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A Preliminary Survey of Norovirus and Astrovirus Antigen in Diarrheic Stools of Children in Borno State, Nigeria

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Abstract- *Background:* Noroviruses and astroviruses are important agents of acute gastroenteritis among children. Acute gastroenteritis (AGE) is a major cause of morbidity and mortality in pediatric populations world-wide. Globally, an estimated 800 000 infants and young children die from diarrhea every year.

Aims: This was a cross-sectional study that included acute diarrheic children presenting in Specialist Hospital, Nursing Home Health Centre and Ngamdu pediatric clinic in Borno state with the aim of detecting norovirus and astrovirus antigen. Two hundred children whose parent/ guardian consented were enrolled in the study.

Methodology: Two hundred acute diarrheic and forty one nondiarrheic fecal samples were collected from children aged 5 or less between June 2013 – May 2014. Samples were screened for norovirus and astrovirus antigen using 3rd generation RIDASCREEN ELISA test kit. Demographic data of the children were obtained.

Results: All non-diarrheic stools were negative for both antigens whileof the two hundred diarrheic stools screened, a prevalence of 8% and 5% were obtained for norovirus and astrovirus respectively. The proportion of males (6/130) positive for norovirus antigen relative to female (10/70) was found to be significant ($p < 0.016199$). No significant difference was observed between male and female positive for Astrovirus ($p = 0.307574$). A significant prevalence of norovirus ($p = 0.00001$) and astrovirus ($p = 0.013321$) based on age group were obtained. Clinical sign and symptoms of infection with norovirus and Astrovirus between male and female showed no significant difference ($p=0.42018$).

Keywords: *norovirus, astrovirus, diarrhea, borno state, nigeria.*

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Conclusion: Norovirus and Astrovirus are a significant aetiology of diarrhea in the study area. Measures to mitigate their sequelae are required to circumvent potential public health crises in future.

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I. Introduction

Viral intestinal infections are the most common cause of acute infectious diarrhea in the pediatric group and accounted for approximately 70% of episodes of acute infectious diarrhea in children (1). Rotavirus, norovirus, adenovirus, and astrovirus are the recognized viral causes of pediatric gastroenteritis (2) and the World Health Organization (WHO) data showed that each child practically has viral diarrhea irrespective of race and socioeconomic status within the first 5 years of life and this has great economic burden for the system of public health services and all society (3)

Epidemiological studies on norovirus gastroenteritis have been conducted in countries such as the United States of America (4, 5), Finland (6), Australia (7), Italy (8), some developing countries such as Brasil (9), Iraq (10). In sub-Saharan Africa, molecular epidemiology studies of norovirus have also been performed in countries such as Malawi (41% prevalence), Ghana (16.4% prevalence), South Africa, Botswana, Cameroon and Burkina Faso (12% prevalence) (11-16). In a study in Lagos Nigeria, norovirus prevalence of 37.3% was obtained among children with acute gastroenteritis (17) while in Ife, Nigeria, norovirus single infections were found in 64.3% (9/14) of the norovirus positive diarrhoea samples (18). Also in Owo, Ondo state Nigeria, norovirus was found in 4/50 (8%) of the diarrheic children examined (19)

The first astrovirus infecting humans was described in 1975 (20). Since then, a total of 8 serotypes closely related to this original astrovirus ("classic human astroviruses" (HAstVs)) have been identified, all of which are believed to cause diarrhea. The prevalence of HAstV infection has been reported to be 2%–16% among children hospitalized with diarrhea and 5%–17% in community studies that used either Enzyme Immunosorbent Assay (EIA) or Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) analysis (21-23). Seroprevalence studies indicate that most children are infected during the first 2 years of life (21, 24, 25). Previous studies in Nigeria show different prevalence of astrovirus. In a study in northwest Nigeria, 5% astrovirus positivity was reported (26) while others (17) and (27) reported 16% prevalence in Lagos and Nasarawa states respectively.

Diarrhea of viral aetiology is under reported in north east region of Nigeria. The aim of this study was to conduct a preliminary survey of norovirus and astrovirus antigen in acute diarrheic stool due to increasing epidemiological significance of viral gastroenteritis in north eastern region of Nigeria and dearth of published data on the existence or otherwise of diarrhea of norovirus and Astrovirus aetiology. It is hoped that the

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information generated in this study will serve as baseline data to inform the relevant authority in Nigeria of the health burden posed by these viruses.

