ORIGINAL ARTICLE

Rotavirus Infection in Four States in North-western Nigeria

[*]Aminu M, [*]Ahmad A A, [**]Umoh J U

[*]Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria

[**]Department of Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Abstract

Background: *Rotaviruses are associated with ~ 611,000 deaths worldwide and with 33,000 deaths in Nigeria in children < 5 years of age annually. However, limited data exit on rotavirus (RV) infection in North-western Nigeria. This study surveyed RV infection in four states in North-western Nigeria.*

Methods: *During July 2002 to July 2004, 1063 (869 diarrhoeic and 194 control) stool samples were collected from children <5 years of age presenting with diarrhoea in clinics/hospitals in Kaduna, Kebbi, Sokoto and Zamfara States. The stools were analysed for RV antigen and the RV positive stools were further characterized by antigenic and genomic methods.*

Results: *Rotavirus was detected in 18% of children with diarrhoea and in 7.2% of the age-matched case controls. Rotavirus antigen was detected more frequently in Kaduna state ($p > 0.05$). The highest RV burden was detected in children aged below six months. The infection occurred throughout the study period. The most common clinical features associated with RV were fever (71%), vomiting (64.1%) and a combination of fever and vomiting (48.2%). Vomiting was strongly associated with RV ($p < 0.01$). There was a statistically significant association between food type and rotavirus infection ($p < 0.05$), with the highest prevalence occurring amongst children exclusively breast-fed. The majority of the RV positive samples revealed long electropherotypes and VP6 subgroup I + II specificity.*

Conclusion: *Rotavirus was shown to be an important cause of diarrhoea in children 0-5 years of age in North-western Nigeria. An effective vaccine would therefore need to be administered at birth for children in the study area since there is no effective way to completely eliminate rotavirus infection other than vaccination. There is also a need for additional studies in Nigeria to provide data required to hasten vaccine introduction.*

Date accepted for publication 12th June 2008

Nig J Med 2008; 285 - 290

Introduction

Rotavirus is responsible for a large proportion of morbidity and mortality associated with diarrhoeal illnesses.[1] Recent estimates attribute 611 000 deaths in children less than five years old to rotavirus annually.[2] In sub-Saharan Africa, rotavirus infection is responsible for the death of approximately 110,000-150,000 children under 5-years annually.[3] In Nigeria, a high incidence of childhood diarrhoea is estimated to account for over 160,000 of all deaths in children less than 5 years of age annually and of this number approximately 20% are associated with rotavirus infection.[1] Improvements in sanitation and the availability of clean water have not decreased the rate of rotavirus diarrhoea and the development and implementation of an effective vaccine into the routine National Programme on Immunization (NPI) schedule is considered the first strategy of prevention.[4] The availability of rotavirus vaccines to children in developing countries may provide a new tool to address childhood mortality in African settings.[5]

Epidemiological studies of rotavirus infection are increasingly revealing a great diversity of rotavirus strains co-circulating in the human population worldwide. Africa, especially West Africa, seems to display the greatest degree of natural rotavirus diversity.[6-8] Studies have shown that there is always an unexpected diversity among human rotavirus strains isolated in northern Nigeria with varied and unusual epidemiology.[9, 10] As in many viral diseases, neutralizing antibodies appear to play an important role in protection against rotavirus associated diseases in a type-specific manner.[11] Therefore, monitoring the rotavirus strains circulating at any one time and the emergence of novel rotaviruses are important for vaccine development and crucial during the implementation of vaccination programme.

There are a number of published articles on rotavirus-associated disease in southern Nigeria [12-16] with a few studies conducted in northern Nigeria, predominantly in the north-eastern [9, 10] and north-central regions.[17-18] This

study expands the rotavirus data available for Nigeria and examines rotavirus strains detected in children less than five years old residing in four states of North-western Nigeria.

Materials and Methods

Study Area

The study was conducted in four states (Kaduna, Kebbi, Sokoto and Zamfara) in North-western Nigeria between July 2002 and July 2004. The region has a tropical climate and Northern dry savannah vegetation with alternating humid to wet and cool to hot dry season. The region is urban with people of different educational and socio-economic background living in neighbourhoods with distinctly different levels of sanitation. Borehole and well water are the major source of drinking water in this area. The main occupations are cattle rearing and subsistence farming. All the state headquarters have Government runhospitals, private clinics and primary health care units (PHCU) that provide health services to the population.

