Detection of Rotavirus Vp6 Antigen in Faeces of Asymptomatic School-Aged Pupils in Gboko, Benue State of Nigeria

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***Abstract*: - Rotaviruses are a leading cause of severe acute gastroenteritis, resulting in approximately 453,000 annual deaths among children especially those less than five years of age with over 85% of these deaths occurring in the less developed countries of Asia and Africa. Children experience multiple rotavirus infections during childhood which almost are asymptomatic. In Nigeria, rotavirus accounts for over 20% of all cases of childhood diarrhoea annually. Transmission is via the faeco-oral route and distribution is worldwide. The study involved 260 asymptomatic school-aged pupils (aged 1-12) from which faeces were collected in sterile tubes between March and September, 2018 and analyzed immediately without storage using the commercially available Rotavirus VP6 antigen test kit from Bioneovan® Diagnostics, China, following the manufacturer's instructions. A questionnaire was designed and administered to obtain data on socio-demography, clinical history and some potential risk factors that might be related to rotavirus infection. Data obtained from this study was analyzed using SPSS version 20 and Pearson Chi-square (χ^2) test of Association was used to test significant association between variables at 95% confidence Interval and *p*-value ≤ 0.05 being statistically significant. Results showed that only 24 were positive, giving a prevalence rate of 9.23%. Results also revealed a prevalence of 3.46% for females while males recorded a higher 5.77%. With respect to the Age range distribution, 26.54% were for ages 1-4 years of which 1.54% was positive; 40.39% for ages 5-8 years with a lower burden of Rotavirus infection, and 33.08% distribution for ages 9-12 years with the highest burden of rotavirus infection and there exist a statistically significant association between Age and the onset of rotavirus infection. Findings further revealed that rotavirus is an important viral pathogen of public health importance among school-aged pupils in Gboko, Benue State. The prevalence rate of 9.23% from this study is a pointer to the urgent need for massive commencement of nationwide vaccination of children as asymptomatic carriers of the virus abound and may be a serious threat to the health of the public as the virus is shed from time to time.**

Keywords: **Rotavirus, Prevalence, Asymptomatic, Vaccination.**

I. INTRODUCTION

Diarrhoeal diseases are a leading cause of child mortality and morbidity, mostly resulting from contaminated food and water sources. Diarrhoea is usually a symptom of an infection in the intestinal tract caused by a variety of bacterial, viral and parasitic organisms among other aetiologies (WHO, 2009). Among these causative agents, viral diarrhoea is associated with several diseases and is one of the major causes of mortality in children especially in sub-Saharan Africa (Estes, 2001).

Rotavirus has long been acknowledged to be a major aetiologic agent of viral gastroenteritis and responsible for a large proportion of morbidity and mortality associated with diarrhoeal illnesses (Parashar *et al.*, 2003). In Nigeria, a high incidence of childhood diarrhoea is estimated to account for over 160,000 of all deaths in children annually and of this number, approximately 20% are associated with rotavirus infection (Parashar *et al.*, 2003). Rotavirus has been recognized for over 30 years as the most common cause of infectious gastroenteritis, in infants and young children. By contrast, the role of rotavirus as a pathogen in adults has long been underappreciated (Evan and Stephen, 2004). Rotaviruses are also associated with gastroenteritis in animals and can infect a broad range of animal species including dogs, cats, cattle, horses and birds (Midgley & Hjusaher, 2011) hence its zoonotic importance.

Discovered in humans by Ruth Bishop and her colleagues in 1973 (Flewett *et al.*, 1974), the virus belong to the family Reoviridae, Genus Rotavirus (Group III – dsRNA). The virus is non-enveloped and icosahedral, triple-layered with a double-stranded RNA genome that is segmented (Che-liang, 2014). Genomic segments encode at least six structural proteins VP1 to VP4, VP6 and VP7, and about 5 or 6 non-structural proteins depending on strain (Che-liang, 2014).

