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The prevalence and predisposing factors of norovirus and astrovirus infection among diarrheic children in north east, Nigeria

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This is a case-control study design that aims to determine the prevalence of norovirus and astrovirus infections in diarrheic children in Northeast region of Nigeria. Six hundred diarrheic stools (which were neither mucoid nor blood tinged) of children of 5 years or below were collected between May 2013 – April 2014. They were screened according to the manufacturer's instruction, using a 3rd generation Ridascreen ELISA kit (R-Biopharm AG, Germany). The control group comprised thirty five non-diarrheic stools. Demographic data were collected via questionnaire administered to parents/guardians of the subjects and analysis was done using online Easy-Chi-square ($p<0.05$) statistical package. An overall norovirus and astrovirus prevalence of 6.7% (40/600) and 5.0% (30/600) respectively across the Northeast region was obtained. The prevalence of norovirus in Taraba, Bauchi and Borno states was 7 (14/200), 5 (10/200) and 8% (16/200), while that of astrovirus was 5.5 (11/200) 4.5 (9/200) and 5% (10/200), respectively. The sex distribution of number of male relative to female children sampled reveals a preponderance of male (336/600) over female (264/600). Prevalence of norovirus and astrovirus antigen was high in children aged 1 to 2 years across the region. Source of drinking water, nearness of toilet to source of drinking water, hand washing after toilet use, playing with toys, attendance of day care center and educational status of parents were statistically ($p<0.05$) and significantly contributory factors to infection. The prevalence of norovirus (6.7%) and astrovirus (5.0%) in diarrhea in Northeast Nigeria has shown that both viruses contribute to childhood diarrhea, with most of the children infected below age 2.

Key words: Prevalence, predisposing factors, norovirus, astrovirus, diarrhea, north-east, Nigeria.

INTRODUCTION

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 each year (Liu et al., 2012). Viruses are the

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major etiological agents of AGE in children of less than 5 years of age. Group A rotavirus, norovirus, enteric adenovirus, human astrovirus, and sapovirus are established etiological agents of AGE (Benschop et al., 2008; Cheng et al., 2008; Arthur et al., 2009; Zhang et al., 2011; Verma et al., 2011).

Norovirus gastroenteritis was reported to be responsible for the mortality of 200,000 children in developing countries (Koopmans, 2008). Norovirus (NoV) is a genus in the family, Caliciviridae and are approximately 38 nm icosahedral viruses with an approximately 7.5 kb single stranded, positive-sense RNA genome that encodes three large open reading frames (ORF2 and ORFs). ORF1 encodes the replicase polyprotein, while ORF2 and ORF3 encode the major and minor capsid proteins, respectively (Lindesmith et al., 2011). NoV exhibits high genetic diversity and can be divided into six genogroups (genogroup I [GI], GII, GIII, GIV, GV and GVI) (Kroneman et al., 2011), while human astroviruses are non-enveloped and positive-sense single-stranded RNA viruses. They belong to the genus Mamastrovirus; family *Astroviridae* (Rivera, 2009; Zhou et al., 2014). The astrovirus taxonomy is mainly based on the species of origin and the serotypes within each species are defined on the basis of twenty-fold or greater cross-neutralization titers (De-Benedictis et al., 2011). The first astrovirus that infected humans was described in 1975 (Madeley and Cosgrove, 1975). 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.

NoV and HAstV transmission occurs via food, water, and airborne routes, hand contact with contaminated surfaces or fomites and through person-to-person contact. It is primarily fecal-oral contamination that drives the spread of both viruses; they cause a severe, sporadic or more than 85% of epidemic diarrhea and vomiting in all age groups especially during the winter (Farkas and Jiang, 2007; Zheng et al., 2010).

A hospital based study in Lagos reported a prevalence of 37.3% norovirus diarrhea (Ayolabi et al., 2010), and Japhet et al., (2012) reported a prevalence of 25.5% in a community based study in Osun State, Nigeria. In Owo, Ondo State, Nigeria, norovirus was found in 4/50 (8%) of the diarrheic children examined (Babalola et al., 2015). Previous studies in Nigeria show different prevalence of astrovirus. In a study in Northwest Nigeria, 5% astrovirus positivity was reported (Aminu et al., 2008), while Ayolabi et al. (2010) and Kuta et al. (2014) reported 16% prevalence in Lagos and Nasarawa states, respectively.

The aim of this study is to determine the prevalence and predisposing factors of norovirus and astrovirus infection among diarrheic children in north east Nigeria. This work was done due to the increasing epidemiological significance of viral gastroenteritis in North eastern region of Nigeria in addition to paucity of literature on the aetiology of norovirus and astrovirus diarrhea in the study area.

