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Prevalence and risk factors of astrovirus gastroenteritis in children in Offa, Kwara State, North Central Nigeria

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ABSTRACT

Background: Astroviruses are small non-enveloped, single stranded, positive RNA viruses belonging to the family astroviridae. Gastroenteritis constitute the main causes of morbidity and mortality among pediatric population world wide. Common symptoms of astrovirus include nausea, vomiting and diarrhea. Human astroviruses are transmitted by contact with infected individuals and fomites, followed by the ingestion of contaminated food and water. This study aimed to determine the prevalence and risk factor of astrovirus in children in offa, kwara state, North central, Nigeria. **Methods :** A cross-sectional study design using random sampling technique. Three hundred (300) consented children with diarrhea were recruited into the study. Stool samples of each children were examined for the presence of astrovirus using Enzyme Linked Immunosorbent Assay (ELISA) technique with commercially available astrovirus kit (BIOTUVA UK). **Results:** This study reported an overall prevalence of 31.3%. astrovirus infection among children with gastroenteritis in Offa, Kwara state, North Central, Nigeria, with majority of the children positive for astrovirus were within the age of 4-5 years (36.2%), followed by those within the age of 2-3 years (34.0%) and the least found in age group 0-1 year (29.8%), 54(57.4%) of the children positive for astrovirus were male and 40 (42.6%) were female. Age and gender were not statistically significant while place of residence and risk factors were statistically significant, which could be as a result of un-hygienic environment. **Conclusion:** This study provides a base line data necessary for the formulation of state and national health policies in mitigating the impact of viral diarrhea, an ubiquitous disease of childhood. Hence, community and hospital- based surveillance are needed to provide an estimate of astrovirus burden in Nigeria.

Introduction

Gastroenteritis constitutes the main cause of morbidity and mortality among children younger than 5 years and are also a major cause of malnutrition and diminished growth [1]. Diarrheal diseases cause 1.6 million deaths, and constitute about 27% of all deaths among children aged less

than 5 years annually [2]. Infantile diarrheas are caused by viruses, parasites, bacteria, and some toxins produced by fungi [3]. Human astroviruses (HAsTVs) are a major cause of viral gastroenteritis after rotaviruses and noroviruses in children, adults and elderly.

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Astroviruses (AstVs) are small, non-enveloped, single stranded, positive-sense RNA viruses belonging to the family Astroviridae, which is divided into two genera, Mamastrovirus and Avastrovirus, whose members infect mammals and birds, respectively [4]. The genome, which is 6.8-7.9 kb long, contains of three open reading frames (ORFs): ORF1a, ORF1b, and ORF2 [5]. Both ORF1a and ORF1b encode the non-structural proteins involved in RNA transcription and replication, while ORF2 encodes the capsid protein precursor, which exhibits the highest diversity among the viral proteins and is used generally as the basis for genotyping [6]. Human astroviruses (HAstVs) are classified into classical HAstV, Melbourne (MLB) clade, and Virginia (VA) clade. Classical HAstV comprises genotypes 1-8 (HAstV-1-8) [7]. The MLB and VA clades are highly genetically distinct from the classical HAstVs and are more closely related to animal astroviruses [7]. The classical HAstVs are circulating globally and associated with 2.9% to 5.0% of acute diarrhea in children [8]. Generally, HAstV-1 is the most common genotype found worldwide, followed by genotypes 2 to 5, which are usually associated with diarrheal outbreaks, whereas HAstV-6 to -8 are seldom detected [9]. However, the predominant genotype varies with time and location [9].

Many countries on the African continent have identified HAstVs in children with diarrhoea. In Mali, 0.8% to 3.2% of HAstV cases were detected in children aged 59 months and younger [10]. Varied prevalence has been reported from Kenya (6.3%, 30/476) [11], Ghana (3.3%, 12/367) [12], Egypt (6.3%, 23/364) [13], Nigeria (40.4%, 65/161) [14], Burkina Faso (2.1%, 1/48) [15], Kenya and The Gambia (9.9%, 94/949) [16] and Tunisia (4%, 2/50) [17].

