

RESEARCH ARTICLE

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Faeco-prevalence of enteric viruses in children under five in Ogun State, Nigeria; a one-year study

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Abstract

Objective: Acute gastroenteritis has been reported as the second largest cause of preventable child mortality, accounting for an estimated annual 1.5 million deaths. In Nigeria, routine clinical diagnosis of infectious diarrhoea is bacterial-focused, leaving out the viral agents. Therefore, this study was designed to investigate the prevalence, seasonality, and risk factors of selected enteric viruses (EVs): human adenovirus (HAdV), norovirus (NoV), hepatitis A virus (HAV), and human astrovirus (HAsV) infection in children under 5 years old with acute gastroenteritis in Ogun State, Nigeria.

Methods: One hundred faecal samples collected from out-patient diarrheal children under five years from six different hospitals between May 2021 and April 2022 were analysed using specific primers for four EVs by nested reverse transcription polymerase chain reaction.

Result: Among the samples analysed, 44% were positive for at least one EV. The highest prevalence was recorded in HAdV (29%), followed by HAsV (9%), and NoV (6%). Mixed infections were only found in 3 cases. Additionally, the occurrence of EV infection was observed to be highest in children below 2 years ($p = 0.002$). A statistically significant ($p = 0.03$) association was observed between the EVs and the source of drinking water. The seasonal pattern of the EVs showed a marked increase in their incidence during the dry season.

Conclusion: This study shows that EVs contribute to the burden of gastroenteritis in Ogun state, hence, continuous monitoring of these pathogens' epidemiology remains fundamental to effective treatment and prevention of this disease.

Keywords: Enteric viruses; children ≤ 5 years; gastroenteritis; human adenovirus; human astrovirus; norovirus; hepatitis A virus

Plain English Summary

Molecular analysis reveals the presence of enteric viruses in faecal samples of diarrheal children under five years. The current study used molecular-based techniques to reveal the presence of human adenovirus (HAdV), norovirus (NoV), and human astrovirus (HAsV) known to cause acute gastroenteritis in children under 5 years. Although, the study did not detect the presence of the hepatitis A virus, however, the occurrence of other enteric viruses calls for serious public health concern. This study shows that EVs contribute to the burden of gastroenteritis in Ogun state, Nigeria.

Background

Acute gastroenteritis has been reported as one of the major causes of preventable child mortality

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accounting for an estimated annual 70 million deaths (1). South-East Asia and Africa account for an estimated 82% of this diarrheal-related mortality (2). The disease burden for diarrhoea was highest among children under five years (2). Among the many aetiologies of infectious diarrhoea, enteric viruses (EVs) often rank highest (3). In Nigeria, rotavirus, human adenovirus (HAdV), norovirus (NoV), human astrovirus (HAstV), and hepatitis A virus (HAV) are some of the leading EVs commonly implicated in cases of viral gastroenteritis (4, 5). In 2016, epidemiological data on the disease burden of EVs in Nigeria showed that rotavirus infection within the country accounted for 14% of deaths in the global children under five mortality indices (6). The annual mortality associated with rotavirus infection within the country resulted in an intervention that led to the introduction of the rotavirus vaccine as part of the mandatory routine immunization program for children in Nigeria (7). Consequently, this same intervention was not extended to other EVs such as HAdV, NoV, HAstV, and HAV.

HAdV (family Adenoviridae) are non-enveloped linear double-stranded DNA viruses implicated in various cases of humans and zoonotic diseases (8, 9). Different serotypes of HAdV have been implicated in varying pathologies, however, only adenovirus species F (types 40 and 41) were noted as the primary aetiology of childhood diarrhoea (10). An essential characteristic of HAdV is its ability to establish latency using the lymphoid tissue of the gut as a reservoir for many species (10). HAdV rapidly spread in close and clustered environments such as hospitals, public swimming pools, and children's homes (11, 12).

Human astroviruses (HAstVs) are non-envelope, positive-sense single-stranded RNA viruses belonging to the family Astroviridae (13). These groups of viruses have been implicated in different cases of childhood gastroenteritis. Cases of human astrovirus-associated pathologies have been reported globally and their severity in children is of public health significance (14). Globally, HAstVs account for 2.9 – 5.0% of all paediatric gastroenteritis instances with an estimated 1.3% - 40.4% prevalence in children under five years old (15). Human astrovirus infections are caused by eight closely related viral genotypes known as HAstV 1–8 (classic human astrovirus) (16).

