



## Association between Secretor status and Norovirus Infection among Children Under 5 years of Age in South-South, Nigeria

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Received: 15.06.2021; Accepted: 11.11.2021

Date of Publication: December, 2021

**Abstract:** Norovirus has been identified to constitute a key biological cause of gastroenteritis worldwide. This study aimed to determine the association between secretor status and norovirus infection among children under 5 years of age with diarrhea in Edo, Bayelsa and Delta States, South-South, Nigeria. Ethical approval was received from participating health institutions before inclusion of patients in this study. A total of 505 participants, consisting of 405 children with diarrhea and 100 apparently healthy children, who served as controls were included in this study. Stool specimens were collected from all participants and analyzed for norovirus using a rapid lateral flow immunoassay kit. Secretor was determined using commercial test kits La and Lb antisera. The overall prevalence of norovirus antigen among children with diarrhea was 18.5%. Secretors were found to significantly have higher prevalence of norovirus in Delta State (OR=0.165; 95% C.I, 0.0048, 0.5609; P=0.0039) when compared to non-secretors, but there were no significant association between norovirus infection and secretor status among subjects in Bayelsa and Edo States. Routine screening for norovirus infection among secretors with diarrhea should be a priority among subjects from Delta State.

**Keywords:** Norovirus, Secretor status, children, South-South, Nigeria.

### 1.0 Introduction

Following the introduction of Rotavirus vaccines, Human noroviruses are now currently hypothesized to be the leading cause of epidemic acute non-bacterial gastroenteritis worldwide [1]. Studies have shown that norovirus can be shed in feces or vomitus from both symptomatic and

asymptomatic infected individuals, with an average duration of 10 to 28 days [2]. The susceptibility to norovirus is now known to be largely dependent on human histo-blood group antigens [3], whose synthesis is under the genetic control of the FUT2 (Secretors) and FUT3 (Lewis) genes [4]. It is hypothesized that non-secretors are resistant

to norovirus infection. This research aims to highlight the association between norovirus infection and secretor status among children in Delta, Bayelsa and Edo states.

## **2.0 Materials and Methods**

### **2.1 Study area**

This study was carried out at five secondary health institutions (Central Hospital, Warri, Primary Health Care Centre, Pessu/Ugbuwangwe, Central Hospital, Benin City, Stella Obasanjo Hospital, Benin City and Federal Medical Centre, Yenagoa) in Delta, Edo and Bayelsa States, South-South, Nigeria. All hospitals are located in the Niger-Delta region of Nigeria, Southern, Nigeria. Warri is a major commercial city in Delta State, Nigeria, with a population of over 311,970 while Yenagoa is the capital city of Bayelsa State with an estimated population of 266,008, and Benin City is the capital of Edo State with an estimated population of 1,495, 800 people [5].

### **2.2 Study population**

This study was conducted between March 2018 and February 2019. A total of 505 participants, consisting of 405 children with diarrhea and 100 asymptomatic apparently healthy age and sex matched children who served as controls, were recruited for this study. Children with at least 3 episodes of diarrhea- with an onset of 1 to 7 days whose parents or guardians consented for their ward/children to participate were included in this study. The exclusion criteria included the refusal of the parents or guardians of wards to give consent, those with other diseases and children on antiviral therapy. A structured questionnaire was administered to collect data bothering on biodata, socio-demographics, and clinical symptoms from parents/caregivers of each subject before specimen collection. The protocol for this study was approved by the Ethics and Research Committees of the Ministry of

Health of Delta, Bayelsa and Edo States with reference numbers CHW/VOL14/130, FMCY/REC/ECC/2017/OC/046 and 732/T/89 respectively.

### **2.3 Inclusion criteria**

Age range 0 to 5 years, Children with at least three episodes of diarrhea- with an onset of one day to seven days. Children whose parents/ guardians consented for their child or ward to be included in the study.

### **2.4 Exclusion criteria**

Refusal of Parents / guardians of wards to give consent to be included in this study, those without diarrhea. Subjects whose age were over 5 years. Subjects on antiviral therapy.

