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Norovirus Infection among Children Under 5 Years of Age In Niger-Delta Zone, Nigeria

Oghomwen F. Osazuwa[1], Rachel Okojie[2], Frederick O. Akinbo[1]

[1]Department of Medical Laboratory Sciences, University of Benin, Benin City, Edo State, Nigeria.

[2]Department of Microbiology, University of Benin, Benin City. Edo State, Nigeria

*Correspondence should be addressed to Oghomwen Osazuwa: oszmarventonic@gmail.com

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Background: Norovirus is now recognized as a major cause of gastroenteritis among children worldwide. This study aimed to determine the prevalence of Norovirus infection among children that are less than five years of age in Niger-Delta zone, Nigeria.

Methods: A total of 263 subjects consisted of 163 children with diarrhea attending Paediatric clinics at the Central Hospital, Warri and Federal Medical Center, Yenagoa as well as 100 aged- and sex-matched healthy individuals that served as controls were recruited for this study. Demographic information was obtained through the aid of a well-structured questionnaires. Norovirus was detected by using a rapid lateral flow immunochromatographic assay kit (Biopanda reagents, Belfast, United Kingdom).

Results: The overall prevalence of Norovirus infection was 15.3%. The prevalence of Norovirus infection was significantly higher in Delta State than in Bayelsa State (22.9 % vs. 6.7%; OR= 3.6615; 95% CI = 1.378, 9.725; P=0.0092). Children that are less than 5 years of age attending day care centers are greatly affected by Norovirus infection (P=0.0044). Also, poor hand hygiene practice was also a significant risk factor for Norovirus infection among study subjects (P=0.0036). Furthermore, secretors were found to have a 1 to 334 fold increased risk of acquiring Norovirus infection in Delta State than in Bayelsa State (OR=19.28, 95% C.I, 1.1102, 334.68, P=0.0422).

Conclusion: The need for routine screening for Norovirus infection among children with diarrhea is advocated. Data from this study as well suggests that genetic and environmental factors play a role in Norovirus infection.

Keywords: Bayelsa and Delta States, Norovirus, Secretor status, Children under 5 years

1.0 INTRODUCTION

Viral gastroenteritis is a major threat with high morbidity and mortality rates, especially in children, elderly and immuno-compromised individuals in developed and developing countries [1]. Noroviruses (NoV) cause an estimated 1.1 million hospitalizations and up to 218,000 deaths among children that are less than 5 years of age annually [1]. NoV has been reported to be responsible for the death of 200,000 children in developing countries every year [2]. Following the introduction of Rotavirus vaccines, Human Noroviruses are now currently hypothesized to be the leading cause of epidemic acute non-bacterial gastroenteritis worldwide [3]. NoV is a genus in the family Caliciviridae and is an icosahedral virus ~38 nm large with an ~7.5 kb single- stranded, positive-sense RNA genome that encodes three large open reading frames (ORF 1, ORF 2 and ORF 3). ORF1 encodes the replicate polyprotein, while ORF2 and ORF3 encode the major and minor capsid proteins, respectively [4]. NoV exhibits high genetic diversity and can be divided into six genogroups (GI, GII, GIII, GIV, GV, GVI) [5].

NoV is by far the most frequent pathogen in epidemic diarrhea in both developing and industrialized countries, with outbreaks frequently occurring in closed settings such as hospitals and day-care centers [6]. Moreover, the high stability and infectivity of the virus makes it a common cause of food and waterborne gastroenteritis [6]. Despite these facts, there are no vaccines or therapeutic treatments available for this disease [6]. Additionally, little is known about the transmission pathway of the virus, and the measures that should be taken in order to prevent contamination of the environment are not in place. These un-clarities are partly due to the complexity of this virus as there are often several strains circulating due to the virus genomic recombination and point mutations. The strains have each of their own intrinsic properties that are related to the differences in severity, transmission pathways, and receptor specificities in the human host [7].

Certain studies have associated NoV susceptibility with secretor status- defined by the presence of an α (1,2) -linked fucose on histo-blood group antigens (HBGAs), which is determined by the FUT2 gene [8]. Individual carrying >1 functional FUT2 allele, and thus expressing α (1,2) fucosyltransferase 2 (FucT-II), are secretor positive (termed secretors) and can express the blood group A and B antigens as well as the H-type 1 and Lewis b (Leb) antigens on mucosa and in secretions. In contrast, people lacking FucT-II (non-secretors) have been found to be highly protected against infections from several NoV genotypes, including the common GII.4, as well as the Norwalk virus prototype strain (GI.1) [9]. There is dearth of information on the association between secretor status and Norovirus infection among children that are less than 5 years of age with diarrhea in Delta and Bayelsa States, against this background, this study was conducted to determine the prevalence of NoV and its association with secretor status among children under-5years old with diarrhea in Delta and Bayelsa States, Nigeria.

