using phylogenetic inferences. Interestingly, the corresponding recombinant genomic region grouped with CVA4 sequences (Figure 3) while the non-recombinant regions clustered with CVA16 sequences (Figure 3).

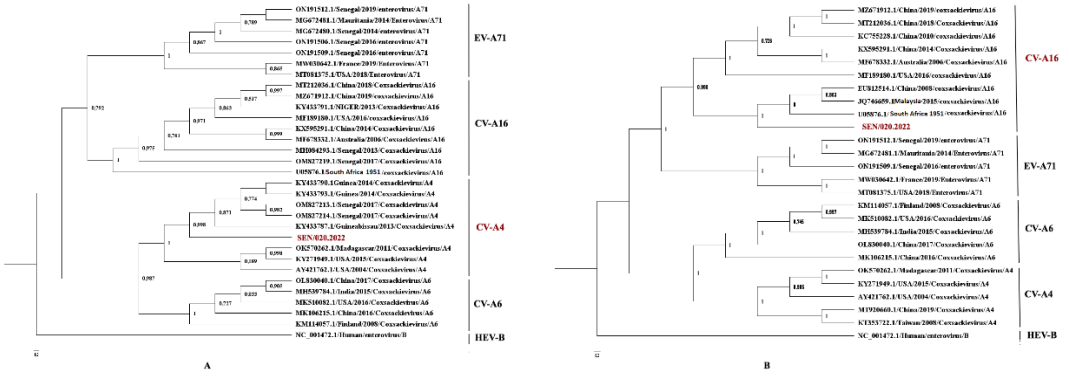

Figure 3: Phylogenetic comparisons trees based on 31 partial VP1 sequences of the recombinant region (position 2212-2978 nt) (A) and 26 concatenate sequences of the non-recombinant regions (position 0-2212 nt and 2979-5637 nt) (B) including the newly characterized CVA16 sequence and EV-A previously available sequences obtained from GenBank. The newly characterized sequences are highlighted in red. Nodes are labelled with local support values computed using the Shimodaira-Hasegawa test for 1,000 replications. The scale bar indicates the distances of the branches. GenBank accession numbers for published sequences are shown in the tree. Previous sequences obtained from GenBank included CVA16, EV-A71, CVA6, CVA4 and EV-B as outgroup.

Recombination Analysis

By analyzing the SEN/020.2022 sequence (CVA16), the SimPlot graph consists of a graphical representation of similarities between the reference group and the remaining groups using a sliding window approach. The Simplot data revealed a difference of nt similarity within genomic partial VP3-VP1 gene, suggesting that this region is almost different to those of the other CVA16 strains (Figure 4A). In addition, the genomic region between nucleotide positions 2212-2978, displayed a relatively high degree of similarity to CVA4 sequences, in particular with a CVA4 strain from Guinea (Accession KY433787) isolated in 2017. The BootScan method was used for the identification and mapping of recombinant sequence regions. Interestingly, the BootScanning data confirmed the existence of a strong intertypic recombination signal within partial VP3-VP1 gene (Figure 4B). In addition, this recombination event was significantly supported by the RDP4 analysis, exhibiting that the newly characterized CVA16 sequence recombined with a CVA4 strain previously isolated from Guinea (KY433787).

Figure 4: Similarity plot (A) and Boot scanning analysis (B) of the CVA16 study strain SEN/020/2022. with closely related strains. The genetic organization of enteroviruses is shown in the top panel. Analyses were conducted by using SimPlot 3.5.1 (Kimura distance model, window size 200 bp moving in 20 nucleotides steps). SEN/020.2022 was used as a query sequence.

Discussion

Gastroenteritis has a major impact on morbidity and mortality worldwide [46]. Besides, a large number of pathogens are known to be aetiological agents responsible for gastroenteritis especially viruses (more than 70%), followed by bacteria (10–20%) and protozoa (less than 10%) [5]. Gastroenteritis remains a serious public health concern among children under 5 years with the highest incidence in children aged between 6–24 months [2,46,47] Particularly, in low-income and middle-income countries [5,48]. The Global Enteric Multicenter Study (GEMS) identified Rotavirus (family Reoviridae) as the most predominant enteric pathogens associated with gastroenteritis in humans [49,50]. However, the relative incidence of Rotaviruses has significantly declined due to the successful implementation of

rotavirus vaccines in many countries during the last decade [51,52] while the prevalence of the other viral etiologies is increasing [53]. Nevertheless, in most of the African countries, microbiological methods for investigation of gastroenteritis are usually restricted to conventional enteric bacteria identification (such as *Salmonella sp, Shigella sp* and *Enteropathogenic Escherichia coli*) and viral etiology is rarely investigated.