### **2.5 Ethical Approval**

The protocol for this study was approved by the Ethics and Research Committees of the Ministry of Health of Delta, Bayelsa and Edo States with reference numbers CHW/VOL14/130,FMCY/REC/ECC/2017/OC/046 and 732/T/89 respectively.

### **2.6 Specimen collection**

Stool specimens were collected into universal containers.

### **2.7 Screening for norovirus**

Norovirus 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.

### 2.8 Determination of 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<sup>+</sup>b<sup>-</sup>) agglutination reaction, the secretors will generate the Le (a<sup>-</sup>b<sup>+</sup>) agglutination reaction.

### 2.9 Statistical Analysis

The data obtained were analyzed using Chi-square (X<sup>2</sup>) 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 Result

The overall prevalence of norovirus antigen among children with diarrhea was 18.5%, there were no norovirus infection among the control subjects. There was a significant difference in the prevalence of norovirus among children from Delta, Bayelsa and Edo States (OR=4.40; 95% C.I, 3.305, 5.857, P=0.0038). The result of this study shows that the prevalence of norovirus among children with diarrhea differs from states to states, the prevalence of norovirus among children in Delta, Edo and Bayelsa States was 27.8%, 15.5% and 7.5% respectively (Table 1).

Secretors were found to significantly have higher prevalence of norovirus in Delta State

(OR=0.165; 95% C.I, 0.0048, 0.5609; P=0.0039) when compared to non-secretors, but there were no significant association between norovirus infection and secretor status among subjects in Bayelsa and Edo States (Table 2).

**Table 1: Prevalence of norovirus antigen among children in Delta, Bayelsa and Edo States**

States	No. Sampled	No. Positive (%)	OR	95%CI	P value
<b>Patient group</b>					
Delta	151	42 (27.8)	4.40	3.305, 5.857	0.0038
Bayelsa	80	6 (7.5)			
Edo	174	27 (15.5)			
<b>Total</b>	<b>405</b>	<b>75 (18.5)</b>			

*P<0.05*

**Table 2: Association between norovirus infection and secretor status among children less than 5 years in Delta, Bayelsa and Edo States**

Characteristic	No. Tested (%)	No. infected	OR	95%CI	P value
<b>Secretor Status</b>					
<b>Delta</b>					
Secretors	103	39(37.9)	0.165	0.0048, 0.5609	0.0039
Non-secretors	48	3 (6.3)			
<b>Bayelsa</b>					
Secretors	71	4 (5.6)	3.944	0.0635, 24.6783	0.1424
Non-secretors	9	2 (22.2)			
<b>Edo</b>					
Secretors	98	17 (17.3)	0.758	0.3286, 1.7510	0.5173
Non-Secretors	76	10 (13.2)			

*P<0.05*

#### 4.0 Discussion

Using antigen detection method, the overall prevalence of norovirus in Delta, Bayelsa and Edo states was 18.5%. Norovirus infection rates were significantly different among states, norovirus infection was significantly higher among children under 5 years of age in Delta State, when compared to subjects from Bayelsa and Edo states. The difference in norovirus prevalence between these three close States with similar climatic, environmental and geographical conditions is unclear. The most likely cause might be due to differences in poor hand hygiene practice [6, 7].

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 [8]. In this study, secretor status affected strongly the prevalence of norovirus in Delta State (OR: 0.165 95% CI: 0.0048, 0.5609 P: 0.0039), but not among children in the other states studied. This justify a potential association between being a secretor and the higher likelihood of susceptibility to norovirus infection among children in Delta State. Significant association of secretor positive status and norovirus infection have been documented in some other previous studies in other parts of the world [9, 10, 11].

In Conclusion, routine screening for norovirus among secretors is essential in Delta State, because of increased possibility of susceptibility to norovirus among this subject group.

**Footnote:** Part of doctoral thesis of first author

**Funding:** Authors received no financial grant for this study.

**Conflicts of Interest:** None exists

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