2.0 METHODOLOGY

2.1 Study Area

This cross-sectional study was carried out in two hospitals: the Central Hospital, Warri (Delta State) and the Federal Medical Centre, Yenagoa (Bayelsa State). Both hospitals are tertiary hospitals located in the Niger-Delta region of Nigeria, which is the major hub for crude oil related business in the South-South Nigeria. Warri is an oil hub in Delta State, with a population of over 311,970, while Yenagoa is the capital city of Bayelsa State with an estimated population of 266,008 [10].

2.2 Study Population

This study included children under the age of 5 suffering from diarrhea that were at the Paediatric Out-patient clinics, Special Care Baby Units, and the In-patient wards of the Central Hospital, Warri and Federal Medical Centre, Yenagoa. Children with at least three episodes of diarrhea with an onset of one day to seven days- were included in the study. Children whose parents or guardians consented to participate, who were within the ages of 0-5 years were recruited in this study. The exclusion criteria included the refusal of the parents/ wards to give consent or when the child was on antiviral therapy. A structured questionnaire was administered to collect data bothering biodata, socio-demographics, and clinical symptoms from parents/caregivers of each subject before specimen collection. A total of 263 faecal specimens, consisting of 163(83 from Delta and 80 from Bayelsa States) children with diarrhea and 100 apparently healthy aged and sex matched as controls were analyzed for NoV. The protocol for this study was approved by the Ethics and Research Committees of the Ministry of Health of both Delta and Bayelsa States with reference numbers CHW/VOL14/30 and FMCY/REC/ ECC/2017/OC/046 respectively.

2.3 Screening for Norovirus

NoV in stool specimens was qualitatively detected using a lateral flow immunochromatography test kit (Biopanda, Belfast, United Kingdom) following the manufacturer's instructions. Briefly, approximately 50 mg or 50 µl stool (solid or liquid) was dispensed into a specimen collection

tube containing the extraction buffer. This was then shaken vigorously to mix the specimen and the extraction buffer. Test cassettes were opened gently and approximately 80ul of the extracted specimen were transferred to the test cassette. Results were read after 15 min. The results of the test were reported as positive, negative or invalid accordingly.

2.4 Secretor status

This was determined using commercial test kits La and Lb antisera (Lorne Laboratories, Danehill Berkshire United Kingdom). Briefly, a 2-3% of washed red blood cells was prepared while 30µl of the washed red cells and Lorne Lewis reagent was placed in a labeled test tube. This was then mixed thoroughly and incubated at room temperature for 15 min. All tubes were centrifuged for 20 sec at 1000 relative centrifugal force. It was read macroscopically for agglutination. Secretor status were then interpreted following manufacturer's instructions, non-secretors generated Le (a^+b^-) agglutination reaction, the secretors will generate the Le (a^-b^+) agglutination reaction.

2.5 Statistical Analysis

Data obtained were analyzed using Chi square (X2) test for frequency data and odd ratio analysis for potential risk factors. The statistical software used for all analyses was SPSS v. 16(IBM Computer Manufacturing Company, NY, USA).

3.0 RESULTS

The overall prevalence of Norovirus among children with diarrhea was 15.3%,there was no Norovirus infection among the control subjects (Table 1).

Table 1. Prevalence of Norovirus among children in Delta and Bayelsa States

States	No of Samples	No. Infected (%)	OR	p-value
Delta	83	19 (22.9)	3.6615	
Bayelsa	80	6 (7.5)		0.0092
Total	163	25 (15.3)		

In this study, age associated with the prevalence of Norovirus infection only among participants in Bayelsa State (P<0.001) (Table 2). There was a significant difference in the infection of Norovirus between subjects from Delta and Bayelsa States (Delta 22.9 % vs Bayelsa 7.5 %; OR 3.6615; 95% CI 1.378, 9.725; P=0.0092).

Table 2. Relationship between age and Norovirus infection among children in Delta and Bayelsa States

	Delta			Bayelsa		
Age (Mnth)	No. Tested	No. infected (%)	p-value	No. Tested	No. Infected (%)	p-value
0-1	38	8 (21.1)	0.253	46	6 (13.0)	<0.001
2-3	25	9 (36.0)		25	0	
4-5	20	2 (10.0)		9	0	

Generally, gender and type of toilet did not affect the prevalence of Norovirus among children under the age of 5 (P>0.05). However, attendance at Day Care Centers significantly affected the prevalence of Norovirus infection (OR=0.2787; 95%CI 0.1157, 0.6714; P= 0.0044). In this study, secretor status significantly affect the prevalence of Norovirus infection in Delta State (OR 19.28, 95%CI 1.1102, 334.68, P=0.0422) but not among subjects in Bayelsa State (Table 3). NoV infection was significantly higher during the rainy season among participants in both Delta and Bayelsa States (Table 3).