Studies on EV infections showed that they can cause a range of illnesses including severe clinical illness, sometimes lethal, and outbreaks of EV-associated diseases vary in specific genotypes. Between 2001 and 2002, the E13 type emerged in the United States and Japan, and spread causing many epidemics of acute meningitis, worldwide [54,55]. However, Since, 2016, members of the species A such as EV-A71, CVA6 and CVA16 and species D such as EV-D68, have been involved in recent outbreaks of fatal Hand Foot and Mouth Disease (HFMD) and respiratory disease, respectively [56]. In 2018, E30 circulation was high, and large-scale E30 meningitis-related upsurges were reported in Denmark, Germany, the Netherlands, Norway, and Sweden [57]. This year, E11 especially are one of the most frequent types responsible for EVs neonatal infection, the World Health Organization (WHO) reported an increasing number of severe neonatal infections associated with echovirus 11 in many European countries, notably in Croatia, Spain, Sweden, and the United Kingdom [58].

Although their replication cycle occurring in the intestine systematically go unnoticed, EV can also affect the gastrointestinal tract and have recently been added to the list of causative agents of gastroenteritis [10,11,59]. In addition, some genotypes have been associated with acute gastroenteritis [60].

Our study reported on the circulation of EV during investigations around a cluster of patients with acute gastroenteritis in Senegal. During these investigations, other pathogens known to be endemic in Senegal such as Dengue virus and *Salmonella spp* were detected with low prevalence [61,62]. Although a detection rate of 15.62% was recorded in our study for EV, prevalence rates between 1.2% and 42% in fecal specimens from patients with acute gastroenteritis have been previously reported worldwide [10,63,64,65]. In fact, previous studies performed in India reported an EV detection rate of 33–40% [11] while another study conducted in Brazil reported the prevalence of 23.3%, of which EV-A, B and C [66]. In addition, a prevalence of 5.8% was reported in Thailand and CVA24 and EV-C96 were the most prevalent genotypes detected [67]. However, another study conducted in the same country exhibited a prevalence of 6.2% and mainly involved the PV-2 genotype [68].

Various EV genotypes including E11, CVA6, CVB2, PV3, CVB4, E18, and CVA2 were detected in stool from children with acute diarrhea in China [63]. Moreover, an outbreak of gastroenteritis caused by E6 was previously reported among children in Japan [69]. E11 was similarly reported to be responsible for outbreaks of diarrhea in Malaysia [70] and southern India [71]. The E11 and E30 species have been found to be the most prevalent genotypes associated with diarrhoea in children in Southern India [60] while the E6 and E14 species were predominant in children with diarrhoea in Western India from 2004 to 2009 [11]. The E11 species was also responsible for an outbreak of acute gastroenteritis in confinement homes in Southern India and Malaysia [70] and acute gastroenteritis caused by CVB6 was previously reported from children in Brazil [72].

In Africa, the CVA24 species was reported as the most predominant genotype identified in children with acute gastroenteritis in Ghana [73]. Although several other species have been previously identified in patients with gastroenteritis, EV were also detected in asymptomatic individuals at a prevalence of 22.8% [74].

In our study, we reported on identification of the first recombinant CVA16 associated with acute gastroenteritis in Senegal. Generally, the EV-A types as EV-A71, CVA16, CVA6, and CVA10 are the most commonly sequenced types in East Asia, and Southeast Asia. However, only few short-length genomic sequences of EV-A are currently available from Africa, North America, and Western Asia.

First isolated in South Africa in 1951, the CVA16 species was subsequently associated with cases of AFP [36]. However, more recent data showed its involvement as a major cause of many HFMD outbreaks [75-77]. Moreover, it was responsible for about 50% of all the confirmed EV infections among HFMD cases in mainland China [78,79]. Although CVA16 infection was usually mild and benign, the emergence of a new clade [80,81] in China indicates that CVA16 is continuing to evolve into more diverse branches, so continued surveillance is warranted. In addition, severe complications such as myocarditis and pericarditis, even death could occur [82,83]. In Africa, the CVA16 species was rarely reported probably due to the lack of active surveillance or limitations in resources and diagnostic tools. Besides, a large part of sequences are linked to AFP cases notified in the frame of the poliovirus eradication efforts, hence this highlights the need for more surveillance in underreported regions.

Widely used for EV taxonomy, identification of new types, and molecular epidemiology [84,85], comparative sequence and phylogenetic analyses based on the VP1 gene revealed that the newly characterized CVA4 and CVA16 strains shared high nucleotide identity with a CVA4 strain from Guinea in 2014, suggesting that this CVA4 strain from Southern Senegal could be probably imported from Guinea which shares borders with the Sédhiou region, involving an important transboundary circulation of human populations and animals.