Noroviruses (NoVs) are viruses belonging to the family Caliciviridae. They are known to cause sporadic and epidemic acute viral gastroenteritis (17, 18). NoVs are well-known aetiology of severe gastroenteritis and have been implicated in outbreaks in places like schools, cruise ships, nursing homes, and communities (17). Human NoV strains are classified into 3 groups: GI, GII, or GIV. Norovirus-associated gastroenteritis can be distinguished from other viral aetiology by the

abrupt onset of diarrhoea and/or vomiting, although it may also be accompanied by nausea, stomach discomfort, fever, headache, and body pains (19). Epidemiological survey of some strains of noroviruses in children shows the presentation of sporadic self-limiting gastroenteritis often requiring hospitalization (20, 21, 22, 23). Conversely, there are reports of severe illnesses and fatalities associated with NoV-associated gastroenteritis. The annual case-fatality of NoV-associated gastroenteritis in children is estimated at 70,000 (24).

Hepatitis A virus (HAV) is a member of the genus Hepatovirus and belongs to the family Picornaviridae (25). The endemicity of HAV infection is highly dependent on hygiene and sanitation practices (26). An annual 1.5 million clinical cases of HAV infections are reported worldwide (27). Many young children who have been infected with HAV are asymptomatic unlike adults and older children who show symptoms such as jaundice (28, 29). HAV infection is found to be common among developing countries with poor sanitary practices and it is endemic in areas like Africa, Asia, South and Central America, the Middle East, and the Western Pacific (30). Although Africa is included as one of the regions with high endemicity of HAV, there has been limited information on the infection of HAV in Africa (27). A range of 80-95% of children less than 5 years have asymptomatic infection (30). HAV infection rarely results in complications but when it does it causes fulminant hepatitis A which only affects less than 1% of patients and is characterized by worse jaundice and encephalopathy (31).

In 2018, an estimated 5.3 million deaths of under-five children were recorded with sub-Saharan Africa accounting for half of these deaths (32). In the following year, Nigeria recorded an estimated 858,000 under-5 deaths, a significant percentage of which was attributed to diarrhoea (33). Additionally, interventions such as the Stop Diarrhea Initiative (SDI) aimed at the prevention and control of diarrheal diseases as proposed by WHO and UNICEF in 2009 have not fully yielded the desired result. In Nigeria, the aetiology of diarrhoea is commonly thought to be bacterial and the presence of enteric viruses is often not clinically investigated (4). Furthermore, the lack of continuous and routine investigation of EV in clinical facilities in Nigeria creates an epidemiological disparity between the body of literature and what is obtainable in the field. Hence, this study was designed to investigate the prevalence, seasonality, associated symptoms, and risk factors of HAdV, NoV, HAV, and HAstV in children under 5 years old with acute gastroenteritis in Ogun State, Nigeria.

Materials and Methods

Study location and population

This study was a cross-sectional hospital-based study conducted between May 2021 and April 2022 at the paediatric wards of six hospitals: Ibafo Health Clinic (6°44'38.2" N 3°25'16.7" E), Mowe Primary Healthcare Centre (PHC) (6°48'28.7" N 3°26'07.9" E), Ofada PHC (6°51'54.7" N 3°25'39.4" E), Mokoloki PHC (6°52'55.8" N 3°22'04.8" E), Sacred Heart Hospital (7°10'00.2" N 3°21'20.2" E) and

Federal Medical Centre (7°08'44.7" N 3°22'41.4" E) as shown in (Figure 1). The demography for this research was noted for low-middle-income earners and high population density. Diarrheal children ≤ 5 years of age attending these hospitals and whose parents gave consent for participation in the research were enrolled.

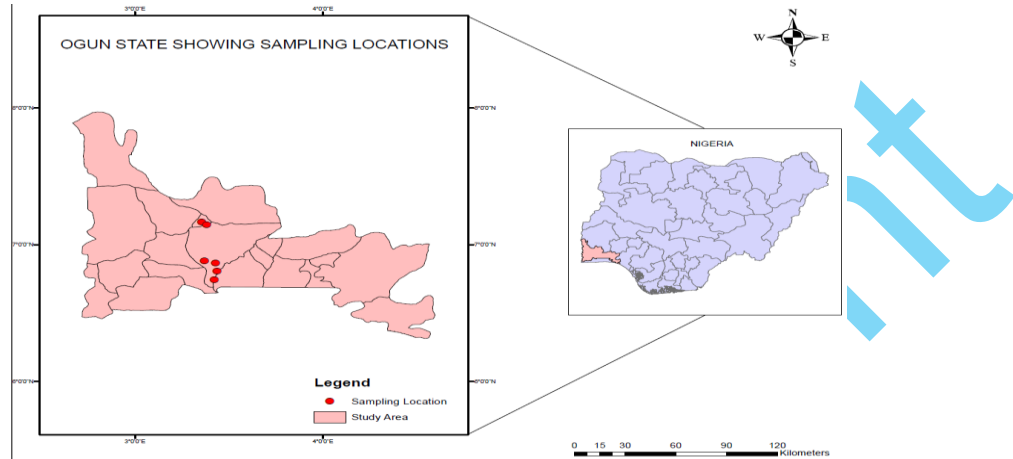


Figure 1: Study area and sampling locations (Self-generated).

