

The Risk Factors Associated with Rotavirus Gastroenteritis among Children Under Five Years at University of Maiduguri Teaching Hospital, Borno State, Nigeria

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Abstract

Background: Diarrhea is a major cause of childhood morbidity and mortality in both developing and developed countries. It remains a common cause of hospitalization worldwide. Rotavirus is a cause of acute watery diarrhea in children under five years of age. The incidence of diarrhea decreases with increasing age. **Aim:** This study aimed at finding some risk factors associated with rotavirus infection in children <five years of age presenting with acute diarrhea at the university of Maiduguri teaching hospital. **Patient, Materials and Methods:** This is a cross-sectional study. A total of 173 children <five years presenting with diarrhea of <two weeks were recruited for the study. Stool samples were collected, and rotavirus antigen was detected using immunochromatographic, and the positive sample was then further analyzed using reverse transcriptase polymerase chain reaction for the VP4 and VP7 genotyping. The risk factors were analyzed using multivariate analysis conditional regression model after collecting data using a well-structured questionnaire. **Results:** The Source of water supply and presence of persons with gastroenteritis in the household were found to be risk factors for acquiring the infection with statistically significant $P < 0.05$. Breastfeeding was found to be protective of rotavirus gastroenteritis. **Conclusion:** Rotaviruses cause morbidity and mortality in children under five years of age. In view of the associated risk factors, making available safe drinking water and encouraging good personal hygiene is important. Promotion of exclusive breastfeeding and vaccination is advocated. Public health strategies like creating awareness to affected communities are a good strategy.

Keywords: Risk factors, rotavirus, under-five children

INTRODUCTION

Human Rotaviruses were discovered in 1973 by electron microscopic examination of a duodenal biopsy specimen from infants with diarrhea.^[1] It resembled a wheel; hence the name Rotavirus was coined by Thomas Henry Flewett. The name was officially recognized by international committee on taxonomy of viruses in 1976.^[2] Rotaviruses belong to the family Reoviridae. Rotaviruses are a major cause of severe gastroenteritis requiring hospitalization. An estimated 454,000–705,000 associated deaths occur worldwide especially in low-income countries.^[3] In Africa, prevalence of 24% was reported by Cunliffe *et al.*^[4] Those affected are mostly children aged <five years with incidence highest in children aged six months to two years,^[5] older children and adults can also be infected. Risk factors associated with adult infection include traveling to

endemic countries, immunocompromised persons, elderly, and adults caring for children with RVGE.^[6] Rotavirus gastroenteritis is a highly communicable disease that is transmitted mainly feco-orally, but the use of fomites, close contact with persons with the disease, and respiratory route have been implicated.^[7] Infectious dose is 10–100 viral particles. Rotaviruses also affect several animal species, but interspecies spread is rare with a

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few documented cases of bovine Rotavirus strains infecting a child.^[8,9] Incubation period is one to three days. Patients present with low-grade fever, vomiting, abdominal cramps followed by copious watery nonbloody diarrhea. The symptoms usually last for four to eight days, but viral excretion can last for about 12 days. The longer shedding of this virus and its naked structure will lead to increase transmissibility and susceptibility of children to this infection. Risk factors in children include age of the child, with the younger children are at greatest risk. Overcrowding, daycare attendance and presence of persons with gastroenteritis in the household increase the risk of acquisition of rotavirus gastroenteritis. Other factors such as educational and employment status of parents or caregivers, nutritional status of the child also affect the transmission of this infection. Infant feeding practices such as exclusive breastfeeding and bottle feeding have been implicated in the acquisition, duration, and immunity to rotavirus gastroenteritis.

There is no treatment known, only supportive therapy using oral rehydration fluids for mild cases, intravenous fluids for severe cases, antiemetics, and antipyretics. Access to healthcare is poor, especially in rural communities thus increasing the morbidity and mortality. Vaccines are not currently available for routine childhood immunization; thus, low-income families lack access to these vaccines.

Rotavirus gastroenteritis have been associated with a high level of morbidity and mortality, especially in developing countries like Nigeria, data generated from this study will identify the common risk factors for the acquisition of this infection and thus will help in developing policies and strategies for prevention and control of the infection. This will in turn ease the burden of cases especially in areas with no access to proper healthcare like the insurgency ravaged areas of northeastern Nigeria.

PATIENT, MATERIALS AND METHODS

Study area/study population: The study was carried out at the University of Maiduguri Teaching Hospital (UMTH), Borno State, north-eastern, Nigeria. The population of interest is the under five children attending UMTH.