Rotavirus infection has assumed a global dimension and African countries are hardest hit owing to risk factors such as person-to-person contact, source of water, zoonotic potential, general sanitation and nutritional challenges (Surajudeen *et al.*, 2011). The burden of this condition is made worse by the fact that available vaccines are not even readily available and accessible, coupled with the fact that no treatment is available as it were besides supportive care.

The widespread occurrence of infectious diarrhoea has become one of the major public health problems worldwide, therefore, a rapid response, which includes identification of the pathogens and prevention of the spread of these pathogens in the community, is crucial for the control of disease outbreak and case investigations (Yuanhai *et al.*, 2008). Early

childhood diarrhoeal episodes have been shown not only to affect growth but also fitness, cognitive function and school performance (Torres *et al.*, 2000). Thus increased attention should be given to its study in children.

There is presently a dearth of information on Rotavirus infection in Benue State of Nigeria, particularly from non-hospital based studies. Thus this study set out to detect the presence and prevalence of Rotavirus VP6 antigen in stool of school-aged pupils in Gboko, Benue State and to identify determinants predisposing the pupils to Rotavirus infection in the study location.

II. METHOD

A Cross sectional study design was adapted for this study. Employing the prevalence rate of 20% reported in a previous study by Parashar *et al.* (2003), the sample size was calculated using a formula by Thrusfield *et al.* (2005) and 260 stool samples were collected from school-aged pupils aged 1-12years old between March and September, 2018, using clean universal sample containers that were handed out to each pupil to produce their faeces. The collected samples were transported in icepack to the Microbiology laboratory of University of Mkar, Mkar and analyzed immediately without preservation as viable viral particles decrease with time, making diagnosis a bit more difficult if there was a delay.

Ethical clearance was sought and obtained from the University of Mkar Ethical Committee, while informed consent was sought and obtained from School management, Parents and the pupils with a pledge of utmost confidentiality.

A diluent buffer vial for each sample was labelled. The cap of the vial was removed and approximately 50-100 mg of faecal sample was taken with a stick affixed to the vial cap. The stick impregnated with sample was introduced into the diluent buffer, while the vial was closed tightly and gently swirled to complete dispersion of faecal matter in the buffer solution. The test kit was removed from the pouch and place on a clean flat surface. The test strip, made primarily of monoclonal antibody conjugated with gold particles and directed against the specific human Rotavirus VP6 antigen, was labelled with each identification number, to correspond with the already buffered sample number. The test strip was dipped into the buffered sample with the arrow pointing to the bottom. Then the test was incubated at room temperature and read after 5-15 minutes.

A questionnaire was designed and administered to obtain data on socio-demography, clinical history and some potential risk factors that might be related to rotavirus infection. The data obtained from this study was analyzed using simple statistics and Pearson Chi-square (χ^2) test of Association was used to test significant association between variables at 95% Confidence Interval and $p \leq 0.05$.

III. RESULTS

A total of 260 samples were analyzed and the results showed that only 24 tested positive, representing a prevalence rate of 9.23% as shown in Fig. 1 below.