MATERIALS AND METHODS

Study area

The North-east region of Nigeria comprises six states: Adamawa, Taraba, Gombe, Yobe and Borno. However, three representative states of Taraba, Bauchi and Borno were selected because they do not have contact with one another. This region is in the Sahel Savannah, with high temperature for almost seven months of the year and with little rainfall. Taraba, Bauchi and Borno states have coordinates 8°00′N 10°30; 10° 18' 57"N, 09° 50' 39"E. and 11° 30'N, 13° 00'E, respectively. Ethical approval was obtained from the respective institutional ethics and research committee.

Study population

Children of less than 1 to 60 months old attending selected primary health centers within the metropolis of the representative states were considered to be the target population. Diarrheic children of both sexes whose parent consented were included in the study. Non-diarrheic and diarrheic children above 5 years old or below and whose parents/guardians declined consent were excluded.

Laboratory method and analysis

Sample preparation

Each stool sample was prepared for analysis according to the manufacturer's instruction without modification. One milliliter (1 ml) RIDASCREEN® sample was placed in dilution buffer in a labeled test tube. Liquid stool was sucked up into a disposable pipette until it rose to just above the second mark (approximately 100 µl). It was suspended in the buffer which was placed in the tube beforehand. The stool suspension was homogenized either by suction and ejection from a disposable pipette or, alternatively, by mixing in a vortex mixer. The specimen was centrifuged at 5000 rpm (approximately 2300 to 2500 G) for 5 min and the resulting supernatant of the stool suspension was used.

Procedure

RIDASCREEN Enzyme linked Immunosorbent assay kit has been used in previous studies (Hugo et al., 2014; Robilotti et al., 2015; Gupta et al., 2015) to assay norovirus and astrovirus. In this study, the analysis was done as described by the manufacturer's (RIDASCREEN, R-biopharm AG, Darmstadt, Germany) instruction without modification: One hundred microliter (100 µl) of positive control, the negative control (specimen-dilution buffer diluent) and the stool supernatant were dispensed in the wells. One hundred microliter (100 µl) of the biotin-conjugated antibody was added to the wells and incubated at room temperature (20 to 25°C) for 60 min after mixing thoroughly (by lightly tapping on the edge of the plate). After this, the plates were washed 5 times in 300µl wash buffer with the aid of 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 (100 µl) of the streptavidin-peroxidase conjugate was added to the wells, incubated at room temperature (20 to 25°C) for 30 min 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 to 25°C) for 15 min in the dark.

Table 1. Prevalence of norovirus and astrovirus in stool of diarrheic children 0-5 years old in north east Nigeria.

State	Total sample	Norovirus positive (%)	Astrovirus positive (%)
Bauchi	200	10 (5.0)	9(4.5)
Borno	200	16 (8.0)	10(5.0)
Taraba	200	14 (7.0)	11(5.5)
Total	600	40 (6.7)	30 (5.0)**

p=0.68728*; p=0.93611**.

Table 2. Sex distribution of norovirus and astrovirus detected in stool of diarrheic children 0-5 years old in north east Nigeria.

State	Male			Female		
	Total	No rovirus*	Astrovirus***	Total	No rovirus*	Astrovirus***
Bauchi	120	8	7	80	2	2
Borno	180	6	5	70	10	5
Taraba	86	7	7	114	7	4

p=0.0005*; Odd Ratio=0.86*; p=0.0005**; Odd Ratio=1.38**.

The reaction was stopped by adding 50 µl of stop reagent to each well. After mixing carefully (by lightly tapping the side of the plate) the extinction was measured at 450 nm using a reference wavelength ≥ 600 nm (optional).

Evaluation and interpretation criteria

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

RESULTS

Out of the six hundred samples from across the three representative states, an overall prevalence of 6.7 (40/600) and 5% (30/600) for norovirus and astrovirus, respectively was obtained (Table 1). The number of male positive for norovirus (21/40) was higher than that of female (19/40), while 19/30 and 11/30 astrovirus positive was for male and female, respectively (Table 2). Odd ratio for norovirus and astrovirus positive was 0.86 and 1.38, respectively (Table 2). Children ≤ 2years were found to be more infected with norovirus (24/40) than those >2 years (16/40) (Table 3), while astrovirus infection was even (15/30; 15/30) for the two aforementioned age categories (Table 4). Table 5 shows that source of drinking water, nearness of toilet to source of drinking water, hand-washing after toilet use, playing with toys, attendance of day care center and educational status of parents were statistically ($p<0.05$) associated with infection (Table 5).