Faecal sample collection and processing

Faecal samples were collected from one hundred out-patient children under 5 years presenting with symptoms of diarrhoea as diagnosed by the resident physician. Stools were collected in a clean sterile leak-proof stool collection bottle. Samples were subsequently labelled with unique laboratory codes specific to each sample, packed, sealed, and transported on ice packs to the Laboratory for analyses within 24 hours. Interviewer-administered questionnaires were used to obtain information about clinical manifestation and baseline sociodemographic characteristics that included age, gender, occupation, and sanitary wares of the diarrheal child from the parent(s). The data obtained were used as determinants for viral gastroenteritis in children within the study population. Stool samples were initially screened for enteric pathogenic bacteria and tested negative by polymerase chain reaction.

Inclusion and Exclusion Criteria for Study Participation

Children of both sexes under the age of 5 years presenting with diarrhoea were enrolled for this research. Children with diarrhoea but older than 5 years were excluded from this study.

Sample size

The sample size was calculated using the estimated prevalence of 7.3% for HAstV in Nigeria (35) and the formula as described by (34). The estimated sample size was one hundred and four.

Viral DNA/RNA extraction (ref)

Viral nucleic acids were extracted from 140 microlitres (μ l) of clarified 10% faecal suspension in phosphate-buffered saline (PBS) using the QIAamp® viral genomic DNA/RNA kits (QIAGEN, Germany) according to the manufacturer's instructions. Briefly, 560 μ l of AVL buffer was mixed with 5.6 μ l of the carrier RNA (cRNA) and gently swirled 10 times. An aliquot of 560 μ l of the prepared buffer AVL-cRNA mixture was then added to 140 μ l of 10% faecal suspension, pulse-vortexed, and incubated at room temperature for 10 min. The tube was then pulse-centrifuged and an aliquot of 560 μ l of absolute ethanol was added to the mixture, pulse-vortexed and centrifuged. An aliquot of 630 μ l of the lysate mixture was transferred to QIAamp mini columns and centrifuged at 6000 x g (8000rpm) for 1 min. The filtrate from the mini-column was discarded (this step was repeated for the remaining lysate mixture). An aliquot of 500 μ l of Buffer AW1 was added to the mini-column and centrifuged at 6000 x g (8000rpm) for 1 min. The flow through was discarded after which an aliquot of 500 μ l of Buffer AW2 was added to each mini-column and centrifuged at 20,000 x g for 3mins. The mini-column was placed in a clean 1.5 ml microcentrifuge tube and the previous collection tubes were discarded. An aliquot of 60 μ l of Buffer AVE was added to the mini-column, incubated at room temperature for 1 min, centrifuged at 6000 x g for 1 min, and stored at -20 °C used for molecular analyses the next day.

Viral detection

Adenovirus detection

The Adenovirus genes were detected using nested polymerase chain reaction (PCR) as previously described by Oh *et al.* (22). The primers used in this analysis are presented in Table 1. The first round of PCR (thermal cycling condition) amplification was performed in a mixture (25 µl) consisting of 5 µl of Red Load Taq Master (Jena Bioscience, Germany), 5 µl of the DNA template, 13 µl of PCR grade water (Qiagen, Germany) and 1 µl of primer mix (0.8 µM of AdV1 and AdV2). The PCR reactions were conducted using a MiniAmp thermal cycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) set at 94 °C for 2 mins followed by 40 cycles of amplification: denaturation at 94 °C for 30 secs, 42 °C for 30 secs, 72 °C for 45 secs, and 72 °C for 5 mins. The nested-PCR amplifications were performed in a 25 µl PCR reaction mixture consisting of 5 µl of Red Load Taq Master (Jena Bioscience, Germany), 2 µl of the DNA template, 16 µl of PCR grade water (Qiagen, Germany) and 1 µl of primer mix (0.8 µM of AdV3 and AdV4). All amplicons were resolved by electrophoresis on 1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator. Amplicons with a band size of 307bp for the first round PCR were selected for the nested PCR while those with a band size of 230bp for the nested PCR were confirmed as positive for Adenovirus.

Detection of HAstV, NoV, and HAV

Three of the viruses (HAstV, NoV, and HAV) were detected by reverse transcription polymerase chain reaction (RT-PCR). RT was conducted with random hexamers using the SCRIPT cDNA Synthesis Kit (Jena Bioscience, Germany) according to the manufacturer's instructions on a MiniAmp thermal cycler (Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany).