Human astroviruses are transmitted by contact with infected individuals and fomites, followed by the ingestion of contaminated food and water. [18] The morbidity caused by HAstVs is influenced by the season, with higher infection rates during the rainy season. The clinical signs of HAstV infection are watery diarrhoea of about 3 days, lasting up to 10 days episodes of non-blood-stained stools in 24 hours, often associated with vomiting, fever, and abdominal pain [19].

Different methodologies have been employed for the detection and characterization of these viruses including electron- and- immune

electron microscopy, enzyme immunoassay, latex agglutination, viral isolation in cell culture, and molecular procedures such as reverse transcription-polymerase chain reaction (RT-PCR) [20]. The RT-PCR has been widely utilized for both viral detection and genotyping using primers designed for each one of genomic regions [19].

To date, there is no vaccine or widely accepted treatments for astrovirus (HAstV) infection. This is partially due to the relatively mild symptoms of HAstV infection, which typically resolve without medical intervention. If treatment is necessary, it is generally only to alleviate symptoms [21]. Moreover, infections due to astroviruses can be prevented by decontamination of fomite and drinking water.

A study on the epidemiology of astro viral infections among children is a prerequisite not only for formulation of appropriate control strategies but also to predict risk for communities under consideration. Moreover, based on our knowledge and findings, there has not been any published study on epidemiology of astrovirus among children in the present study area. Thus, the need to update the general public, World Health Organization (WHO) and other agencies on epidemiological of astrovirus in children in Offa, Kwara state, North central, Nigeria. This study aimed to determine the prevalence and risk factors of astrovirus in children in Offa, Kwara state, North central, Nigeria.

Materials and Methods

Sampling technique

A cross-sectional and epidemiological study design and a simple random sampling technique was used to recruit children presenting with diarrhea for this study. A semi structured questionnaire was administered to collect demographic data of participants. A total number of three hundred (300) children were recruited for this study. The study participants consisted of children presenting with diarrhea admitted to hospitals, clinics and Primary Health Centre in Offa, Kwara State. **SAMPLE COLLECTION**

Fecal samples were collected from the 300 concented participants into sterile screw cap universal bottles and were transported to the virology laboratory for Astrovirus antibody screening using ELISA technique.

Laboratory analysis

Astrovirus screening using Enzyme Linked Immunosorbent Assay (ELISA)

Assay procedure

All reagents for the assay were brought to room temperature (20°C-25°C) from a temperature of 2°C-8°C and thoroughly mixed by swirling gently before use. Samples were numbered according to the microtitre wells. The desired number of coated strips were placed into the holder. Five microlitre (5µl) of the test samples, negative control, positive control and calibrator control were added to 200 µl of the sample diluents to make 1 in 40 dilutions. One hundred microlitres (100 µl) of diluted sera, calibrator and controls were dispensed into appropriate wells. For the reagent blank, 100µl sample diluents were dispensed into 1A well position. The holder was tapped so as to remove air bubbles, mixed well and incubated for 30 minutes at room temperature. The liquid from all the wells were removed and wells washed three times with washing buffer. One hundred microlitres (100 µl) of the enzyme conjugate was also dispensed into each well and the plate incubated for 30 minutes at room temperature. Excess enzyme conjugate was removed by washing each well with washing buffer three times. Approximately, 100µl of TMB chromogenic Substrate was dispensed into each well and incubated at room temperature for 30 minutes. One hundred microlitres (100 µl) of 2 N HCl was finally added to stop the reaction. There was a colour change from blue to yellow and within 15 minutes the plates were read at an absorbance of 450nm using a microwell reader.