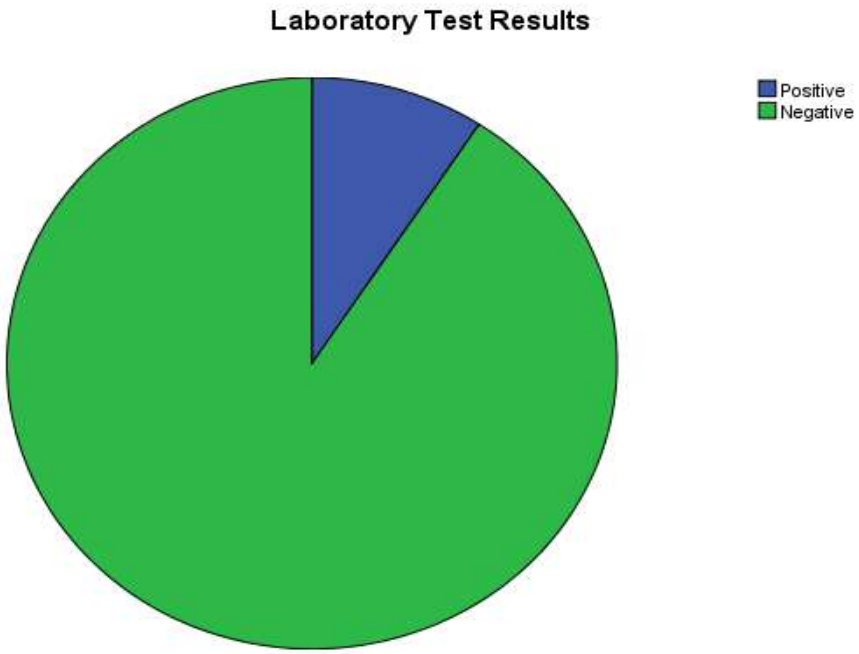

Fig. 1: Prevalence of Rotavirus infection in school-aged pupils in Gboko, Benue state.

Table 1: Prevalence of Rotavirus in relation to participant's demography in Gboko, Benue state

Parameter	Total (%)	Positive (%)	Negative (%)	χ^2	*p*-value
Age range (Yrs)					
1-4	69 (26.54)	4 (1.54)	65 (25.00)	26.540	0.000
5-8	105 (40.39)	1 (0.39)	104 (40.00)		
9-12	86 (33.08)	19 (7.31)	67 (25.77)		
Gender					
Female	151 (58.08)	9 (3.46)	142 (54.62)	4.598	0.032
Male	109 (41.92)	15 (5.77)	94 (36.15)		
Parent Occupation					
Business	77 (29.62)	14 (5.38)	63 (24.24)	10.654	0.014
Civil servant	110 (42.31)	6 (2.31)	104 (40.00)		
Farmer	68 (26.15)	4 (1.54)	64 (24.61)		
Others	5 (1.92)	0 (0.00)	5 (1.92)		
Class					
Nursery 1	20 (7.69)	0 (0.00)	20 (7.69)	47.287	0.000
Nursery 2	44 (16.92)	4 (1.54)	40 (15.38)		
Primary 1	41 (15.77)	0 (0.00)	41 (15.77)		
Primary 2	44 (16.92)	0 (0.00)	44 (16.92)		
Primary 3	30 (11.54)	1 (0.39)	29 (11.15)		
Primary 4	25 (9.62)	5 (1.92)	20 (7.70)		
Primary 5	15 (5.77)	0 (0.00)	15 (5.77)		
Primary 6	41 (15.77)	14 (5.38)	27 (10.39)		

In terms of demography presented in Table 1, the breakdown showed that 41.92% males and 58.08% females were sampled. The result however revealed a prevalence of 3.46% for females while males recorded a higher 5.77% prevalence rate. Statistical analysis revealed a chi-square value of 4.598, with a corresponding p-value for the test statistic being 0.032. The statistical inference from this study reveals that gender is a significant factor to incidence of Rotavirus infection ($p < 0.05$).

With respect to the Age range distribution, 26.54% were for ages (1-4)years of which 1.54% was positive; 40.39% for ages (5-8)years with a lower burden of Rotavirus infection, and 33.08% distribution for ages (9-12)yrs with the highest burden of rotavirus infection. The test statistic is given as ($\chi^2 = 26.540$, df = 2, $p < 0.001$). There is a statistically significant association between Age and the onset of rotavirus infection.

Going by Parent occupation of participants, civil servants constitute the larger chunk standing at 42.31%, with Business persons and farmers at 29.62% and 26.15% respectively. The results showed a higher positive result with pupils whose parents were into business (5.38%), then followed by Civil servants standing at 2.31%. The test statistic for this parameter is given as ($\chi^2 = 10.654$, df = 3, $p < 0.05$).