Human astrovirus PCR

The HAstV genes were detected using nested polymerase chain reaction (PCR) as described by Japhet *et al.* (36). The primers used in this analysis are presented in Table 1. The first round of PCR amplification was performed in a mixture (12.5 µl) consisting of 2.5 µl of Red Load Taq Master (Jena Bioscience, Germany), 2.5 µl of the cDNA, 6.5 µl of PCR grade water (Qiagen, Germany) and 0.5 µl of primer mix (0.8 µM of AV92a and AV91). The semi-nested PCR was done in a 20 µl reaction mixture consisting of 4 µl of Red Load Taq Master (Jena Bioscience, Germany), 2 µl of the first round PCR template, 12.4 µl of PCR grade water (Qiagen, Germany) and 0.8 µl of primer mix (0.8 µM of AV93 and AV91). Thermal cycling was done as follows: 94 °C for 2 minutes followed by 40 cycles of amplification; 94 °C for 30 seconds, 42 °C for 30 seconds, 72 °C for 90 seconds, and 72 °C for 5mins. All amplicons were resolved by electrophoresis on

1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator. The amplicons with a band size of 826bp for the first round were selected for the semi-nested PCR. Amplicons with a band size of 726bp for the semi-nested PCR were considered positive for HAstV.

Norovirus PCR

The NoV genes were detected using nested polymerase chain reaction (PCR) as described by Oh *et al.* (22). The primers used in this analysis are presented in Table 1. The first round of PCR amplification was performed in a mixture (20 µl) consisting of 4 µl of Red Load Taq Master (Jena Bioscience, Germany), 4 µl of the cDNA, 10.2 µl of PCR grade water (Qiagen, Germany) and 0.6 µl of primer mix (0.6 µM of NV32, NV32A, and NV 36). The nested PCR was done in a 20 µl reaction mixture consisting of 4 µl of Red Load Taq Master (Jena Bioscience, Germany), 2 µl of the first round PCR template, 11.6 µl of PCR grade water (Qiagen, Germany) and 0.6 µl of primer mix (0.6 µM of NV33, NV33a, NV35, and NV35a). Thermal cycling was done as follows: 94 °C for 2 mins followed by 35 cycles of amplification; 94 °C for 30 secs, 42 °C for 30 secs, 72 °C for 45 secs, and 72 °C for 3mins. All amplicons were resolved by electrophoresis on 1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator. The amplicons with a band size of 481bp for the first round were selected for the semi-nested PCR. Amplicons with a band size of 331bp for the semi-nested PCR were considered positive for NoV.

Hepatitis A virus PCR

The HAV genes were detected using nested polymerase chain reaction (PCR) as described by Beji-Hamza *et al.* (37). The primers used in this analysis are presented in Table 1. The first round of PCR amplification was performed in a mixture (20 µl) consisting of 4 µl of Red Load Taq Master (Jena Bioscience, Germany), 4 µl of the cDNA, 10.2 µl of PCR grade water (Qiagen, Germany) and 0.5 µl of primer mix (0.5 µM of S3978 and S3979). The nested PCR was done in a 20 µl reaction mixture consisting of 4 µl of Red Load Taq Master (Jena Bioscience, Germany), 2 µl of the first round PCR template, 11.6 µl of PCR grade water (Qiagen, Germany) and 0.6 µl of primer mix (0.6 µM of S397A and S397D). Thermal cycling was done as follows: 95 °C for 5 mins followed by 35 cycles of amplification; 94 °C for 30 secs, 50 °C for 30 secs, 72 °C for 35 secs, and 72 °C for 7 mins. All amplicons were resolved by electrophoresis on 1.8% agarose gel stained with ethidium bromide and viewed using the UV-transilluminator. The amplicons with a band size of 511bp for the first round were selected for the semi-nested PCR. Amplicons with a band size of 313bp for the semi-nested PCR were considered positive for HAV.

Statistical Analyses

Data obtained from the questionnaires were analysed using SPSS version 20 (IBM Corp., Armonk, NY). Descriptive statistic was used to

describe the results of this research. Chi-square was used to evaluate the association of the tested variables with the prevalence of the virus. The p-value of <0.05 was considered to be statistically significant.

Table 1: Primer sequences used in the nested RT-PCR assay and their required base pairs products.