Statistical analysis

The data was analysed using IBM-Statistical Package for Social Sciences (SPSS) version 25.0 and descriptive statistics (frequencies, percentage) was used to describe the data. The chi-square was used to test for the hypotheses. In this study, we assumed 5% significance levels i.e. alpha = 0.05 implying $p < 0.05$.

Ethical approval

Clearance and approval for this study were collected from the Ministry of Health, Kwara State with reference nu)MOH/KS/EU/777/525. At an individual level, informed consent was received from the parents of each participants before data collection. Respondents received a detailed description of the study, confidentiality provisions and the fact that their participation is voluntary and

they could withdraw at any point if they wish. The principles privacy and confidentiality were upheld.

Results

Socio-demographic characteristics of the study participants

Table 1 shows the socio-demographic characteristic of study participants. Majority of children enrolled for the study were within the age of 4-5 years 144(48.0%), followed by those within the age of 0-1years 80 (26.7%) and least among 2-3years 76 (25.3%). While majority of the children were male 172 (57.3%) and female 128 (42.6%).Based on educational level, majority of the children were in primary class 122 (40.7%), followed by those in crèche 80 (26.7%), Nursery class 52 (17.3%) and least were kindergarten class 46 (15.3%)

Prevalence of astrovirus infection among study participants

Table 2 showed that out of the 300 stool samples analyzed for presence of Astrovirus infection among children 94(31.3%) were positive while 206(68.7%) were negative for the presence of astrovirus infection among children.

Age distribution of astrovirus among children in Offa

Table 3 showed the age groups of school children with astrovirus, majority of the children positive for astrovirus were within the age of 4-5 years (36.2%), followed by those within the age of 2-3 years (34.0%) and the least positive were found in age group 0-1 year (29.8%).There was no statistical significance association of age groups ($p=0.861$) with positive prevalence of astrovirus infection at $p < 0.05$.

Gender distribution of astrovirus among children in Offa

As shown on **table (4)**; majority 54(57.4%) of the children positive for astrovirus were male and 40 (42.6%) were female. There was no statistical significance association of gender ($p=0.307$) with prevalence of astrovirus infection at $p > 0.05$.

Distribution of astrovirus among children in Offa based on educational level

As shown on **table (5)**; majority 30 (31.9%) of the children positive for astrovirus were in Creche, followed by those in primary 28 (29.8%), kindergarten 20 (21.3%) and nursery 16 (17.0%).There was no statistical significance association of educational level ($p=0.426$) with prevalence of astrovirus infection at $p < 0.05$.

Distribution of astrovirus among children in Offa based on residence

As shown on **table (6)**; majority 72(76.6%) of the children positive for Astrovirus reside in urban area, followed by Semi urban 12 (12.8%) and rural 10 (10.6%). There was statistical significance association of residence ($p=0.001$) with prevalence of Astrovirus infection at $p<0.05$.

Risk factors associated with prevalence of astrovirus among children

As shown on **table (7)**, children who use water from well (53.2%) had highest prevalence of astrovirus infection. This is followed by those who use borehole (27.7%), Treated tap water (10.6%) and

river water (8.5%). There was statistical significance association of water used for domestic use ($p=0.001$) with prevalence of astrovirus infection at $p<0.05$.

Majority (53.2%) of the children make use of pit toilet, followed by those who make use of bush (34.0%) and water closet (12.8%). There was statistical significance association of type of toilet used ($p=0.003$) with prevalence of astrovirus infection at $p<0.05$.

More than half (70.2%) of the children did not wash their hands after using toilet while 29.8% do wash their hands. There was statistical significance association of hand washing habit ($p=0.006$) with prevalence of astrovirus infection at $p<0.05$.

Table 1. Socio-demographic characteristics of the participants.

