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Prevalence of rotavirus among children under five years of age with diarrhea in Kaduna State, Nigeria

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Abstract*:* Rotavirus (RV) is a major etiological agent of acute infantile gastroenteritis and is associated with 20%-25% of diarrhea cases in infants. Nigeria continues to be among the first five countries with greatest number of RV disease associated deaths per year. The objective was to determine some demographic factors that might be associated with rotavirus diarrhea among children in Kaduna State. From September 2013-August 2014, 401 diarrheic stool samples were collected from children under 5 years of age in Kaduna State, Nigeria and analyzed for RV antigen using ELISA. An overall RV prevalence of 32.2% (129/401) was obtained with the infection occurring throughout the study period. The infection was higher in males (33.0%:63/111) compared to females (31.4%:66/210). The highest burden was detected in children 25 -36 months of age (37.3%:22/59). Highest prevalence was detected in children whose parents had primary education (35.8%:19/53) and those whose parents were civil servants (35.6%:36/101). There was no statistically significant association between breast feeding and RV infection (P> 0.05). The study has revealed that rotavirus remains an important cause of acute diarrhea in children under five years in Kaduna State, Nigeria. Hence the need to introduce the vaccines into the childhood immunization program in the country

Keywords: Prevalence, Rotavirus, Children, Kaduna State, Nigeria

Introduction

Diarrheal disease kills 1.8 million children under five years of age yearly.[1] It is the second leading cause of death, and accounted for 9.9% of the 6.9 million deaths in this age group in 2011.[2] In Nigeria it is encountered both in urban and rural areas.[3] It is estimated that 1.3 billion episodes and 4 million deaths occur each year in children under five years old with about 80% of deaths occurring in the first two years of life.[4]

Rotavirus (RV) gastroenteritis is a mild to severe disease, with incubation period of about 1-2 days.[5] The symptoms often starts with fever, nausea, and vomiting, followed by abdominal cramps and frequent watery diarrhea, which may last for 3-8 days. Infected children may also have a cough and runny nose.[5,6] Rotavirus infection is more frequent in Africa, especially West Africa including Nigeria in the cooler, drier winter months.[7,8]

Rotavirus is a major etiological agent for acute infantile gastroenteritis and is associated with 20%-25% of diarrhea cases in infants.[9,10] The number of deaths caused yearly by rotavirus has been estimated to be 453,000 in children less than five years old annually worldwide.[11] Nigeria continues to be among the 10 countries with greatest number of RV disease associated deaths per year. Estimates attribute up to 33,000 deaths annually to RV disease in Nigerian children less than 5 years old.[7,9] Prevalence of 11.0%-56% have been reported in Nigeria.[5,12,13,14, 15,16,17,18,19,20]

The introduction of two effective RV vaccines (Rota Teq and Rotarix), licensed in 2006 and have been recommended for use in all countries by WHO, particularly in those countries with high diarrhea-related mortality in children younger than 5 years.[21] Substantial declines in morbidity and mortality attributable to RV and all-cause diarrhea have been recorded in high-income and middle-income countries that have introduced RV vaccines so far.[10] Therefore, there is the need to introduce RV vaccine into the National Immunization Program.

Rotavirus infection is not routinely diagnosed in most Nigerian hospitals probably due to the cost of its diagnosis and because clinical spectrum of signs and symptoms are similar to other gastroenteritis. There is the need for regular detection of RV strains, because this information is needed to interpret the results of vaccine studies and epidemiologic surveillance.

Rotavirus surveillance has been going on in Nigeria since 2010 at the Institute of Child Health, University of Nigeria Teaching Hospital, Enugu.[20] The aim of the study was therefore to determine the prevalence of Rotavirus associated diarrhea among children under 5 years with diarrhea in Kaduna state Nigeria.