The distribution of the class of participants revealed that pupils in the nursery classes make up approximately 24% of the study while the primary school section represents approximately 76% of the study. The results show that pupils in Primary 6 were mostly positive, representing 5.38% of the laboratory results obtained, closely followed by pupils in primary 4 with a 1.92% prevalence. The test statistic is given as ($\chi^2 = 47.287$, df = 7, $p < 0.001$).

The predisposing factors to Rotavirus infections such as Personal Hygiene, Environmental factors, Medical history, Nutritional factors and Zoonotic potential are presented as shown in Table 2.

Table 2: The predisposing factors to Rotavirus infection in Gboko, Benue state

	Positive (%)	Negative (%)	Total	χ^2	*p* value
MEDICAL HISTORY					
How often have you been ill in the last three months					
NA	23 (8.85)	148 (56.92)	171 (65.77)	10.615	0.001
Once	1 (0.38)	88 (33.85)	89 (34.23)		
What symptoms did you present with?					
NA	23 (8.85)	138 (53.07)	161 (61.92)	15.161	0.056
Body rash	1 (0.38)	4 (1.54)	5 (1.92)		
Fever, Stomach discomfort	0 (0.00)	11 (4.23)	11 (4.23)		
Fever, Vomiting	0 (0.00)	10 (3.85)	10 (3.85)		
Malaria	0 (0.00)	5 (1.92)	5 (1.92)		
Stomach discomfort	0 (0.00)	47 (18.08)	47 (18.08)		
Stomach dis./fever/headache	0 (0.00)	16 (6.16)	16 (6.16)		
Stomach dis./fever/vomiting	0 (0.00)	5 (1.92)	5 (1.92)		
NUTRITION					
Where do you get your food from?					
Canteen	0 (0.00)	15 (5.77)	15 (5.77)	2.813	0.245
Commercial	0 (0.00)	10 (3.85)	10 (3.85)		
Home – made	24 (9.23)	211 (81.15)	235 (90.38)		
Do you eat fresh or cooked food?					
Fresh food	0 (0.00)	99 (38.08)	99 (38.08)	16.267	0.000
Cooked food	14 (5.38)	81 (31.15)	95 (36.53)		
Both	10 (3.85)	56 (21.54)	66 (25.39)		
How many times do you eat in a day?					
Two times	0 (0.00)	54 (20.77)	54 (20.77)	9.741	0.008
Three times	24 (9.23)	166 (63.85)	190 (73.08)		
Four times	0 (0.00)	16 (6.15)	16 (6.15)		
PERSONAL HYGIENE					
How many times do you take your bath in a day?					
Two times	15 (5.77)	180 (69.23)	195 (75.00)	2.203	0.138
Three times	9 (3.46)	56 (21.54)	65 (25.00)		
Do you bite your finger nails?					
Yes	19 (7.31)	126 (48.46)	145 (55.77)	5.868	0.015
No	5 (1.92)	110 (42.31)	115 (44.23)		
Do you wash hands after using toilet?					
Yes	9 (3.46)	67 (25.77)	76 (29.23)	1.770	0.413
No	15 (5.77)	169 (65.00)	184 (70.77)		
ENVIRONMENT					
What is your source of drinking water?					
Bore – hole water	9 (3.46)	121 (46.54)	130 (50.00)	9.871	0.020
Satchet water	0 (0.00)	21 (8.08)	21 (8.08)		
Tap water	15 (5.77)	78 (30.00)	93 (35.77)		
Well water	0 (0.00)	16 (6.15)	16 (6.15)		
Do you treat your water before drinking?					
Yes	5 (1.92)	41 (15.77)	46 (17.69)	0.179	0.672
No	19 (7.31)	195 (75.00)	214 (82.31)		
What type of toilet system do you use at home?					
Open defecation	4 (1.54)	36 (13.85)	40 (15.39)	0.070	0.966
Pit toilet	5 (1.92)	54 (20.77)	59 (22.69)		
Water Cistern (WC)	15 (5.77)	146 (56.15)	161 (61.92)		