Factors	Frequency	Percentage
Age		
0-1	80	26.70%
2-3	76	25.30%
4-5	144	48.00%
<i>Mean age</i>	2.21	
Gender		
Male	172	57.30%
Female	128	42.70%
Residence		
Urban	36	12.00%
Semi Urban	240	80.00%
Rural	24	8.00%
Educational status		
Crèche	80	26.70%
Kindergarten	46	15.30%
Nursery	52	17.30%
Primary	122	40.70%

Table 2. Prevalence of astrovirus infection among study participants.

Participants	Total number screened	Positive (%)	Negative (%)
	300	94(31.3%)	206(68.7%)
Total	300	94(31.3%)	206(68.7%)

Table 3. Age distribution of astrovirus among children in Offa.

Age (years)	Frequency (N=94)	(%)	X ²	p-value	Remarks
0-1	28	29.8	0.29	0.861	NS
2-3	32	34.0			
4-5	34	36.2			
Total	94	100			

Key:

NS= Not significance

Table 4. Prevalence of astrovirus based on gender among the infected children.

Gender	Frequency (N=94)	(%)	X ²	p-value	Remarks
Male	54	57.4	1.04	0.307	NS
Female	40	42.6			
Total	94	100			

Key:

NS=No significance

Table 5. Distribution of astrovirus among children in Offa based on educational level.

Educational level	Frequency (N=94)	(%)	X ²	p-value	Remarks
Creche	30	31.9	2.79	0.426	NS
Kindergarten	20	21.3			
Nursery	16	17.0			
Primary	28	29.8			
Total	94	100			

Key:

NS=No Significance

Table 6. Distribution of astrovirus among children in Offa based on location of residence.

Residence	Frequency (N=94)	(%)Positive	X ²	p-value	Remarks
Rural	10	10.6	36.62	0.001*	S
Urban	12	12.8			
Semi urban	72	76.6			
Total	94	100			

Key:

S=Significance

Table 7. Risk factors associated with sero-positive prevalence of astrovirus among children.

Variables	Sero-positive prevalence of astrovirus		X ²	p-value	Remarks
	Frequency N=94	% Positive			
Source of water for domestic use			24.06	0.001*	S
Treated tap water	10	10.6			
Borehole	26	27.7			
Well	50	53.2			
River	8	8.5			
Type of toilet used			11.53	0.003*	S
Water closet	12	12.8			
Pit	50	53.2			
Bush	32	34.0			
Handwashing habit			7.68	0.006*	S
Yes	28	29.8			
No	66	70.2			

Key:

S = Significance

Discussion

The human astroviruses was first identified in 1975 during an outbreak of gastroenteritis in a maternity ward, have been identified worldwide since then [23]. Several epidemiological studies have tried to determine the exact prevalence of classical astrovirus, but there is a high heterogeneity among studies depending on the studied population, the diagnostic method used, the timing of sampling, and the geographic area [23]. Thus, this study determined the prevalence and risk factor distribution of astrovirus among children in Offa, Kwara state.

The overall prevalence of astrovirus infection among children in Offa was 31.3%. Human astroviruses are transmitted by contact with infected individuals and fomites, followed by the ingestion of contaminated food and water [18]. The morbidity caused by human astroviruses is influenced by the season, with higher infection rates during the rainy season. The prevalence from this study is higher than 5% among children in Northwestern Nigeria [22], 7.6% among children in Niger State [24], 5.0% among diarrheic children in north east, Nigeria [25], 6.3% among Children with Diarrhoea in young Kenyan [26], 14.6% among children in Ouagadougou (Burkina Faso) [27], 19.4% among children with gastroenteritis in southwestern Nigeria [28] and 10.3% among Congolese children in Brazzaville, Republic of Congo [29]. Also, the prevalence from this study is

lower than 40.4% among children in Lagos, Nigeria [14], 84.0% among children from a Birth Cohort in Nepal [30]. The difference in prevalence rates may be due to sample size, study population, participants' age, geographical location, climatic factors, and different diagnostic methods used in various studies.