Materials and Methods

Study Area

The study was carried out in Kaduna state, Nigeria. The state has a total number of 23 Local Government Areas (LGAs) and three senatorial districts; that include south, north and central senatorial zones. Six of the LGAs were selected by simple random sampling for this research. These LGAs include Kachia and Kagarko (south); Chikun and Giwa (central) and Soba and Sabon gari (north). The health care facilities selected were Primary Health Care Unit (PHCU) Ladduga for Kachia LGA, PHCU Buruku for Chikun LGA, PHCU Maigana for Soba LGA, General Hospital Kagarko for Kagarko LGA, PHCU Gangara for Giwa LGA and Major Abdullahi Memorial Hospital Sabon gari for Sabon gari LGA.

Study Design

The research was a descriptive cross sectional study, and was conducted in a hospital or PHCU in each of the LGA. Children under 5 years of age were studied. A diarrhea case was defined as a child passing 3 or more loose, liquid, watery stool in a 24 hour period.

Ethical Approval

Ethical clearance was obtained from the ethical committee of Kaduna state Ministry of Health.

Sample Size

A prevalence rate of 36.5% reported in a previous study by Wada-Kura[15] was used to calculate the sample size using the equation by Sarmukaddam and Garad.[22] The calculated sample size was 356.15. However to have a good representation of the target population and to increase the chances of having positive samples, a total of 401 diarrheic stool samples were used for the study.

Sample Collection

A total of 401 stool samples were collected from children 0-5 years of age across the six selected LGAs. About 5ml of fecal sample was scooped with a wooden spatula or decanted respectively into clean, labeled screw capped tubes with the assistance of the laboratory technologist. All samples were transported in ice box to the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria and stored frozen at -20^0C until analyzed.

Specimen Preparation

Exactly 1 ml of sample diluent was added to properly marked tube using a pipette. For solid stool, the sample was pressed into transfer pipette to the first mark. For liquid stool, samples were aspirated into transfer pipette to the first mark. Samples were re-suspended in 1 ml of sample diluents. This makes a 10% fecal suspension.

Detection of Human Rotavirus

Each 10% fecal suspension was screened for the presence of rotavirus antigens using commercially available enzyme immunoassay (EIA) kit (Premier Rotaclone Meridian Bioscience, Inc. USA). All assays were performed according to the manufacturers' instructions. The samples, microtiter wells and reagents were brought to room temperature before the test was carried out. Wells for samples and controls were inserted into the microtiter well holder. About 2 drops (100µl) each of diluted fecal sample, positive control and negative control (sample diluents) was added to the bottom of separate wells. About 2 drops (100µl) of enzyme conjugate was added to each well, mixed by gently swirling on tabletop and was incubated at room temperature for 60 ± 5 minutes. After incubation the liquid was poured out of the wells into a discard vessel, and the microtiter well holder was tapped upside down vigorously against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled to overflow with washing buffer and the liquid was poured out. The microtiter well holder was tapped upside down vigorously against absorbent paper to ensure complete removal of liquid from the wells. The washing procedure was repeated two more times for a total of three washes.
About 2 drops (100µl) of substrate A solution was added to each well, and 2 drops (100µl) of substrate B solution was added to each well, and it was incubated for 10 minutes at room temperature. Visual determination was made after 10 minutes incubation. Samples with blue color greater than negative control are taken to be positive, while samples showing equal or less color than negative control are taken to be negative. Spectrophotometric determination was done by adding 2 drops (100µl) of stop solution (Sulphuric acid) to each well after the incubation, and the absorbance of each well was read at 450nm against an air blank within 60 minutes. Specimens with absorbance units (A_{450}) greater than 0.150 were considered positive, while Specimens with absorbance equal to or less than 0.150 are considered negative.

Analysis of Results

Data obtained from the questionnaire and the EIA was analyzed using statistical package for the social sciences (SPSS) version 21. Chi-square and odds ratio was used as test of association and risk respectively at 95% confidence interval with $p \leq 0.05$ taken as statistically significant.

Results

Out of the 401 fecal samples screened for the presence of human rotavirus in children, 32.2% (129/401) were positive for rotavirus antigens (Figure 1).
The distribution of rotavirus infection in children with respect to location is shown in Table 1. There was statistically significant difference between prevalence of rotavirus infection and location (χ^2=13.651, df=5 p=0.031).