ZOONOTIC POTENTIAL					
Do you have a companion animal?					
Yes	9 (3.46)	138 (53.08)	147 (56.54)	3.900	0.048
No	15 (5.77)	98 (37.69)	113 (43.46)		
How close are you to the animal?					
Very close	4 (1.54)	48 (18.46)	52 (20.00)	5.080	0.079
Not close	5 (1.92)	95 (36.54)	100 (38.46)		
NA	15 (5.77)	93 (35.77)	108 (41.54)		
How are the animals kept?					
Caged	0 (0.00)	57 (21.92)	57 (21.92)	8.065	0.018
Free – range	9 (3.46)	81 (31.16)	90 (34.62)		
NA	15 (5.77)	98 (37.69)	113 (43.46)		

IV. DISCUSSION

This study was undertaken to detect the viral antigen and to determine the prevalence of rotavirus infections among pre-primary and primary school pupils in Gboko, Benue State of Nigeria. Rotavirus infection was found in 24 out of the 260 samples taken from the study participants. A prevalence rate of 9.23% observed in this study was lower than reports of previous study by Udeani *et al*. (2018) in Enugu with a reported prevalence of 25%. This lower prevalence for this study may most likely be as a result of the fact that this study is a non-hospital study that sampled asymptomatic children. The previous studies have all been hospital-based studies in which clinically ill patients were screened. Since this is a non-hospital based asymptomatic study, clinically healthy children were also included. Another factor that may be responsible for the low prevalence recorded in this study compared to others may be due to the age disparity. While previous studies concentrated on infants, this study focused on school-aged pupils ranging from 1-12. This wider range in age may also be responsible for the lower prevalence recorded in this study. A third factor for this disparity in prevalence could be due to the geographical difference in the respective studies. Outside of Nigeria, a prevalence rate of 25.5% and 28.9% was reported from Ghana and Abidjan (Armah *et al.*, 2000; Akran *et al.*, 2010) respectively, while in Uganda, Bwogi *et al*. (2016) reported 37% high occurrence of rotavirus infection.

This study revealed a predominance of rotavirus infection in male subjects with 5.77% rate compared to the females with 3.46% rate. This is in consonance with the report of Tagbo *et al*. (2014) in Enugu, Nigeria, Bonkoungou *et al*. (2010) in Burkina-Faso, Nakawesi *et al*. (2010) in Uganda and Zaccottic *et al*. (2010) in Italy. The reason for this predominance remains unclear though a possible explanation might be the suggestion that boys are twice more likely to be more active than girls hence explaining the slight predominance by males as they get more predisposed to potential risk factors. A study from Cameroon by Ndze *et al*. (2012) have however reported conversely a higher detection rate for females than for males. Statistical analysis also revealed that the gender is a significant factor to Rotavirus infection as $p<0.05$. This finding is similar to the findings of Junaid *et al*. (2011) in Jos, who reported statistically significant association between gender and rotavirus infection.

Rotavirus was detected in all age groups in this study as of the 24 positive samples, 16.67% belongs to age range 1-4years while only 4.17% was from age range 5-8years and then 79.16% belonged to the 9-12years age range. This observation is noted to be in contrast to studies in Brazil by Fernandes *et al*. (2000). The reason for this observation is not known, however asymptomatic rotavirus as earlier reported by Abiodun *et al*. (1985) in Benin-city, Nigeria in 1985 is known to be associated with older children above the age of 5 without the manifestation of a clinical diarrhoea (Phillips *et al*., 2010). There is a statistically significant association between Age and the onset of rotavirus infection from this study.