II. Materials and Methods

a) Study Population

Samples were collected at random from children aged below 5years presenting with acute diarrhea at the In and Out Patient Departments and the Pediatric Wards of Specialist Hospital, Nursing Home Health Centre and Ngamdu pediatric clinic in Borno State, Nigeria.

b) Exclusion Criteria

Children above age of 5 years and those below 5 years, whose parents/guardians declined consent, were excluded from the study.

c) Inclusion Criteria

Diarrheic children aged below 5 years whose parents/guardians consented to participate in the research were included in the study. Diarrhea was defined as passage of three or more watery stool within the last 24-hour period.

d) Study Design

In this research, a cross sectional design was employed in order to allow for stool sample collection from every other child presenting at any of the selected hospital in the study area.

e) Ethical Approval

Ethical approval was sought and obtained from the Ethical Committees of the respective hospitals involved in the study.

f) Analyses of stool sample

Stool samples collected were assayed to detect norovirus, and astrovirus antigen using RIDASCREEN® ELISA Test Kit.

g) Detection of Norovirus, and Astrovirus by Enzyme Linked Immunosorbent Assay (ELISA)

i. *Sample preparation*

Each stool sample was prepared for analysis according to manufacturer's instruction:

One milliliter (1ml) RIDASCREEN® sample was placed in dilution buffer in a labelled test tube. Liquid stool was sucked up into a disposable pipette until it rose to just above the second mark (approx. 100 µl) and was suspended in the buffer which was placed in the tube beforehand. The stool suspension was homogenised either by suction and ejection from a disposable pipette or, alternatively, by mixing in a vortex mixer. The specimen was centrifuged at 5000 rpm (approx. 2300 – 2500 G) for 5 minutes and the resulting supernatant of the stool suspension was used.

ii. *ELISA Procedure*

One hundred microliter (100µl) of positive control, the negative control (specimen-dilution buffer diluent) and the stool supernatant were dispensed in the wells. One hundred microliter (100µl) of the biotin-conjugated antibody was added to the wells and incubated at room temperature (20 – 25 °C) for 60 minutes after mixing thoroughly (by lightly tapping on the edge of the plate), after this, the plates were washed 5 times using 300µl wash buffer each time using an automated machine. (The wells were emptied completely by knocking them out after each wash on a part of the absorbent paper which is dry and unused). One hundred microliter (100µl) of the streptavidin-peroxidase conjugate was added to the wells and incubated at room temperature (20 – 25 °C) for 30 minutes and washed as described above. One hundred microliter (100µl) of substrate was added to each well. Then the plate was incubated at room temperature (20°C - 25°C) for 15 minutes in the dark. The reaction was stopped by adding 50µl of stop reagent to each well. After mixing carefully (by lightly tapping the side of the plate) the extinction was measured at 450nm using a reference wavelength ≥ 600 nm (optional).

h) Evaluation and interpretation

Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = Extinction for the negative control + 0.15

Test result

Samples are considered **positive** if their extinction is more than 10 % above the calculated cut-off.

Samples are considered **equivocal** and must be repeated if their extinction is within ± 10 % of the cut-off. If repeating the test with a fresh stool sample again yields a value in the grey range, the sample must be considered negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered **negative**.

III. Results

Of the two hundred diarrheic stool screened, a prevalence of 8% (16/200) and 5% (10/200) were obtained for norovirus and astrovirus respectively (Table 1). The proportion of males (6/130; 4.62%) positive for norovirus antigen relative to female (10/70; 14.29%) was found to be significant ($P < 0.016199$; Table 1) but not so for Astrovirus ($p = 0.307574$; Table 1). A significant prevalence of norovirus ($P = 0.00001$) and astrovirus ($P = 0.013321$) based on age group were obtained in this study (Table 1). Clinical sign and symptoms of infection with norovirus and Astrovirus between male and female showed no significant difference ($P = 0.42018$; Table 2).

Table 1 : Norovirus and Astrovirus distribution among children in Borno State according to Age and Sex

Variables	No. of Sample	Number Positive		p-value	
Age group (month)		Norovirus (%)	Astrovirus (%)	Norovirus	Astrovirus
1-6	13	0(0)	1(7.69)		
7-12	44	3(6.80)	1(2.27)		
13-24	49	6(12.20)	5(10.20)	0.00001	0.013321
25-36	35	4(11.40)	2(5.71)		
37-48	36	2(5.60)	1(2.78)		
49-60	23	1(4.30)	0(0.00)		
Total	**200**	**16(8.0)**	**10(5.0)**		
Sex					
Male	130	6(4.62)	5(3.85)	0.016199	0.307574
Female	70	10(14.29)	5(7.14)		
Total	**200**	**16(8.0)**	**10(5.0)**		

Table 2 : Norovirus and Astrovirus clinical sign/symptom in relation to sex

Sign and Symptom	No. of Sample	Norovirus positive		Astrovirus positive		p-value of M / F
		Male (M)	Female (F)	Male	Female	
Fever (F) only	67	1	2	1	1	0.42018
Vomiting (V) only	23	2	2	1	0	
Fever and Vomiting	39	1	4	3	3	
Abdominal Cramp	31	2	2	0	1	
Mucoid/bloody stool	40	0	0	0	0	

IV. Discussion

The results from the present study suggest that norovirus and astrovirus contribute significantly to the disease burden of childhood diarrhea in Borno state, Nigeria.