Interestingly, similarity plot graphs and Bootscan analyses based on whole genome sequences revealed that the new CVA16 strain from Sédhiou recombined with a CVA4 sequence at the VP3-VP1

region, suggesting a potential intertypic recombination during CVA16 evolution and confirmed data from the incongruent ML tree topologies between the VP1 gene and others genomic regions. The newly characterized sequence represents the first-ever reported strain of recombinant CVA16 identified in Senegal.

Viral recombination is a major evolutionary mechanism driving adaptation processes, such as the ability of host-switching. It generates mosaic genomes containing genomic material from different viruses, and it can occur within the same (homologous) or different (non-homologous) sites from the parental strains [86,87].

Recombination is a well-known phenomenon for EV. Therefore, such a mechanism has been considered as the major driving force in EV evolution, allowing the virus to rapidly explore a larger sequence space than the slow accumulation of point mutations. In addition, it could create new combinations of pre-existing polymorphisms resulting in an increased virulence of EV, which could trigger serious public health problems [88,89]. Indeed, when different EV infect and replicate in the same cell, recombination between parts of the genome of different types may occur. This mechanism is adopted by the virus to generate genetic variation and thereby, could potentially influence the replication, tissue tropism, and virulence [90]. Further it allows EV to create and maintain their genetic diversity [89]. Recombination in EV was first reported in polioviruses [91-93]. Subsequently, in many analyses of epidemic characteristics of EV, co-circulation of many types may also provide favourable conditions for recombination between different types of EV [94-96], more frequently in EV-B species [94,97,98]. While many recombinant EV have been reported in isolates derived from vaccine polioviruses and co-circulating EV-C viruses [91,99], only few examples of recombinant EV-A viruses have been previously described [100-102]. Thereby, complete genome analysis or sequencing of multiple regions for circulating strains could represent an effective approach for assessment of mechanisms of viral evolution such as recombination.

Previous analyses of complete genome sequences of many EV species suggested that intertypic or intratypic recombination frequently occur in the non-structural regions [95, 100,103], suggesting that these genomic regions represent relatively stable units. However, our data exhibited a recombination in the structural region corresponding to the VP1 gene. The VP1 gene has been reported to possess several antigenic determinants and induce the production of neutralizing antibodies [104]. However, genetic variations in the VP1 gene also allow the virus to escape neutralizing antibodies. Recombination in the VP1 gene has been previously identified in a type 2 vaccine-derived recombinant poliovirus isolated from a child with vaccine-associated paralytic poliomyelitis in Romania [105]. More recently, evidence has been found that type 1 poliovirus strains isolated from poliomyelitis patients in China from 1991 to 1993 contained a 367-nucleotide block of sequence derived from the Sabin 1 oral polio-vaccine strain spanning the 3′ end of VP1 and the 5′ half of the protease 2A inserted in a wild type 1 poliovirus genome background [106]. Our data are noteworthy by not only exhibiting the rarity of this phenomenon of a recombinant of CVA16 and CVA4, but also that of enterovirus recombination within the capsid region. Although intertypic recombination within the Capsid region of EV-B has been previously described [98], its occurrence in the Capsid region seems therefore to be very rare in nature. However, a CVA16 recombination with CVA4 in the P2 region was previously reported from a patient with HFMD in China in 2010 (Accession KU163608.1); suggesting that the CVA4 strain has recombined with CVA16 prior to the isolation of a CV16/A4 recombinant in Guinea (KY433787) in 2017 [107]. This earlier recombination reported from a CVA16/A4 recombinant circulating before 2022 is more likely than recombination within VP1 between the same two types of enterovirus A within the same region of the Capsid as found in our study.

Due to the lack of complete genome sequences for many EV types from neighboring African countries such as Guinea, phylodynamic analysis could not be performed to estimate the time to the recombinant CVA16 introduction to Senegal and determine its geographical origin. Therefore, continuous programs focusing on continuous complete genome sequencing of more of EV-A strains could be promoted in Africa, particularly in West Africa, for a better understanding of the impact of recombination in the fitness of non-polio EV species. Further clinical characterization of the CVA16 infection and more insights in its genetic diversity and pathogenesis will provide more information and help to the development of a future CVA16 vaccine.