Enteric virus (genes)	Primer name	Sequence (5'–3')	Amplicon size (bp)	Reference	
Human Astrovirus (ORF1b)	AV92a	GGTCARTGYGGGTGGTCACC			
	AV91	TTTGGWCCICCCCTCCA	826	Japhet et al. (34)	
	AV93	GAYTGGACICGMTWTGATGG	726		
Hepatitis A virus (VP1/2A)	S3978	GACAGATTCYACATTTGGATTGGT			
	S3979	CCATYTCAAGAGTCCACACACT	511	Beji-Hamza et al., (35)	
	S397A	CTATTCAGATTGCAAATTAYAAT	313		
	S397D	AAYTTCATYATTTTCATGCTCCT			
Norovirus (ORF1)	NV 32	ATGAATATGAATGAAGATGG			
	NV 32A	ATGAACACAATAGARGATGG			
	NV 36	ATTGGTCCTTCTGTTTTGTC	481	Oh et al. (22)	
	NV 33	TACCACTATGATGCAGATTA	331		
	NV 33A	TATCACTATGATGCTGACTA			
	NV 35	TATCACTATGATGCTGACTA			
	NV 35A	ACAATYTCATCATCICCAT			
Adenovirus (hexon gene)	AdV1	CAAGATGGCCACCCCTCG			
	AdV2	CGATCCAGCAGCGCGGGATGTC	307		Oh et al. (22)
	AdV3	AATGGTCTTACATGCACAT	230		
	AdV4	ACCCGGTTGTCGCCACGGCCAG			

Results

Among the 100 faecal samples, 44 % contained at least one enteric virus (EV). HAdV (29%) was the most prevalent EV detected, followed by HAstV (9%) and NoV (6%). HAV was not detected in any

of the faecal samples. Mixed EV infections were found in 3 samples (3%), with HAstV and HAdV (2%) being the most detected. Also, three different EVs (HAstV, NoV, and HAdV) were detected in one faecal sample (Figure 2).

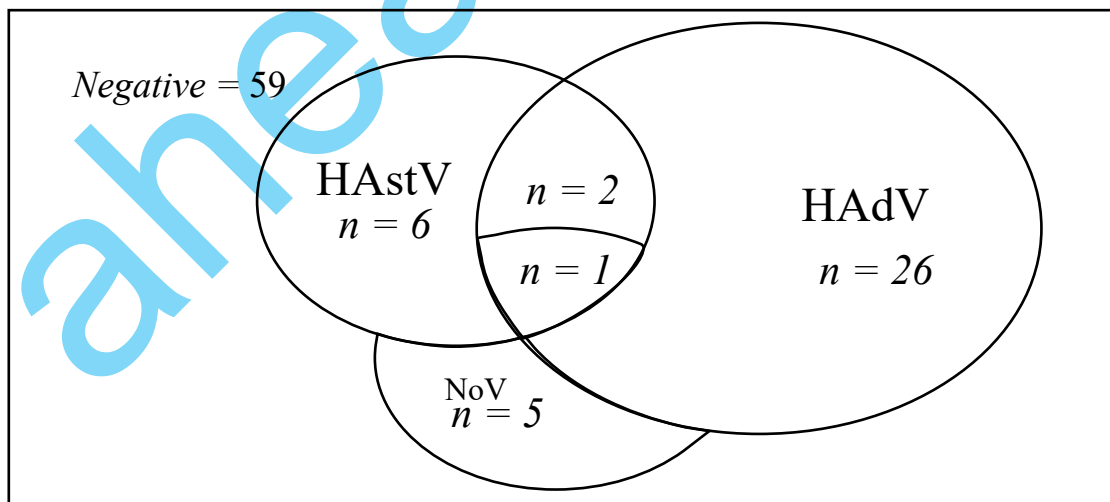


Figure 2: Venn diagram of enteric viral distribution in children under five years with viral gastroenteritis in Ogun State, Nigeria

The occurrence of EVs in the children showed that the highest burden of infection was amongst the age group < 2 years (59.3%), followed by the age

group ≥2 years (22%). The occurrence of EVs infection was observed to be higher in males (51.6%) compared to females (40.6%) (Table 2).

Table 2: Prevalence of enteric viruses in children under five years with viral gastroenteritis in Ogun State, Nigeria (N = 100)

Variable	NoV, n (%)	HAdV, n (%)	HAsV, n (%)	Total, n (%)	P-value
<i>N</i>	6	29	9	44	
Sex					
Female (<i>n</i> = 69)	4 (5.8)	17 (24.6)	7 (10.2)	28 (40.6)	0.25
Male (<i>n</i> = 31)	2 (6.5)	12 (38.7)	2 (6.5)	16 (51.6)	
Age (year)					
< 2 (<i>n</i> = 59)	5 (8.5)	22 (37.3)	8 (13.6)	35 (59.3)	0.002*
≥2 (<i>n</i> = 41)	1 (2.4)	7 (17.1)	1 (2.4)	9 (22.0)	

*p-value is significant

The distribution of EVs in the clinical manifestations was statistically significant ($p < 0.05$). In addition, the occurrence of EVs was highest in children exposed

to the use of pit latrines (Table 3). Conversely, children whose mother was stay-at-home spouse had the least detected EVs infection (28.6%).