DISCUSSION

The norovirus prevalence of 6.7% (Table 1) is lower than the prevalence (21%) found for children in the United States of America and that reported in a pooled analysis of studies conducted in seven developing countries (12.1%), spanning from Malawi to Thailand and Peru (Dove et al., 2005). Also, the figure in the present study was lower than 37.5% found for children in Lagos, South-west, Nigeria (Ayolabi et al., 2010). The prevalence of astrovirus in the North-east region of Nigeria in this study was 5% (Table 1). It is within the prevalence range of 2 to 16% of human astrovirus (HAstV) infection reported among children hospitalized with diarrhea and 5 to 17% in community studies that used either EIA or RT-PCR analysis (Blanton et al., 2006; Pang and Vesikari, 1999; Kirkwood and Bishop, 2001). This prevalence is also similar to those obtained in previous studies in other regions in Nigeria. The prevalence of astrovirus in this study was observed to be similar to 5% prevalence in Northwest Nigeria (Aminu et al., 2008); and 4.9%

Table 3. Age based prevalence of norovirus in diarrheic children 0-5 years old in north east Nigeria.

Age group (months)	Taraba		Bauchi		Borno	
	Total	Positive (%)*	Total	Positive (%)*	Total	Positive (%)*
0-6	18	1(5.6)	23	3(13)	13	0(0)
7-12	58	3(5.2)	25	2(8)	44	3(6.8)
13-24	41	5(12.2)	22	1(4.5)	49	6(12.2)
25-36	15	1(6.7)	57	2(3.5)	35	4(11.4)
37-48	35	3(5.6)	43	2(4.7)	36	2(5.6)
49-60	33	1(3)	40	0(0)	23	1(4.3)

p=0.015985*.

Table 4. Age based seroprevalence of astrovirus in diarrheic children 0-5 years in north east Nigeria.

Age group (months)	Taraba		Bauchi		Borno	
	Total	Positive (%)*	Total	Positive (%)*	Total	Positive (%)*
0-6	18	0(0)	23	0(0)	13	1(7.69)
7-12	58	3(5.2)	25	1(4.0)	44	1(2.27)
13-24	41	2(4.9)	22	2(9.1)	5	5(10.20)
25-36	15	2(13.3)	57	1(1.8)	35	2(5.71)
37-48	35	1(2.9)	43	1(2.3)	36	1(2.78)
49-60	33	3(9.1)	40	4(10)	23	0(0)

p=0.003574*.

prevalence reported in Mexico; it was lower than 10.8% reported in the United States, and 16% prevalence in Nasarawa State, North Central, Nigeria (Kuta et al., 2014).

Different prevalence for both viruses was observed across the study area. This may have been due to the period the samples were collected from each patient relative to the duration of the diarrhea. Norovirus shedding generally peak within the first week of illness but can last for nearly two months (Aoki et al., 2010). This reflects how the duration of the illness can affect the outcome of the 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 age of illness of patients was not recorded implying that some samples might have been collected perhaps after the peak period of the infection. This limitation is similar to the reports which neglected to list limits in the duration of illness (Mattison et al., 2010; Ayukekbong et al., 2011; Nordgren et al., 2013).

The sex distribution of number of male relative to female children sampled reveals a preponderance of male (336/600) over female (264/600). This was found to be significant (p=0.005) at p<0.05. Also, the number of males positive for norovirus (21/336) (Table 2) and astrovirus (19/336) (Table 2) was higher than female for both viruses (19/264; 9/264 respectively). These figures were marginally significant (p=0.104) at p=0.10. While Odd ratio of norovirus male positivity rate was found to be 0.86 (O.R=0.86) (Table 2), implying that males were 0.86 more likely to be infected than female, for astrovirus. Odd ratio was 1.38 (Table 3), implying males were 1.38 more likely to be infected with astrovirus than female. Males' greater susceptibility to rotavirus infection has been attributed to genetic and immunological factors (Fischer et al., 2007). By extension, this may also be applicable to norovirus and Astrovirus, hence the result obtained in this study.

In the present study, the age-based prevalence of norovirus across the three states was found to be significant (p=0.015985) affecting a greater proportion (24/40) of children less than age 2 (Table 3). This finding is similar to that of other studies (Bucardo et al., 2008; Payne et al., 2013). Interestingly, 4/24 (1 in Taraba; 3 in Bauchi) of these children were of 1 to 6 months old (Table 3). Possible reason for this observation is that children begin to learn to crawl at age 3 to 4 months; hence the tendency to pick and transfer items on the ground into their mouth is rife. Since the virus transmission is through fecal-oral route, such contaminated items picked on the ground would then serve as potential mechanical vector. For astrovirus, age-based prevalence in all three states was also significant (p=0.003574). However, equal proportion of children between those ≤2 years and older was infected (Table 4).

Table 5. Predisposing factors of norovirus and astrovirus infection among diarrheic children in north east Nigeria.