Conclusions

In this study, we reported on the first identification of a recombinant CVA16 in Senegal to date. This recombination is noteworthy as it occurred within the Capsid region. Our data exhibited also the importance of genomic surveillance for identification of emerging EV genotypes driven by selection pressures such as recombination. In addition, our data provided an important insight into developing integrated public-health interventions for outbreak's investigation and control. Therefore, it will be important to implement programs focusing on continuous genomic surveillance of EV for monitoring of the circulation dynamic of this newly characterized recombinant strain and rapid identification of emerging EV species. It is further recommended to intensify non-polio EV diagnosis from clinical samples across Africa since these are important global endemic and epidemic pathogens and further perform a survey on molecular evolution of the recombinant strains as well as their evolution over time.

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References

1. Chhabra P, Payne DC, Szilagyi PG, Edwards KM, Staat MA, et al. (2013) Etiology of viral gastroenteritis in children< 5 years of age in the United States, 2008–2009. J Infect Dis. 208: 790-800.
2. Dennehy PH. (2011) Viral gastroenteritis in children. Pediatr Infect Dis J. 30: 63-64.
3. Rivera-Dominguez G, Ward R (2018) Pediatric gastroenteritis. StatPearls [Internet].
4. Leung AK, Hon KL. (2021) Paediatrics: how to manage viral gastroenteritis. Drugs Context. 10: 2020-11-7.
5. Fernandez-Cassi X, Martínez-Puchol S, Silva-Sales M, Cornejo T, Bartolome R, et al. (2020) Unveiling viruses associated with gastroenteritis using a metagenomics approach Viruses 12: 1432.
6. Eckardt JA, Baumgart CD. (2011) Viral gastroenteritis in adults. Recent Patents Anti-Infect Drug Disc 6: 54-63.
7. Hellysaz A, Neijd M, Vesikari T, Svensson L, Hagbom M. (2023) Viral gastroenteritis: sickness symptoms and behavioral responses. Mbio 14: e03567-22.
8. Al-Ali RM, Chehadeh W, Hamze M, Dabboussi F, Hlais S, et al. (2011) First description of gastroenteritis viruses in Lebanese children: a pilot study. J Infect Public Health 4: 59-64.
9. Bányai K, Estes MK, Martella V, Parashar UD. (2018) Viral gastroenteritis. The Lancet. 392: 175-186.
10. Rojjanadumrongkul K, Kumthip K, Khamrin P, Ukarapol N, Ushijima H, et al. (2020) Enterovirus infections in pediatric patients hospitalized with acute gastroenteritis in Chiang Mai, Thailand, 2015–2018. PeerJ 8: e9645.
11. Patil PR, Chitambar SD, Gopalkrishna V. (2015) Molecular surveillance of non-polio enterovirus infections in patients with acute gastroenteritis in Western India: 2004–2009. J Med Virol. 87: 154-161.
12. Tomatis Souverbielle C, Erdem G, Sánchez PJ. (2023) Update on nonpolio enterovirus and parechovirus infections in neonates and young infants. Curr Opin Pediatr. 35: 380-389.
13. Nikonov OS, Chernykh ES, Garber MB, Nikonova EY. (2017) Enteroviruses: classification, diseases they cause, and approaches to development of antiviral drugs. Biochem Mosc 82: 1615-1631.
14. Pons-Salort M, Parker EP, Grassly NC. (2015) The epidemiology of non-polio enteroviruses: recent advances and outstanding questions. Curr Opin Infect Dis 28: 479-487.
15. Rudolph H, Schroten H, Tenenbaum T. (2016) Enterovirus infections of the central nervous system in children: an update. Pediatr Infect Dis J 35: 567-569.
16. Sinclair W, Omar M. (2022) Enterovirus. In: StatPearls [Internet]. StatPearls Publishing;
17. Chaimongkol N, Khamrin P, Suantai B, Saikhreang W, Thongprachum A, et al. (2012) A wide variety of diarrhea viruses circulating in pediatric patients in Thailand. Clin Lab 58: 117-123.
18. Maslin J, Kohli E, Leveque N, Chomel JJ, Nicand E, et al. (2007) Caracterisation des virus agents potentiels des diarrhees chez l'adulte a Djibouti. Médecine Trop 67: 249-254.
19. Pham NTK, Trinh QD, Chan-It W, Khamrin P, Shimizu H, et al. (2010) A novel RT-multiplex PCR for detection of Aichi virus, human parechovirus, enteroviruses, and human bocavirus among infants and children with acute gastroenteritis. J Virol Methods 169: 193-197.
20. Phan TG, Nguyen TA, Shimizu H, Yagyu F, Okitsu S, et al. (2005) Identification of enteroviral infection among infants and children admitted to hospital with acute gastroentritis in Ho Chi Minh City, Vietnam. J Med Virol 77: 257-264.
21. Ramachandran SE, Joseph J, Sasidharanpillai S, Shahin S, Michael CJ, et al. (2022) Molecular Epidemiology of Enteroviruses Associated with Hand, Foot and Mouth Disease in South India during 2015 to 2017. Published online 167: 2229-2238.
22. Puenpa J, Wanlapakorn N, Vongpunsawad S, Poovorawan Y. (2019) The history of enterovirus A71 outbreaks and molecular epidemiology in the Asia-Pacific region. J Biomed Sci. 26: 1-11.
23. Wu Y, Yeo A, Phoon MC, Tan EL, Poh CL, et al. (2010) The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. Int J Infect Dis 14: e1076-e1081.
24. Han Z, Song Y, Xiao J, Jiang L, Huanget W, et al. (2020) Genomic epidemiology of coxsackievirus A16 in mainland of China, 2000–18. Virus Evol 6: veaa084.
25. Takahashi S, Metcalf CJE, Arima Y, Fujimoto T, Shimizu H, et al. (2018) Epidemic dynamics, interactions and predictability of enteroviruses associated with hand, foot and mouth disease in Japan. J R Soc Interface 15: 20180507.
26. Hassel C, Mirand A, Farkas A, Diedrich S, Huemer HP, et al. (2017) Phylogeography of coxsackievirus A16 reveals global transmission pathways and recent emergence and spread of a recombinant genogroup. J Virol 91: e00630-17.
27. Sickles GM, Mutterer M, Feorino P, Plager H. (1955) Recently classified types of Coxsackie virus, group A. Behavior in tissue culture. Proc Soc Exp Biol Med 90: 529-531.
28. Pöyry T, Hyypiä T, Horsnell C, Kinnunen L, Hovi T, et al. (1994) Molecular analysis of coxsackievirus A16 reveals a new genetic group of enteroviruses. Virology 202: 982-987.
29. Dunn JJ. (2016) Enteroviruses and parechoviruses. Diagn Microbiol Immunocompromised Host. Published online 273-296.
30. Xing W, Liao Q, Viboud C, Zhang J, Sun J, et al. (2014) Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. Lancet Infect Dis 4: 308-318.
31. Jiang P, Liu Y, Ma HC, Paul AV, Wimmer E. (2014) Picornavirus morphogenesis. Microbiol Mol Biol Rev 78: 418-437.
32. Zell R. (2018) Picornaviridae—the ever-growing virus family. Arch Virol 163: 299-317.