4.0 DISCUSSION

Norovirus (NoV) has been identified to constitute a key biological cause of gastroenteritis worldwide [11]. To the best of our knowledge, this is the first study to determine the prevalence of Norovirus in Delta and Bayelsa States, Nigeria. An overall prevalence of 15.3% Norovirus infection was observed among children with diarrhea in Bayelsa and Delta States, Nigeria. This prevalence is similar to 13.5% reported by Mans *et al* [12], in a multicenter study in fourteen different African countries. The prevalence observed in this study is higher than the 6.7% reported in North East, Nigeria [13], but less than the reported prevalence of 37.3% among children in Lagos [14] and 25.5% in a study at Ile-Ife, Osun State [15]. The prevalence of Norovirus in this study when compared to other African countries was less than 29.6 % obtained in Cameroon [16], but higher than 11.3 % and 11.8% in community based studies in Malawi [17] and Tanzania [18] respectively. This observation suggests that Norovirus infection is a significant cause of diarrhea in Delta and Bayelsa States. The difference in Norovirus infection in this study and that of other studies might be due to differences in hand washing practices, food and water sources, sanitization practices, climatic factors, environmental/household characteristics.

Norovirus prevalence between these two close States with similar climatic, environmental and geographical conditions is unclear. Norovirus infection is higher in

Table 3. Association between Norovirus infection and risk factors among children less than 5 years children in Delta and Bayelsa

	Characteristic	No. Tested	No. Infected (%)	OR	p-value
Gender	Males	98	17 (17.3)	0.709	0.453
	Females	65	8 (12.3)		
Types of toilet in use	Water Cistern	69	7 (16.7)		
	Pit Laterine	88	17 (19.3)		0.3927
	Open (bush) Defecation	06	1 (16.7)		
Hand washing with soap after defecation	Yes	69	3 (4.3)		
	No	94	22 (23.0)		
Attendance at Day Care Centre	Yes	54	16 (29.6)	0.2787	0.004
	No	109	9 (8.3)		
Secretor status Delta	Secretor	62	19 (30.6)	19.280	0.042
	Non secretor	21	0		
Secretor status Bayelsa	Secretor	71	4 (5.6)	0.210	0.1003
	Non secretor	9	2 (22.2)		
Season (Delta)	Rain	53	18 (33.9)		0.0012
	Dry	30	1 (3.3)		
Season (Bayelsa)	Rain	49	3 (6.1)		0.556
	Dry	31	3 (9.7)		

early childhood with children < 2 years old more likely to be infected; this finding has also been confirmed in studies done in North-eastern, Nigeria [13], and in Vhembe district, South Africa [19].

Gender did not significantly associate with Norovirus infection in this study (OR= 0.7095, 95% CI 0.2893, 1.7937 P= 0.4533). This finding is in tandem with that of Babalola and colleagues [20]. This suggests that gender may have no role in shaping Norovirus dynamics in the areas under this study. Urbanization and work regulations make mothers and caregivers source for day care centers for their children/wards. Thus, inappropriate hand cleanliness [21] and close contact with infected persons at these Centers must have accounted for the significant NoV infection recorded in this study at these locations [22, 23]. Hypothesis has been drawn from some proof of concept study that secretors are more likely to be Norovirus positive while non-secretors are highly protected from infections with several Norovirus genotypes [24]. In this study, secretor status affected strongly the prevalence of NoV in Delta State (P=0.0422). Though a previous study has reported a secretor independent Norovirus outbreak in Sweden [25]; the reason for Norovirus being more commonly detected among secretors in Delta State than in Bayelsa State is unclear.

This study shows that improper hygiene practice is a significant risk factor for NoV infection. Caregivers and parents should therefore institute proper environmental cleaningness and hand washing practice most especially for their wards after defecation. Government and non-governmental organizations should as well invest in WASH facilities development in the Niger-Delta region.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Authors Contribution

FOA conceptualized the study, performed data collection, sample analysis, statistical analysis and drafted the manuscript. **FOA, RO, NOE**, carried out study design, provided study materials, validate result and revised manuscript . All authors approved the final manuscript.

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