This is a cross-sectional study. The sample size is 173 using Fisher's formula. Nonprobability conservative sampling method was used. The study was reviewed and approved by the Ethical Review Committee of UMTH.

The fresh diarrheal stool samples (about 5–10 mls) were then collected in a sterile wide container and properly labeled and transported immediately to the laboratory stored at 4°C before processing. Well-structured questionnaire was administered to the children. Rotavirus antigen detection was done using the Rota – dipstick (Mascia Brunelli S. p. a, Milano, Italy). This test was carried out according to the manufacturer's instruction.

The Rota – dipstick is a lateral flow immunoassay for the detection of Rotavirus antigen type A in human stool samples.

Table 1: Sociodemographic characteristics of respondents

Variable	Frequency (%)
Age (months)	
0-11	88 (50.9)
12-36	80 (46.2)
37-59	5 (2.9)
Total	173 (100.0)
Gender	
Male	91 (52.6)
Female	82 (47.4)
Total	173 (100.0)
Parent educational status	
Secondary	21 (12.1)
Tertiary	30 (17.3)
No formal education	122 (70.6)
Total	173 (100.0)
Level of dehydration	
Mild	134 (77.5)
Moderate	27 (15.6)
Severe	12 (6.9)
Total	173 (100.0)
Persons with gastroenteritis in the household	
Yes	62 (35.8)
No	111 (64.2)
Total	173 (100)
Employment status of caregivers	
Employed	133 (76.9)
Unemployed	40 (23.1)
Total	173 (100.0)
Breastfed infant	
Yes	164 (94.8)
No	9 (5.2)
Total	173 (100)

Table 2: Prevalence of rotavirus infection

Molecular test	Frequency (%)
Positive	25 (14.5)
Negative	148 (85.5)
Total	173 (100)

Methods

Diarrheal stool sample was collected in universal container and transported to the laboratory immediately in reverse cold chain and stored in the refrigerator at 2–8°C before processing.

Fifteen drops (0.7 mls) of extraction buffer were placed in an extraction tube. Liquid and semi-solid stools were pipetted and about 6–7 drops were put into extraction tubes containing the extraction buffer and mixed well by vortexing. The specimen was transferred to the extraction tube containing the extraction buffer and vortexed. The strip was immersed into the prepared sample in the direction indicated by the arrows on the strip for 10 min before reading.

Table 3: Risk factors associated with rotavirus infection using a Chi-square test

Variable	Molecular test Positive (n=173), n (%)	P
Gender		
Male	14 (56.0)	0.713
Female	11 (44.0)	
Total	25 (100)	
Presence of persons with gastroenteritis		
Yes	18 (72.0)	0.000*
No	7 (28.0)	
Total	25 (100)	
Water supply source		
Borehole	13 (52.0)	0.000*
Water vendor	12 (48.0)	
Total	25 (100)	
Nutritional status		
Under-nutrition	17 (68.0)	0.907
Normal	7 (28.0)	
Overweight	1 (4.0)	
Total	25 (100)	
Employment status of caregivers		
Employed	17 (68)	0.255
Unemployed	8 (32)	
Total	25 (100)	
Breastfed		
Yes	23 (92)	0.279
No	2 (8)	
Total	25 (100)	
Daycare attendance		
Yes	4 (16)	0.453
No	21 (84)	
Total	25 (100)	
Nutritional status		
Under-nutrition	17 (68.0)	0.907
Normal	7 (28.0)	
Overweight	1 (4.0)	
Total	25 (100)	
Age		
0–11	16 (64)	0.404
12–36	9 (36)	
37–59	0	
Total	25 (100)	
Breastfed		
Yes	23 (92)	0.279
No	2 (8)	
Total	25 (100)	
Daycare attendance		
Yes	4 (16)	0.453
No	21 (84)	
Total	25 (100)	
Socioeconomic class		
Lower class	18 (72)	0.224
Middle class	6 (24)	
Upper class	1 (4)	
Total	25 (100)	

*Statistically significant (i.e., $P < 0.05$)

Interpretation, negative test: Only the green band appeared on the control region assuring the correctness of the test, for positive test: a clearly distinguishable red band appeared in addition to the control green band. Invalid test was seen in samples in which the control green band did not appear.