33. Knowles NJ, Hovi T, Hyypiä T, et al. (2012) Family-Picornaviridae. Published online 2012.

34. Kang HJ, Yoon Y, Lee YP, Kim HJ, Lee DY, et al. (2021) A different epidemiology of enterovirus A and enterovirus B co-circulating in Korea, 2012–2019. J Pediatr Infect Dis Soc 10: 398-407.

35. Fernandez-Garcia MD, Kebe O, Fall AD, Ndiaye K. (2017) Identification and molecular characterization of non-polio enteroviruses from children with acute flaccid paralysis in West Africa, 2013–2014. Sci Rep 7: 3808.

36. Ndiaye N, Fall A, Kébé O, Kiory D, Dia H, et al. (2022) National Surveillance of Acute Flaccid Paralysis Cases in Senegal during 2017 Uncovers the Circulation of Enterovirus Species A, B and C. Microorganisms. 10: 1296.

37. Gunson RN. (2008) The Development, Implementation and Evaluation of a Real-Time PCR-Based Diagnostic Service for Viral Causes of Infectious Intestinal Disease. PhD Thesis. University of Glasgow; 2008.

38. Nix WA, Oberste MS, Pallansch MA. (2006) Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 44: 2698-2704.

39. Kroneman A, Vennema H, Deforche KVD, Avoort HVD, Peñaranda S, et al. (2011) An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol 51: 121-125.

40. Hall TA. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium Series. Vol 41. Oxford 95-98.

41. Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ. (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32: 268-274.

42. Rambaut A. (2014) FigTree, a graphical viewer of phylogenetic trees (tree. bio. ed. ac. 691 uk/software/figtree). Inst Evol Biol Univ Edinb 1: 2.

43. Lole KS, Bollinger RC, Paranjape RS, Gadkari D, Kulkarni SS, et al. (1999) Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J Virol. 73: 152-160.

44. Martin DP, Murrell B, Golden M, Khoosal A, Muhire B. (2015) RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evol 1: vev003.

45. Price MN, Dehal PS, Arkin AP. (2010) FastTree 2–approximately maximum-likelihood trees for large alignments. PloS One 5: e9490.