In this present study, age group was not significantly associated with prevalence of astrovirus infection. Astrovirus was found common among 4-5years in this study. This was in contrast with study carried out in 2014 [25], who found prevalence of astrovirus higher among 0-2 years. Astroviruses affect predominantly the pediatric population although infections in elderly people and immunocompromised hosts are also reported [31]. The age of children infected with classic astrovirus is highly variable, ranging from newborns to over 5 years [1], however, infection is more common among those younger than two [32]. Classic astroviruses are distributed all over the world and are associated with 2 to 9% of cases of acute, non-bacterial diarrhea in children [33]. Possible reason for this observation was behavioral since the virus transmission is through fecal-oral route, fecal-contaminated items picked from the ground into the mouth by crawling children could serve as potential mechanical vector. Also, this could be due to premature nature of the body defenses [14].

The prevalence of astroviruses among male children 27(57.4%) was higher compared to their female 20(42.6%) counterparts. Though there was

no statistical difference between gender in the study ($P=0.307$). The study found similarly in gender distribution with the work of **Ayolabi et al.** (2012) [14]. and **Kuta et al.** (2014) [24]. also found higher prevalence in male than female participants. But found no significant association with prevalence of astrovirus.

The prevalence of astrovirus was common among children in creche, although not statistically significant. ($p=0.426$) which was similar to **Oyinloye et al.** (2016) [25]. in lagos, they found high prevalence among daycare children. Such children might have been affected due to crawling or playing around in a dirty environment the children can easily swallow infected soiled because the infection in been transmitted majorly through faecal-oral route..

The rate of infection with astrovirus in the semi-urban 36(76.6%) areas was observed to be high. This was in contrast to the report of **Kuta et al.** (2014) [24], who found higher prevalence among those leaving is rural area. Location of residence was found to be associated with prevalence of astrovirus. The reason for this may be attributed to poor hygienic practices coupled with poor water quality occurring in some semi-urban settlements. However there is statistical significant prevalence of Astrovirus in offa with location of residence. (p -value 0.001).

Associated risk factors to high prevalence of astrovirus infection in this study are water used for domestic purposes, type of toilet used and handwashing habit. This agrees with study carried out by **Oyinloye et al.** (2016) [25]., who found source of drinking water, nearness of toilet to source of drinking water, hand washing after toilet use were statistically significantly contributory factors to infection. Since wastewater treatment practices do not ensure the complete removal of viral pathogens, astrovirus present in untreated and treated wastewaters are discharged into the environment and may become contaminants of marine water, freshwater, and groundwater [34]. Groundwater may also receive viral contamination through the injection of reclaimed wastewater [35] Surface water and groundwater are employed as sources of drinking water for public consumption, and poor water quality is recognized as a major health threat. The WHO estimates that almost 90% of the global burden of gastroenteritis is attributable to unsafe water and poor sanitation and hygiene, with the pediatric population in developing areas being the

most vulnerable group [36]. Food and water may act as vehicles for human enteric virus transmission. Several large HAstV outbreaks have been associated with consumption of contaminated food by improper handwashing [37].

Conclusion

The prevalence of astrovirus among children in Offa, Kwara state was high. Age and place of residence was found to be contributing factor to high prevalence of astrovirus infection.

The high prevalence of astrovirus infection amongst children recruited in this study may be as a result of the socio demography of the participants as well as the route of transmission of the virus within rural communities.

Other contributing risk factors associated with the high prevalence in this study include the use of contaminated water for domestic and agricultural purposes which have been identified and a prtemt means of viral transmission. The nature of toilet facilities, level of health education as well as personal hygiene practices are also worthy of mention as risk factors associated with the spread of astrovirus gastroenteritis.

It is therefore recommended that Government pays attention to improving the standard of living of individuals within rural communities, facilitate frequent health education and publicity on good hygiene practices and improved water supply as well as establishing policies to routinely screen for Astrovirus among children who present to health facilities with acute gastroenteritis.

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