Table 1: Distribution of Rotavirus among Children in Kaduna State, Nigeria

Location (LGA)	No examined	No positive (%)	No negative (%)
Chikun	58	21(36.2)	37(63.8)
Giwa	131	36(27.5)	95(72.5)
Kachia	41	25(61.0)	16(39.0)
Kagarko	62	14(22.6)	48(77.4)
Sabon gari	82	23(27.7)	60(72.3)
Soba	26	10(38.5)	16(61.5)
Total	401	129(32.2)	272(67.8)

(χ^2=13.651, df=5, p= 0.031)

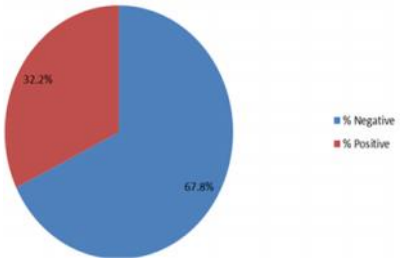

Fig 1: Prevalence of Rotavirus among Children in Kaduna State, Nigeria

The monthly distribution of rotavirus infection in children in parts of Kaduna State was determined as shown in Figure 2. The highest prevalence was recorded in March (75%:3/4), and least in July (14.0%:3/21) (χ^2=30.661, df=11, p=0.002).
The result was analyzed according to some demographic factors, and the result is shown in Table 2.There was no statistically significant difference between sex, age and parents' educational level and the presence of rotavirus infection.

Table 2: Prevalence of Human Rotavirus in Relation to Demographic Factors in Children in Parts of Kaduna State

Parameter	Examined No	Positive No (%)	Negative No (%)	p-value
Age group (months)				
0-12	101	34(33.7)	67(66.3)	
13-24	285	60(32.4)	125(67.6)	
25-36	59	22(37.3)	37(62.7)	0.039
37-48	290	9(31.1)	20(68.9)	
40-60	27	4(14.8)	23(85.2)	
Sex				
Male	111	63(33.0)	128(67.0)	
Female	210	66(31.4)	144(68.6)	1.002
Parents' educational level				
Informal	170	51(30.0)	119(70.0)	
Primary	53	(35.8)	34(64.2)	
Secondary	95	33(34.7)	62(65.3)	0.635
Tertiary	83	26(31.3)	57(68.7)	
Parents' occupation				
Self-employed	288	90(31.3)	98(68.7)	
Civil servant	101	36(35.6)	65(64.4)	0.043
Unemployed	12	3(25.0)	9(75.0)	

The result of the distribution of rotavirus with respect to mode of feeding is shown in Table 3. There was no statistically significant difference between breast feeding and the prevalence of rotavirus (χ^2=3.124,df=1, p=0.562).

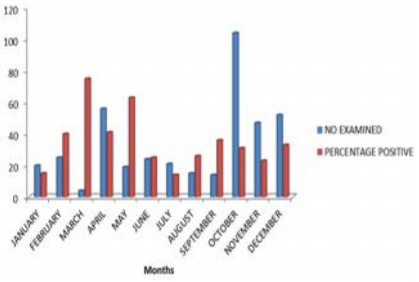

Fig 2: Monthly Distribution of Rotavirus Among Children in Kaduna State, Nigeria

Table 3: Prevalence of Rotavirus in Children with Respect to Breast Feeding and Introduction of Other Food

Mode of feeding	Tested No	Positive No (%)	Negative No (%)	OR95% CI on OR	p-value
Breast feeding					
Yes	198	72(36.4)	126(63.6)	2.453 2. 500-2.732	0.562
No	203	57(28.1)	146(71.9)		
Introduction of other food					
Yes	328	100(30.5)	228(69.5)	3.1322.9 83-3.365	0.025
No	73	29(39.7)	44(60.3)		