46. Hartman S, Brown E, Loomis E, Russell HA. (2019) Gastroenteritis in children. Am Fam Physician. 99: 159-165.

47. Farfán García AE, Imdad A, Zhang C, Arias-Guerrero MY, Sánchez-Álvarez NT, et al. (2020) Etiology of acute gastroenteritis among children less than 5 years of age in Bucaramanga, Colombia. Published online.

48. Weghorst AA, Bonvanie IJ, Holtman GA, De Boer MR, Berger MY. (2022) Course of uncomplicated acute gastroenteritis in children presenting to out-of-hours primary care. BMC Prim Care. 23: 125.

49. Diarrhoea LBD, Reiner RC, Wiens KE, Deshpande A, Meretoja TJ, et al. (2020) Mapping geographical inequalities in childhood diarrhoeal morbidity and mortality in low-income and middle-income countries, 2000-17: analysis for the Global Burden of Disease Study 2017. Lancet. Published online.

50. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH,et al. (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet 382: 209-222.

51. Rackoff LA, Bok K, Green KY, Kapikian AZ. (2013) Epidemiology and evolution of rotaviruses and noroviruses from an archival WHO Global Study in Children (1976–79) with implications for vaccine design. Plos One 8: e59394.

52. Bányai K, László B, Duque J, Steele AD, Nelsonet EAS, et al. (2012) Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. Vaccine 30: A122-A130.

53. Ouédraogo N, Kaplon J, Bonkoungou IJO, Traoré AS, Pothier P, et al. (2016) Prevalence and genetic diversity of enteric viruses in children with diarrhea in Ouagadougou, Burkina Faso. PLoS One. 11: e0153652.

54. Bingjun T, Yoshida H, Yan W, Tsuji T, Shimizu H, et al. (2008) Molecular typing and epidemiology of non-polio enteroviruses isolated from Yunnan Province, the People's Republic of China. J Med Virol. 80: 670-679.

55. Maan HS, Chowdhary R, Shakya AK, Dhole TN. (2013) Genetic variants of echovirus 13, northern India, 2010. Emerg Infect Dis 19: 293.

56. Antona D, Kossorotoff M, Schuffenecker I, Mirand A, Leruez-Ville M, et al. (2016) Severe paediatric conditions linked with EV-A71 and EV-D68, France, May to October 2016. Eurosurveillance 21: 30402.

57. Suresh S, Rawlinson WD, Andrews PI, Stelzer-Braid S. (2020) Global epidemiology of nonpolio enteroviruses causing severe neurological complications: A systematic review and meta-analysis. Rev Med Virol. 30: e2082.

58. Mondiale de la Santé O (2023) Organization WH. WHO's Health Emergencies Programme: acute emergencies monthly summary–May 2023–Programme OMS de gestion des situations d'urgence sanitaire: résumé mensuel des situations d'urgence aiguë–mai 2023. Wkly Epidemiol Rec Relevé Épidémiologique Hebd. 298: 281-286.

59. Oppong TB, Yang H, Amponsem-Boateng C, Kyere EKD, Abdulaiet T, et al. (2020) Enteric pathogens associated with gastroenteritis among children under 5 years in sub-Saharan Africa: a systematic review and meta-analysis. Epidemiol Infect 148.

60. Rao DC, Babu MA, Raghavendra A, Dhananjaya D, Kumar S, et al. (2013) Non-polio enteroviruses and their association with acute diarrhea in children in India. Infect Genet Evol. 17: 153-161.

61. Dieng I, Ndione MHD, Fall C, Diagne MM, Diop M, et al. (2021) Multifoci and multiserotypes circulation of dengue virus in Senegal between 2017 and 2018. BMC Infect Dis. 21:1-11.

62. Dieye Y, Hull DM, Wane AA, Harden L, Fall C, et al. (2022) Genomics of human and chicken Salmonella isolates in Senegal: Broilers as a source of antimicrobial resistance and potentially invasive nontyphoidal salmonellosis infections. PLoS One. 17(3):e0266025.

63. Shen XX, Qiu F zhou, Li G xia, Meng-Chuan Z, Wang J, et al. (2019) A case control study on the prevalence of enterovirus in children samples and its association with diarrhea. Arch Virol. 164:63-68.

64. Alcalá AC, Pérez K, Blanco R, González R, Ludert JE, et al. (2018) Molecular detection of human enteric viruses circulating among children with acute gastroenteritis in Valencia, Venezuela, before rotavirus vaccine implementation. Gut Pathog. 10(1):1-12.

65. Bubba L, Pellegrinelli L, Pariani E, Primache V, Amendola A, et al. (2015) A novel multiplex one-step real-time RT-PCR assay for the

simultaneous identification of enterovirus and parechovirus in clinical fecal samples. J Prev Med Hyg. 56(2): E57.