Table 3: Sociodemographic and clinical findings in children under five years with viral gastroenteritis in Ogun State, Nigeria (N = 100).

Sociodemographic/ clinical manifestation	NoV, n (%)	HAdV, n (%)	HAsV, n (%)	Total, n (%)	P value
<i>N</i>	6	29	9	44	
Fever					
No (<i>n</i> = 31)	4 (12.9)	13 (41.9)	4 (12.9)	21 (67.7)	0.01*
Yes (<i>n</i> = 69)	2 (2.9)	16 (23.2)	5 (10.9)	23 (33.3)	
Vomiting					
No (<i>n</i> = 53)	4 (7.5)	18 (34)	9 (17)	31 (58.5)	0.02*
Yes (<i>n</i> = 47)	2 (4.3)	11 (23.4)	0 (0)	13 (27.7)	
Loss of Appetite					
Yes (<i>n</i> = 54)	3 (5.6)	9 (16.7)	3 (5.6)	15 (27.8)	0.01*
No (<i>n</i> = 46)	3 (6.5)	20 (43.5)	6 (13)	29 (63.0)	
Abdominal pain					
No (<i>n</i> = 73)	5 (6.9)	26 (35.6)	7 (9.6)	38 (52.1)	0.03*
Yes (<i>n</i> = 27)	1 (3.7)	3 (11.1)	2 (7.4)	6 (22.2)	
Attendance of nursery/school					
Yes (<i>n</i> = 26)	2 (7.7)	10 (38.5)	2 (7.7)	14 (53.9)	0.10
No (<i>n</i> = 74)	4 (5.4)	19 (25.7)	7 (9.5)	30 (40.5)	
Street food consumption					
No (<i>n</i> = 73)	6 (8.2)	20 (27.4)	8 (11)	34 (46.6)	0.41
Yes (<i>n</i> = 27)	0 (0)	9 (33.3)	1 (3.7)	10 (37.0)	
Type of toilet used					
Water closet (<i>n</i> = 91)	6 (6.6)	25 (27.5)	7 (7.7)	38 (41.8)	0.71
Pit Latrine (<i>n</i> = 5)	0 (0)	3 (60)	2 (40)	5 (100)	
Potty (<i>n</i> = 4)	0 (0)	1 (25)	0 (0)	1 (25.0)	
Source of drinking water					
Bottled water (<i>n</i> = 27)	0 (0)	6 (22.2)	3 (11.1)	9 (33.3)	0.03*
Sachet water (<i>n</i> = 26)	3 (11.5)	14 (53.9)	5 (19.2)	22 (84.6)	
Wells (<i>n</i> = 47)	3 (6.4)	9 (19.2)	1 (2.1)	13 (27.7)	
Maternal occupation					
Office worker (<i>n</i> = 27)	5 (18.5)	7 (25.9)	3 (11.1)	15 (55.6)	0.19
Merchant/trader (<i>n</i> = 44)	1 (2.3)	14 (31.8)	2 (4.6)	17 (38.6)	
Artisan/craftsman (<i>n</i> = 22)	0 (0)	6 (27.3)	4 (18.2)	10 (45.5)	
Housewife (<i>n</i> = 7)	0 (0)	2 (28.6)	0 (0)	2 (28.6)	

The HAdV was detected throughout the year (except in June and July) with 82.8% of the positive cases detected between the dry season (October and April) with a peak of prevalence in November

and February. Similarly, HAstV and NoV were found to peak in December and May respectively (Figure 3).