Discussion

This study shows that rotavirus remains an important cause of infantile diarrhea in children. Rotavirus antigen was detected in children 0-5years in parts of Kaduna state with a prevalence of 32.2% in this study. The result is similar to the 36.5% recorded in Kano Northern Nigeria[15] and 35.0% recorded in Lagos Southern Nigeria.[18] However the prevalence is higher than the 15.6%[13] and 23.8%[16] previously reported in Zaria, the 11.0%[14] and 13.8%[5] reported in Jos, 18.0% in North-western Nigeria,[7] 19.2% in Benin City[19] and 56.0% in Enugu.[20] In other African Countries, similar prevalence of 36.0%, 28.9% and 32.8% have been reported in Madagascar,[23] Cote d'voire[24] and Malawi[25] respectively. In other parts of the world, prevalence of 31.0% and 34.4% have been reported in Bangladesh[26] and Italy[27]

The result however contradicts higher values of 57.4% reported in Oman,[28] 39.8% in Ghana,[29] 45.5% in Uganda[30] and 61.0% in Indonesia.[31] Lower values of 14.0%,[10] 26.4%[32] and 26.2% [33] have been recorded in Namibia, South Africa and Tunisia respectively.
This observed difference in prevalence could be due to the method used, time and season of sample collection, sample storage, geographical location or environmental factors. The hospital-based WHO global networks for surveillance of rotavirus diarrhea report estimated the rotavirus rate to range from 39-52% in the African region.[30] The 32.2% obtained in this study, though high, did not fall in this WHO African region range probably because the sample were collected mainly from primary health care units and not the main hospitals where serious cases are referred or taken to.

Rotavirus was detected in children throughout the study period occurring with a slightly higher rate during the dry season. This agrees with the observation of previous studies[7,8,10,15,23,25,34] who found the circulation of rotavirus lower during the wet season with slightly increased seasonal peaks during the cooler, dryer months from October to March. The higher prevalence of rotavirus infection in the dry season may be attributed to geographical and environmental factors with low relative humidity being the most important environmental factor.[34]

Rotavirus was recorded in all age groups in this study. Although highest prevalence was recorded among children in age group 25-36 months, there was statistically significant difference between age and the prevalence of rotavirus. This agrees with the findings of Wada-Kura[15] who reported higher prevalence in children between 41-50 months in Kano and Coluchi *et al.*[35] who recorded higher prevalence in children within age group 24-35 months in Paraguay. The result however, contradict the report of Junaid *et al.*[5] and Aminu *et al.*[36] in studies conducted in Jos and Zaria respectively, who reported that the detection of rotavirus infection is more in children under the age of two years. The higher prevalence recorded in children among this age group (25-36 months) in this study could be due to behavioral activities of children at this age, who tend to play outside with possibly fecally contaminated materials. Least prevalence was recorded in children above 40 months of age. This could be due to the fact that older children tend to become protected from severe form of rotavirus infection as a result of protection acquired from multiple re-infection.[13]

Sex was found not to be associated with rotavirus diarrhea in children, this agrees with previous findings conducted in Nigeria.[12,13,15,16,34] Even though in this study, males had a slightly higher prevalence than females. This difference could be due to chance because at this age there is no difference in life styles between the boy and girl child. The result contradicts the finding of Junaid *et al.*[5] in Jos, who reported statistically significant association between sex and rotavirus infection.

Educational level of parents was not statistically significant associated with the prevalence of rotavirus, this agrees with the findings of Aminu *et al.*[12] and Junaid *et al.*[5] Children whose parents had primary education had the highest prevalence while those whose parents had informal education had the least.

Children whose parents were unemployed had the least prevalence and civil servants 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.

Breast feeding was not associated with the prevalence of rotavirus in children. This contradicts the findings of Aminu *et al.*[35] who reported that breast feeding confer some protection against rotavirus infection. In this study, children that were breast feed, and did not start any food had higher prevalence; which could be due to the fact that at this age, children are more vulnerable to rotavirus infection. Breast feeding may only be protective if it is practiced with intensity and frequency that allows continuous high level protection of the intestinal mucosa rather than sporadic or low volume feeds.[30]

Conclusion

The study has revealed that rotavirus remains an important cause of acute diarrhea in children under five years in Kaduna. There is the need for the introduction of the licensed rotavirus vaccine in the study area as a means of preventing rotavirus infection.

Limitations

VP6 subgroup specificity could not be detected because of lack of monoclonal antibodies specific for VP6.

Conflict of interest: None
Funding: None

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