Children whose parents are farmers had the least prevalence and those whose parents are into business had highest prevalence of rotavirus, there exist statistically significant association. This observation, could be due to chance and not a certainty, and this implies that rotavirus infect children regardless of parents demographic characteristics.

In terms of Nutrition of children enrolled in the study, although it was noted that respondents consume foods from a variety of sources including commercially and homemade, it was discovered that only respondents that consume homemade food returned positive for rotavirus infection. Statistical significance however does not show any kind of association between the two variables. All of the positive cases for rotavirus infection confirmed that they eat three times a day and there seem to be a statistically significant association between the variables but this may simply be attributable to a chance occurrence.

For the personal hygiene variable, respondents who bite their fingernails reported a higher prevalence rate for rotavirus infection with 79% (this is to be expected though as micro-organisms are known to populate under the nails), while respondents that do not bite their nails reported a 21% rate. Statistical analysis further supported an association between biting of fingernails and the presence of infecting rotavirus particles. It was interesting to note that while more

respondents who do not wash their hands after using the toilet had a higher prevalence value, statistical significance did not support an association between this variable and the chances of rotavirus infection. Parashar *et al*. (2003) noted that while diarrhoeal disease incidence has reduced in recent years, due in part to improved hygienic practices and sanitation, the incidence of rotavirus infection seemingly continues to increase.

The source of drinking water was shown to be significantly associated with the onset of rotavirus infection ($p<0.05$). This is in consonance with previous studies carried out in Katsina state, Nigeria by Lawal *et al*. (2017) which emphasizes the transmission of rotavirus by the faeco-oral route. The result from this study concerning source of drinking water is however in contrast with reports from a previous study in Yemen by Al-Badani *et al*. (2014) where they reported that the source of water seems to have no effect in the transmission of rotavirus. The type of toilet used by respondents show no significant association with rotavirus infection as discovered in this study. This report agrees with a previous study by Junaid *et al*. (2011) in Jos, who reported no significant association between this variable and rotavirus infection.

Finally, this study shows that having a companion animal is associated with rotavirus infection ($p<0.05$) but this finding was in disagreement with the Yemeni studies which did not report any statistical significance between the two variables (Al-Badani *et al*., 2014).

V. CONCLUSION

The findings of this study showed that rotavirus is an important viral pathogen of public health importance among school-aged pupils in Gboko, Benue State of Nigeria. The prevalence rate of 9.23% obtained from this study portends a serious call for concern, especially considering the fact that the study is a non hospital-based study. This therefore implies that asymptomatic carriers of the virus abound and may be a serious threat to the health of the public as the virus is shed from time to time. This is thus a call for urgent measures to commence nationwide vaccination of our children. The study also revealed that personal hygiene is a critical factor in preventing and controlling the spread of this pathogen. Handwashing should be encouraged among children and they should be educated on the dangers of biting their fingernails every now and then. The source of drinking water is also another factor that is a very significant finding from this study. All hands must be on deck to ensure our children are only allowed to consume clean and portable drinking water. Further studies on molecular typing of rotavirus strains in this study area will create a window for novel findings going forward.

VI. RECOMMENDATION

The study recommends the regular screening of rotavirus infection in children, whether they are symptomatic or asymptomatic, especially to make it a part of the routine laboratory tests in our hospitals. Children should be taught to observe strict personal hygiene including proper hand washing prior to as well as after engaging in some activities such as eating and playing. The study also strongly advocates the nationwide deployment of vaccination against rotavirus for children to be part of the National Programme on Immunization and for the government to ensure the vaccine's availability and at low cost too, especially in schools. Finally, further investigation should be carried out to determine the prevalent serotypes of rotavirus in Gboko which could lead to the identification of appropriate and more effective vaccine to be developed and deployed in the near future.

CONFLICT OF INTEREST

The authors hereby state that there is no conflict of interest in the course of this study.

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