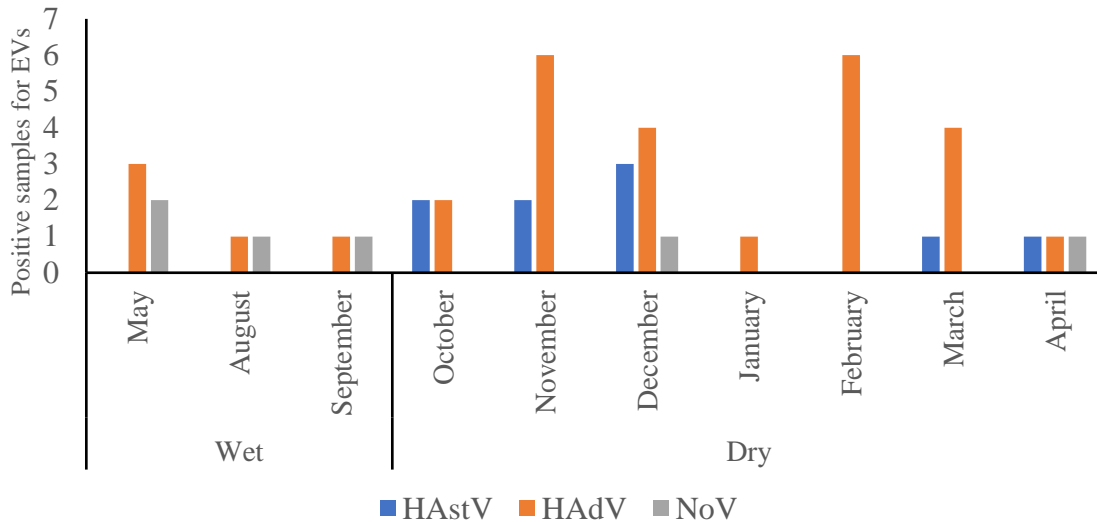


Figure 3: Temporal pattern of HAstV, HAdV, and NoV in children with gastroenteritis in Ogun State, Nigeria from May 2022 to April 2023. HAdV, human adenovirus; HAstV, human astrovirus; NoV, norovirus

Discussion

Diarrhoea is one of the major causes of preventable childhood mortality worldwide (38). The disease burden for diarrhoea was highest among children under five years living in sub-Saharan Africa (2). Among the many aetiologies of infectious diarrhoea, enteric viruses (EVs) often rank highest (3). The prevalence of EVs in the current study was 44%. Other studies – that also evaluated children from the same age range found 43.6% (India) (39), 30% - 40% (Europe) (40), 29.6% (Northern Italy) (41), 75.83% (Nigeria) (42), 85.6% (Burkina Faso) (43) and 60.9% (Gabon) (44). This implies that viral gastroenteritis remains a burden for Ogun state, another state in Nigeria and developing countries alike.

The observed frequency of coinfection in this study was 3%. Other studies had previously reported EV coinfection in China (5.2%) (45), Brazil (14%) (46), Enugu – Nigeria (13.19%) (42), Spain (21%) (15), Burkina Faso (35.7%) (43), and Northern Italy (2.3%) (41). This observed disparity in cases of coinfection may be due to the low faecal sample collected and predisposing practices such as the consumption of poorly treated water within these regions. In this study, the most common coinfections were between HAdV and HAstV which is similar to what was previously reported in Nigeria however with a higher prevalence (11.8%) (4). The occurrence of HAstV, HAdV, and NoV coinfection in a solitary case deviate from what was previously

reported in other studies (4, 41, 43, 46) with dissimilar co-infection pattern. Viral co-infection has been shown to result in a probable reduction in the efficacy of enteric virus vaccines, the emergence of new viral strains through viral reassortment or recombination, and viral interference (47).

HAdV was the most prevalent EVs detected in this study. This suggests that this virus is actively circulating and significantly contributes to the burden of diarrheal disease in Ogun State, Nigeria. This finding is in contrast with previous studies where HAdV prevalence was reported to be lower than other EVs such as NoV and HAstV (4, 48). The prevalence of HAdV in this study is higher than in the previous report from Republic Democratic of Congo (49), Ogun state – Nigeria (4), Albania (50), Burkina Faso (43), and Northern Italy (41). This discrepancy might be due to the emergence and evolution of HAdV as the next leading cause of viral gastroenteritis in this region. However, this assertion requires more epidemiological investigation.

In this study, HAstV was the second most prevalent EV after HAdV. This shows that this virus is circulating and contributes at a lower frequency to gastroenteritis compared to HAdV in Ogun State, Nigeria. This finding is in contrast with other studies from Ogun and Kwara state – Nigeria (4, 51) that had previously reported a higher prevalence rate for HAstV and other studies from Lebanon (52), Germany (53), and Gabon (44) with similarly high

prevalence. However, this result falls within the global HAdV prevalence range among children (1.3% - 40.4%). Factors responsible for this decrease in prevalence might be because of the positive effects of some of the diarrhoea prevention interventions and the period of sample collection.

NoV was found in 6% of children with diarrhoea which is slightly higher than 5.1% previously reported in other regions of Ogun state, Nigeria (4) and similar to studies in the Republic Democratic of Congo (49) but lower compared with studies in Albania (50), Burkina Faso (43), Northern Italy (41) and another region (Ondo and Borno state) of Nigeria (54, 55, 56). This observed disparity might be due to the seasonal variation of viruses.