Molecular testing was done on the samples found to be positive by Rota-dipstick antigen detection test. Ribonucleic acid (RNA) was extracted using both phenol-chloroform ether method and RNaid extraction kit. The supernatant containing the extracted RNA was carefully transferred to a sterile Eppendorf tube and stored at -20°C for reverse transcription-polymerase chain reaction (RT-PCR) reaction.

The Reverse transcriptase-polymerase chain reaction was performed by amplification of VP7 and VP4 genes using the Vera Gouvea/Itturiza-Gomara/Banerjee primer cocktail.^[10]

The primer cocktail consists of the following primers, Beg9, end9, RVG9 plus aBT1, aCTI, aET3, aDT4, aAT8, aFT9, G10, G12. This is to determine genotypes G1–G4, G8–G10, and G12, respectively.

The VP4 genes were detected using the Gentsch primer cocktail which consists of the following primers, Con2, Con 3 plus 1T1, 2T1, 3T1, 4T1, 5T1. This determines genes P4, P6, P8, and P10, respectively.^[11]

A well-structured questionnaire was administered to the caregivers of the participants who signed the consent form. The questionnaire contained sociodemographic details of the participants as well as risk factors that have been linked to the infection in previous studies done elsewhere.

The data collected from this study were entered into a computer program Microsoft excel. Data analysis was carried out using the statistical package for social science (SPSS™) version 25.0 Chicago, IL, USA, computer software. Data were cleaned and checked for missing and double entries. Univariate analysis was done to generate frequencies and proportions. Bivariate analysis was done to generate odds ratios (ORs), 95% (Confidence interval [CI]), and P values. Variables demonstrating positive association on bivariate were modelled into a logistic regression model to generate adjusted ORs and 95% CI. P values were set at <0.05 for statistical significance.

RESULTS

One hundred and seventy three (173) children ranging from 0 to 59 months were recruited for the study, out of which 91 (52.6%) were males and 82 (47.4%) were females. The median age of the children was 11 months as shown in [Table 1]. Forty-eight (28%) samples were positive for rotavirus antigen using the rapid detection screening test. The 48 positive samples were further subjected to genomic RNA extraction and Rotavirus RNA detection by PCR. Twenty five (14.5%) samples showed PCR positivity while twenty three samples were negative on PCR. This is depicted in [Table 2]. Factors associated with Rotavirus infection as shown in [Table 3], include the presence of persons with gastroenteritis in the

Table 4: Conditional logistic regression analysis of risk factors for rotavirus gastroenteritis

Risk factor	OR (AOR 95%CI)	P
Persons with gastroenteritis	4.233 (0.121-0.332)	0.000
Source of water	-4.399 (-0.211-0.080)	0.000

OR: Odds ratio, AOR: Adjusted OR, CI: Confidence interval

household, with $P < 0.000$. The source of water supply was also assessed, those fetching from borehole 13 (52%) and water vendors 12 (48%), $P < 0.000$. nutritional status showed 17 (68%) of the children were underweight, 7 (28%) had normal weight, and 1 (4%) were overweight, with $P = 0.907$. Daycare attendance had $P = 0.453$. Other factors analyzed include breastfeeding, employment status, socioeconomic status of caregivers, and sex of respondents with $P = 0.297$, 0.255, 0.244, and 0.713, respectively.

A conditional logistic regression model was used to assess for the strength of association between the variables for rotavirus gastroenteritis. Variables were considered significant if $P < 0.05$ and OR with 95% CI. This is as outlined in [Table 4].

DISCUSSION

The study aimed at finding risk factors associated with rotavirus gastroenteritis in children. Risk factors associated with RVGE include the presence of siblings with gastroenteritis 18 (72%) which is more than those whose siblings did not present with gastroenteritis 7 (28%), and this relationship shows strong statistical association ($P < 0.000$). The presence of persons with gastroenteritis in the household with diarrhea was found to be a significant risk factor in this study with $P < 0.05$, which was also reported by Pennap and Umoh in Zaria^[5] and Sethi *et al.* in the United Kingdom.^[12] This risk factor can be explained by the fact that Rotavirus is an infectious disease that is transmitted through the feco-oral route that means that close contact can help in the transmission of the infection. The virus is a naked virus and thus can withstand some harsh environmental conditions when it contaminates surfaces and fomites, this coupled with the excretion of the virus for up to 12 days will further explain this particular risk factor being significant. Hand washing and proper disposal or disinfection of contaminated items is important for prevention.