66. Machado RS, de Sousa IP, Monteiro JC, Ferreira JL, dos Santos Alves JC, et al. (2020) Detection and identification of enteroviruses circulating in children with acute gastroenteritis in Pará State, Northern Brazil (2010–2011). Virol J. 17(1):1-5.

67. Kumthip K, Khamrin P, Ushijima H, Maneekarn N (2017) Multiple enterovirus genotypes circulating in children hospitalized with acute gastroenteritis in Thailand. Infect Genet Evol. 55:324-331.

68. Chansaenroj J, Tuanthap S, Thanusuwannasak T, Duang-In A, Klinfueng S, et al. (2017) Human enteroviruses associated with and without diarrhea in Thailand between 2010 and 2016. PLoS One. 12(7): e0182078.

69. Abe O, Kimura H, Minakami H, Akami M, Inoue M, et al. (2000) Outbreak of gastroenteritis caused by echovirus type 6 in an orphanage in Japan. J Infect. 41(3):285-286.

70. Rai SB, Mansor HW, Vasantha T, Norizah I, Chua KB. (2007) An outbreak of echovirus 11 amongst neonates in a confinement home in Penang, Malaysia. Med J Malaysia. 62(3):223.

71. Patel JR, Daniel J, Mathan VI. (1985) An epidemic of acute diarrhoea in rural southern India associated with echovirus type 11 infection. Epidemiol Infect. 95(2):483-492.

72. Carvalho R de S, Kirchner E, Moura R de A, Murahovschi J (1977) Acute gastroenteritis associated with coxsackie B-6 virus in infants and children, in São Paulo, Brazil. Rev Inst Med Trop Sao Paulo. 19(1):73-76.

73. Silva PA, Stark K, Mockenhaupt FP, Reither K, Weitzel T, et al. (2008) Molecular characterization of enteric viral agents from children in northern region of Ghana. J Med Virol. 80(10):1790-1798.

74. Di Cristanziano V, Böttcher S, Diedrich S, Timmen-Wego M, Knops E, et al. (2015) Detection and characterization of enteroviruses and parechoviruses in healthy people living in the South of Cote d'Ivoire. J Clin Virol. 71:40-43.

75. Bitnun A, Yeh EA. (2018) Acute flaccid paralysis and enteroviral infections. Curr Infect Dis Rep. 20:1-15.

76. Assessment RR (2016) Enterovirus detections associated with severe neurological symptoms in children and adults in European countries, 8 August 2016. Eur Cent Dis Prev Control Access Httpecdc Eur EuenpublicationsPublications01–08–2016-RRA-Enterovirus.

77. Yang F, Zhang T, Hu Y, Du J, Li Y, et al. (2011) Survey of enterovirus infections from hand, foot and mouth disease outbreak in China, 2009. Virol J 8: 508.

78. Li L, He Y, Yang H, Zhu J, Xu X, et al. (2005) Genetic characteristics of human enterovirus 71 and coxsackievirus A16 circulating from 1999 to 2004 in Shenzhen, People's Republic of China. J Clin Microbiol 43: 3835-9.

79. De W, Changwen K, Wei L, Monagin C, Jin Y, et al. (2011) A large outbreak of hand, foot, and mouth disease caused by EV71 and CAV16 in Guangdong, China, 2009. Arch Virol 156: 945-53.

80. Chen L, Yao XJ, Xu SJ, Yang H, Lu J, et al. (2019) Molecular surveillance of coxsackievirus A16 reveals the emergence of a new clade in mainland China. Arch Virol 164: 867-874.

81. Xu SJ, Yang H, Yao XJ, Zhang HL, Ren Y, et al. (2017) Complete genome sequences of four coxsackievirus A16 strains isolated from four children with severe hand, foot, and mouth disease. Genome Announc 5: e00760-17.

82. Wang CY, Lu FL, Wu MH, Lee CY, Huang LM (2004) Fatal coxsackievirus A16 infection. Pediatr Infect Dis J 23: 275-6.

83. Legay F, Lévêque N, Gacouin A, Tattevin P, Bouet J, et al. (2007) Fatal coxsackievirus A-16 pneumonitis in adult. Emerg Infect Dis 13: 1084-6.

84. Kenmoe S, Sadeuh-Mba SA, Vernet MA, Penlap Beng V, Vabret A, et al. (2021) Molecular epidemiology of Enteroviruses and Rhinoviruses in patients with acute respiratory infections in Yaounde, Cameroon. Influenza Other Respir Viruses 15: 641-650.

85. Oberste MS, Maher K, Kilpatrick DR, Pallansch MA (1999) Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. J Virol 73: 1941-8.