HAV was not detectable in any of the stool samples. The lack of detection of HAV within the study population shows a non-existence of circulating strains. However, studies from other regions of Nigeria had reported seroprevalence of HAV to be between 2-24.7% (57, 58). These differences might be due to methods used for virus detection as serological assays are still able to detect antibodies for HAV even when there is no active virus circulating within the host.

EVs were observed more prevalent ($p = 0.002$) in children less than 2 years old when compared to those with greater than 2 years old. This report agrees with previous findings from Angola (59), Ogun state, Nigeria (4), and Gabon (44). Children within this age group do not have a fully developed immune system and a heightened risk of exposure due to physiological vulnerability. Although not statistically significant ($p = 0.25$), the burden of EVs in this study was higher in males compared to females. Interestingly, the occurrence of EVs was significantly higher ($p = 0.03$) in sachet water compared to other sources of drinking water. An explanation for this may be due to improperly treated water and ineffective water treatment techniques against EVs (60). Furthermore, EVs are known to evade commonly used water treatment techniques and the presence of these viral pathogens is not assayed for in water quality check compared to enteric bacterial pathogens (61). Interestingly, children who had their mother with them all day (stay-at-home spouse) had a lower prevalence of EVs compared to other groups. This contrasts with a report from another study (62) where children whose mothers were stay-at-home spouses had a higher occurrence of diarrhoea. Mothers who are always present with their children at this early stage of life serve to provide watchfulness which helps in preventing them from potential sources of EV infection. In addition, the occurrence of EVs was observed to be highest in children exposed to pit latrine. According to a study (63), EV household spread can occur through groundwater contaminated with spillage from the pit latrine. No causal relationship was established

between EVs and the other sociodemographic/risk factors.

Children who were EV negative were found to have more frequencies of fever, vomiting, loss of appetite and abdominal pains and these findings were statistically significant. Although these symptoms are known clinical manifestations of viral gastroenteritis (4), however, findings from this study showed that they cannot be relied on in empirical diagnosis of this infection as these symptoms are commonly associated with other pathologies.

The seasonal pattern of the HAdV, HAdV, and NoV showed that frequency in incidence was highest during the dry season. Prevailing environmental conditions during this season create conditions favourable for transmission, spread, and maintenance in the environment. This pattern is consistent with previous reports from Nigeria and other countries in West Africa (43). HAdV was detected throughout the year except in June and July, with two major peaks in November and February. This observed seasonality is consistent with a previous report from Northwestern Nigeria (64). HAdV incidence peaked in December as against what was previously reported in Ogun state, Nigeria (4) and Spain (65).

Study limitation

The limitation of this study is the inability to analyse more faecal samples and other enteric viral pathogens due to financial constraints. Nonetheless, the samples analysed covered all age groups, seasonality, and other variables.

Conclusions

In conclusion, this study shows that HAdV is the main aetiology of viral gastroenteritis in the studied population, and its prevalence is highest among children younger than 2 years of age. Enteric viruses are more prevalent during the dry season. Studies that monitor the epidemiological progression of EVs are constantly required. Whether the introduction of the RVA vaccine into the routine immunization program will impact other EVs epidemiology remains uncertain. Hence, continuous monitoring of enteric viruses' epidemiology remains crucial to the treatment and effective management of diarrheal diseases.

List of abbreviations

EVs: Enteric viruses
HAdV: Human adenovirus
NoV: Norovirus
HVA: Hepatitis A virus
HAdV: Human astrovirus
RVA: Rotavirus group.

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved by ethics committee in the Sacred Heart Hospital Ethical Committee (SHH/EC/EA/03/07/20), Ogun State Primary Health Care Development Board (OGHECADEB 02/10/19/041), and Federal/Medical Centre Research Ethics Committee (FMCREC), Idi-Aba, Abeokuta, Ogun state (FMCA/243/HREC/03/2016/14). The study aims were thoroughly explained to the parents of the participating children after which informed consent was obtained. The autonomy to participate or withdraw (without facing consequences) from this research was diligently communicated to the parents after which they decided whether to participate or provide information about their children. Written informed consent has been obtained from the patient(s) to publish this paper.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license. We otherwise convey all copyright ownership, including all rights incidental thereto, exclusively to the journal when published.

Availability of data and materials

Data generated in this study are available from the authors upon reasonable request.

Competing interests

The authors declare that there are no conflicts of interest.

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Author Contributions

OTS, ACI, and ODK conceived, designed, and developed the methodology of the study. OTS, OC, ADI, OAE, OEA, and UAE analysed and interpreted the data, and TSO drafted the manuscript. ACI and ODK revised the manuscript. All authors read and approved the final manuscript.

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