Risk factors	Norovirus and astrovirus antigen in stool						p-value
	Norovirus +ve			Astrovirus +ve			
	TR	BA	BO	TR	BA	BO	
Source of drinking water							0.00001
Tap	4	1	5	1	3	3	
Borehole	1	0	2	4	1	2	
Well	7	6	7	5	3	4	
Stream	2	3	0	0	2	0	
Sachet	0	0	2	1	0	1	
Type of toilet used							0.483932
Water closet	1	2	3	2	0	3	
Pit	4	3	4	3	3	2	
Bucket	3	2	4	2	5	2	
Bush	3	5	4	4	1	3	
Nearness of toilet to source of water							0.001271
Near	10	5	12	8	4	7	
Far	4	5	4	3	5	3	
Hand wash after toilet use							0.000209
Yes	2	1	4	2	2	1	
No	12	9	12	9	7	9	
Exclusive breast Feeding							0.093113
Yes	3	2	7	5	2	2	
No	11	8	9	6	7	8	
Play with Toys							0.037559
Yes	10	7	10	4	7	5	
No	4	3	6	7	2	5	
Attendance of day-care centre							0.00001
Yes	5	6	11	8	4	7	
No	9	4	5	3	5	3	
Educational status of parents							0.00001
Non-formal	6	2	8	5	1	4	
Primary	4	2	1	3	1	2	
Secondary	1	1	3	2	2	2	
Tertiary	3	5	4	1	5	2	

TR: Taraba; BA: Bauchi; BO: Borno.

All but two (type of toilet used and exclusive breast feeding) of the predisposing factors examined were found to have significant impact on the occurrence of infection with norovirus and astrovirus (Table 5). The source of drinking water (tap, borehole, well, stream or sachet) was significantly associated with infection with norovirus and astrovirus infection (p=0.00001). Table 5 reveals that well water was largely implicated as 20/40 norovirus positive and 12/30 of the astrovirus positive patients had well as their source of drinking water. One significant (p=0.001271) factor attributable to this is the nearness of toilet to source of drinking water of the sampled population because 470/600 respondents (Table 5) had their toilets less than 30 meters away from the wells which served as the source of drinking water. This is contrary to the World Health Organization recommended distance of 30 meters between toilets and any source of drinking water (WHO, 1996). Such nearness makes

seepage of contaminants into such wells possible, leading to contamination of the water body. Coupled with the nearness of toilet to source of drinking water is the observed poor toilet habit of the respondents manifesting as lack of hand-washing after use of toilet. A total of 364/600 parents or wards did not wash hand after using the toilet. Out of these, 87.5% (35/40) of norovirus positive and 83.3% (25/30) of astrovirus positive (Table 5) patients did not wash hand after toilet use. This serves as a significant (p=0.000209) contributory factor to infection with both viruses due to possible transfer of the viruses through oral route. With respect to attendance of Day-Care Centre, 22/40 of norovirus and 16/30 of astrovirus positive patients were among the 173/600 (Table 5) who attended day-care centre. Their attendance was associated (p=0.00001) with positivity rate. Understandably, such children might have been victims of either unhygienic behavior of nannies attending to them or lack of adequate attention given to the children. There is likelihood of children transferring soiled items into their mouths. Items such as toys are often played with either at homes or schools. Such are prone to contamination as they often fall to the ground, are picked with bare hand, rubbed on clothing (which sometimes are put into mouth) or even put such toys in their mouth directly; these lead to infection through oral route. Interestingly in the present study, playing with toys was found to be a significant factor of infection (p=0.037559) (Table 5). The educational status of parents/guardians was significant factor of infection with both viruses (p=0.00001). However, amazingly in this study, the prevalence of norovirus and astrovirus positive children/wards of parents with non-formal education (15.6%; 26/167) was less than for parents who had tertiary education (19.8%; 20/101) (Table 5). This is contrary to expectation. This observation may be due to chance or may have been caused by the care-free attitude of supposedly educated parents/guardian to common practice of personal hygiene such as washing of hand after toilet use before handling or attending to their children.

This study is however not without some limitations, not least the number of controls relative to sample size. Also, the predisposing factors advanced in this study were not tested for in the controls. In addition, the choice of enzyme linked Immunosorbent assay technique rather than a more sensitive polymerase chain reaction technique might have influenced low prevalence reported in this study. Also, research grant was not secured for this study.

Conclusion

The prevalence of norovirus (6.7%) and astrovirus (5.0%) in children presenting with diarrhea in Northeast Nigeria presented in this study, based on available published literature, is the latest report if not the first. In addition, this study has shown that both viruses contribute to disease burden of childhood diarrhea in north-east, Nigeria with most of the children infected below age 2. Source of drinking water, nearness of toilet to source of drinking water, hand washing after use of toilet, playing with toys, attendance of daycare center and educational status of parents/guardians of children were found to be statistically ($p<0.05$) and significant contributory factors to infection. Therefore, a more comprehensive research to establish a broader representative prevalence and confirm norovirus and/or astrovirus as primary aetiology of pediatric diarrhea using polymerase chain reaction technique is advocated.

Conflict of Interests

The authors have not declared any conflict of interests.

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