The source of water supply was also a positive risk factor for RVGE, statistically significant with $P < 0.05$. This is in keeping with a study carried out by Mukhtar *et al.* in Katsina state found out there is an associated between source of drinking water and infection.^[13] This further buttresses the fact that Rotavirus is spread through the feco-oral route. A post rotavirus vaccination study carried out in Mexico showed the presence of potable water was protective of RVGE.^[14] Other studies in Kenya and Yemen made similar observations, but also linked the presence of pit latrine in the home as a source of water contamination.^[15,16] This is found in this study which shows that lack of access to clean water is a major risk factor,

and this can be attributed to the breakdown in social amenities in era of insurgency in Maiduguri.

The frequency of rotavirus gastroenteritis was highest in children <11 months of age, which is also reported by Akoua-Koffi *et al.* and Pennap and Umoh in Ivory Coast and Zaria, respectively.^[5,17] Similarly in Sudan, children <12 months had the highest infection rate.^[18] This might be explained by the fact that with repeated rotavirus infection, immunity is acquired, and subsequent infections are mild or asymptomatic. Rotavirus vaccine for this study area will therefore be best administered in early infancy.

Other risk factors also analyzed include, school or daycare attendance, employment status, socioeconomic status, nutritional status, and gender, but these were not found to be significant as the $P > 0.05$.

There is no significant association between daycare attendance and Rotavirus gastroenteritis. This is also reported by Pennap and Umoh in Zaria and Jos, respectively.^[5,25] This finding may be due to improve awareness and sanitation in daycare and schools, as we know that overcrowding and close contact is associated with transmission of infectious diseases.

There was no association between nutritional status and RVGE ($P > 0.05$), this is also reported by Nakawesi *et al.* in Uganda, where they reported that nutritional status, daycare attendance and HIV status was not linked to rotavirus infection.^[19] So also, it was reported in Indonesia that there is no relationship between nutritional status and severity of RVGE.^[20]

The proportion of rotavirus infection is higher in male children than female children, though it was not statistically significant in this study. It has been observed in some studies that male children have higher infection rates compared to female children, implying that males have a more stool excretion rate of rotavirus infection and are more susceptible to infection.^[21,25]

Breastfeeding has been reported to be decrease the severity of RVGE in infants, but there is no difference in the attack rate of Rotavirus between breastfed and bottle-fed babies, this was demonstrated by Duffy *et al.*^[22] This same finding was also reported by Clemens *et al.*^[23] This study also showed there is no statistically significant difference between the prevalence of rotavirus gastroenteritis (RVGE) and in infants who have been breastfed, and nonbreastfed with $P > 0.05$, even though it's not a comparative study between breastfed and bottle-fed infants, but this study found breastfeeding to be protective of rotavirus infection, because the frequency of Rotavirus gastroenteritis is less in the breastfed children. This may be because of presence of maternal antibodies, lactoferrin, lactadherin, and mucin in the breast milk which inhibits rotavirus replication.^[24] Poor hygiene practices when preparing complementary feeds may also be responsible the diarrhea seen in children. These observations have been reported by Al-Badani *et al.* in Yemen.^[15]

There is no significant association between daycare attendance and Rotavirus gastroenteritis. This is also reported by Pennap and Umoh in Zaria and Jos, respectively.^[5,25] This finding may be due to improve awareness and sanitation in daycare and schools, as we know that overcrowding and close contact is associated with transmission of infectious diseases.

Limitation

This study only included those children with diarrhea seen at the UMTH, even though it is a referral center, it may not reflect the true prevalence and risk factors associated with the disease, thus a community-based surveillance is needed to get wider coverage of the extent of the disease burden.

CONCLUSION

Risk factors associated with rotavirus infection include the presence of persons with diarrhea in the household, and source of water supply, breastfeeding is found to be protective. In view of the risk factors associated with the infection, there is increased need for the provision of safe available drinking water. Promotion of good handwashing practice, use of effective disinfection, and proper disposal of contaminated items will help to reduce the identified risk factors for RVGE. The government should promote policies that encourage exclusive breastfeeding, and regulation of marketing of breast milk substitutes. To ensure proper vaccination of children at risk, especially those <one year of age by including rotavirus vaccines as part of childhood immunization is important. Awareness campaign prevention package of all causes of diarrhea as outlined by WHO/UNICEF should be adopted by the government and individuals. The prevention package entails interventions to reduce diarrhea by providing safe drinking water, improved sanitation, promote handwashing, and prompt management of diarrheal cases using oral rehydration salts.

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Conflicts of interest

There are no conflicts of interest.

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