The norovirus prevalence of 8% (Table 1) is similar to 8% obtained in Owo, Nigeria (19) but lower than the prevalence (21%) found for children in the United States of America (28), and that reported in a pooled analysis of studies conducted in seven developing countries (12.1%), spanning from Malawi to Thailand to Peru (29). Also, the figure in the present study was lower than 37.5% found for children in Nigeria (17). The prevalence of astrovirus in Borno state of Nigeria found in this study was 5% (Table 1). It is within the prevalence range of 2%–16% of human astrovirus (HAstV) infection reported among children hospitalized with diarrhea and 5%–17% in community studies that used either EIA or RT-PCR analysis (21-23). The prevalence of astrovirus in this study was observed to be similar to 5% prevalence in northwest Nigeria (26); and 4.9% prevalence reported in Mexico, it was lower than 10.8% reported in the United States, and 16% prevalence in Nasarawa Nigeria (27).

These disparities in norovirus and astrovirus prevalence across different studies may have been caused by different reasons. One reason may be due to the period samples were collected relative to the duration of diarrhea. Norovirus and astrovirus shedding generally peak within the first week of illness but can last for nearly two months (30). This reflects how the duration of illness can affect the outcome of each study because samples collected after the peak period of viral shedding will, as expected, present a possible outright negative or false negative result thereby impacting on the prevalence to be reported. In this study, the duration of illness of patients was not recorded implying that some samples might have been collected perhaps after peak period of infection. This limitation is similar to a report in 2006 which neglected to list limits in the duration of illness (31). Another factor contributing to the disparities among results was the variance in age-based inclusion criteria among the various study populations. In the present study, children had to be less than five years of age, which is the age range most affected by diarrhea.

Sex stratification of the children sampled revealed that the proportion of males (6/130) positive for norovirus antigen relative to female (10/70) was found to be significant ($P < 0.016199$; Table 1). However, the prevalence of male (6/130: 4.62%) positive for norovirus antigen was found to be lower than that of female (10/70: 14.29%). This is contrary to previous study which had reported a greater male susceptibility rate. The greater susceptibility of male to norovirus infection had been attributed to genetic and immunological factors (32). Susceptibility to infection with human Astrovirus showed no association to gender ($P = 0.307574$; Table 1). Overall, the occurrences of infection between the sexes indicate that either male or female could be

infected. This information, if and when corroborated by other studies, can serve to guide possible vaccination policy in future to target children not more 5 years old.

In the present study, the age-based prevalence of norovirus in Borno state was found to be significant (P=0.0001; Table 1) affecting a greater proportion (9/16) of children less than or equal to age of 2 years. This finding is similar to that of other studies (28,33). Interestingly, 3/9 of these children were of age 7-12 month (Table 1). Possible reason for this observation is behavioural. 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. For astrovirus, age-based prevalence was also significant (P=0. 0.013321; Table 1). We also observed in this study that the number of children less than or equal to age of 2 years positive for Astrovirus antigen were more (7/10) than children older than age 2 (3/10) (Table 1). Above reasons are applicable. However, from Table 1, for both norovirus and Astrovirus, the number of infected individual declined from age above 2 years. Boosted immunity and reduced tendency to consume soiled edibles might be attributable. This assertion has yet to be proven scientifically, though.

The most recurring clinical symptom observed among both sexes for norovirus and astrovirus (Table 2) respectively was fever with vomiting (5/39 and 6/39 respectively) though it was not significant between male and female (P=0.42018). Since vomiting (with or without fever) featured prominently as a clinical symptom of infection with both viruses, it implies that the sequelae will be dehydration. Although this study did not determine the association of norovirus and astrovirus diarrhea with dehydration, a previous study in Zaria, Nigeria, reported a rotavirus prevalence of 21% among those not dehydrated and 78.4% among those that were dehydrated (34). Norovirus and astrovirus are also implicated in diarrhea cases; therefore one could infer that the sequelae of infection among the children in this study would also be dehydration arising from vomiting. Hence, the ensuing dehydration warrants public enlightenment/education on the use of oral rehydration solution in order to reduce number of deaths due to possible severe dehydration.

V. Conclusion

This study has established the presence of norovirus and astrovirus in diarrheic stools of children in the Borno state. The prevalences obtained in this study reveal that, though it is under-reported, it could be a debilitating childhood disease due to dehydration. And since definitive diagnosis/examination is hardly done to ascertain causes of death in this part of the developing world, deaths which have been reported to be due to diarrhea related causes in neonates and children might have been caused by either or both of these enteric pathogens. Due to limited funds, we could not undertake immediate molecular characterization of the positive samples.

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