86. Pérez-Losada M, Arenas M, Galán JC, Palero F, González-Candelas F (2015) Recombination in viruses: mechanisms, methods of study, and evolutionary consequences. Infect Genet Evol 30: 296-307.

87. Pond SLK, Murrell B, Poon AFY (2012) Evolution of viral genomes: interplay between selection, recombination, and other forces. Evol Genomics Stat Comput Methods Vol 2. Published online 856: 239-272.

88. Kyriakopoulou Z, Pliaka V, Amoutzias GD, Markoulatos P (2015) Recombination among human non-polio enteroviruses: implications for epidemiology and evolution. Virus Genes. 50: 177-88.

89. Simmonds P, Welch J (2006) Frequency and dynamics of recombination within different species of human enteroviruses. J Virol 80: 483-493.

90. Wang H, Cui X, Cai X, An T (2022) Recombination in positive-strand RNA viruses. Front Microbiol 13: 870759.

91. Jegouic S, Joffret ML, Blanchard C, Riquet FB, Perret C, et al. (2009) Recombination between polioviruses and co-circulating Coxsackie A viruses: role in the emergence of pathogenic vaccine-derived polioviruses. PLoS Pathog 5: e1000412.

92. Gmyl AP, Belousov EV, Maslova SV, Khitrina EV, Chetverin AB, et al. (1999) Nonreplicative RNA recombination in poliovirus. J Virol 73: 8958-65.

93. Ledinko N (1963) Genetic recombination with poliovirus type 1: studies of crosses between a normal horse serum-resistant mutant and several guanidine-resistant mutants of the same strain. Virology 20: 107-119.

94. Lukashev AN (2010) Recombination among picornaviruses. Rev Med Virol 20: 327-337.

95. Oberste MS, Maher K, Pallansch MA. (2004) Evidence for frequent recombination within species human enterovirus B based on complete genomic sequences of all thirty-seven serotypes. J Virol 78: 855-867.

96. Hu YF, Yang F, Du J, Dong J, Zhang T, et al. (2011) Complete genome analysis of coxsackievirus A2, A4, A5, and A10 strains isolated from hand, foot, and mouth disease patients in China revealing frequent recombination of human enterovirus A. J Clin Microbiol 49: 2426-2434.

97. Al-Hello H. (2012) Pathogenic and Molecular Characteristics of Two Isolates of HEV-B Species.

98. Bouslama L, Nasri D, Chollet L, Belguith K, Bourlet T, et al. (2007) Natural recombination event within the capsid genomic region leading to a chimeric strain of human enterovirus B. J Virol 81: 8944-8952.

99. Rakoto-Andrianarivelo M, Guillot S, Iber J, Balanant J, Blondel B, et al. (2007) Co-circulation and evolution of polioviruses and species C enteroviruses in a district of Madagascar. PLoS Pathog 3: e191.

100. Oberste MS, Peñaranda S, Maher K, Pallansch MA (2004) Complete genome sequences of all members of the species Human enterovirus A. J Gen Virol 85: 1597-1607.

101. Ylä-Pelto J, Tripathi L, Susi P (2016) Therapeutic use of native and recombinant enteroviruses. Viruses 8: 57.

102. Brouwer L, Benschop KS, Nguyen D, Kamau E, Pajkrt D, et al. (2020) Recombination Analysis of Non-Poliovirus Members of the Enterovirus C Species: Restriction of Recombination Events to Members of the Same 3DPol Cluster. Viruses 12: 706.

103. Nikolaidis M, Mimouli K, Kyriakopoulou Z, Tsimpidis M, Tsakogiannis D, et al. (2019) Large-scale genomic analysis reveals recurrent patterns of intertypic recombination in human enteroviruses. Virology 526: 72-80.

104. Liu Y, Fu C, Wu S, Chen X, Shi Y, et al. (2014) A novel finding for enterovirus virulence from the capsid protein VP1 of EV71 circulating in mainland China. Virus Genes 48: 260-72.

105. Georgescu MM, Delpeyroux F, Crainic (1995) Tripartite genome organization of a natural type 2 vaccine/nonvaccine recombinant poliovirus. J Gen Virol 76: 2343-8.

106. Liu HM, Zheng DP, Zhang LB, Oberste MS, Pallansch MA, et al. (2000) Molecular evolution of a type 1 wild-vaccine poliovirus recombinant during widespread circulation in China. J Virol 74: 11153-61.

107. Xu T, Wang W, Lyu S (2016) Identification of a novel Coxsackievirus A16 strain isolated from the clinic HFMD sample in Hubei Province in 2010. Adv Biosci Bioeng 4: 51-58.