Sample Collection and Storage

Faecal samples were collected from 869 children under the age of 5 years who were presented or admitted at clinics or hospitals for acute diarrhoeal illness in the four states. In addition, 194 control (non-diarrhoeic) samples were collected from children matched for age and time of year. The number of samples collected per state was largely dependent on its population and the number of health facilities in the state. Each State Ministry of Health and the Ethics Committee of each clinic/hospital approved the study. A diarrhoeic case in this study was defined as a child passing loose, liquid, and watery or a bloody loose stool three or more times in a 24-hour period as reported by parents. The control was any child presented for an illness other than diarrhoea and with no history of it on the day of, or in the three weeks preceding sampling.

At the respective health care facility, arrangements were made with a doctor, matron or laboratory technician whereby the parent/caregiver of any child who satisfies the study inclusion criteria (age 0-5 years of both sexes and diarrhoeic) was asked to provide the child's stool sample after consultation. Prior to collection of sample, the parent/caregiver after consenting was interviewed using structured questionnaire designed to obtain basic demographic data, history of illness and clinical information concerning the child. Information on the clinical features and the dehydration status were provided by the Doctor/matron on duty or obtained from the hospital folder of each child. Faecal samples were collected in clean, labelled screw capped tubes and were stored frozen (-20°C) at the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria, until transportation. Only one stool sample was collected per child. Samples within iceboxes were transported to the **MRC/MEDUNSA** Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, Pretoria, South Africa, where a 10% faecal suspension of each sample was prepared using a balanced salt solution before analysis.

Rotavirus detection

Rotavirus antigens were detected utilizing the 10% faecal suspensions previously prepared and a commercially available Rotavirus IDEIA™ Kit (DakoCytomation, UK) according to the manufacturers' instructions.

Polyacrylamide Gel Electrophoresis (PAGE)

All rotavirus-positive samples were analyzed by PAGE as previously described [19]. Briefly, RNA was extracted from the 10% faecal suspensions previously prepared utilizing phenol-chloroform deproteinization and ethanol precipitation, eletrophorezed overnight and dsRNA bands were visualized by silver staining according to the method described by Herring *et al*. [20]

Subgroup specificity (VP6)

All rotavirus-positive samples were analyzed utilizing an 'in-house' VP6 ELISA as described by Steele and Alexander.[21] Group-specific [22] and subgroup-specific monoclonal antibodies [23] were a kind donation from H.B. Greenberg, Stanford University, USA.

Statistical Analysis

Analysis of RV infection in children according to age, sex and state was done using **Statistical Programme for Social Sciences** (SPSS) version 11.0. Differences with p-values >0.05 were considered not significant at 95% confidence intervals (CI).

Results

The overall prevalence of RV infection was found to be 16% (170/1063) of the total samples analysed. Rotavirus antigen was detected in 18% (156/869) of the diarrhoeic samples and in 7.2% (14/194) of the control samples. Rotavirus antigen was detected more frequently in Kaduna state ($p > 0.05$) (Table I). Infection occurred throughout the study period with slightly higher peaks in the drier months (Figure 1).

Rotavirus infection was significantly associated with children under 2 years of age with a peak incidence in children 0-6 months old ($p < 0.01$) in both the study (25.8%: 40/155) and diarrhoeic (30%: 39/130) populations (Table 2). The virus occurred with an incidence of 18.7% (133/712) and 21% (125/595) in

children under 24 months in the study and diarrhoeic populations respectively compared to 10.5% (37/351) and 11.3% (31/274) in children above 25 months. There was no significant difference ($p > 0.05$) in the prevalence of rotavirus among males and females in the study population, although viral shedding was slightly higher in males (16.4%: 100/608) than females (15.4%: 70/455). This was also true for the diarrhoeic population where the prevalence of rotavirus infection was 17.8% (91/511) in the males and 18.2% (65/358) in the females.

The prevalence of rotavirus infection was significantly ($p < 0.01$) higher (75/340: 22%) in children who passed stool continuously compared to those who did not (95/723: 13%). The most common clinical features associated with rotavirus were fever (71%: 121/170), vomiting (64.1%: 109/170) and symptom of upper respiratory tract (URT) infection (13.5%: 23/170). Combinations of two or three of these symptoms were also observed with fever and vomiting being the most frequently occurring (48.2%: 82/170). Vomiting was strongly associated with RV ($p < 0.01$). Dehydration was found to be mild in 40.0% (68/170) of RV positive children. About 23% (31/136) of children who were admitted for diarrhoea on hospital visit were shedding rotaviruses ($p < 0.05$) while 66% (42/65) of hospitalized diarrhoeic patients were shedding RV antigen.

There was a statistically significant association between food type and rotavirus infection ($p < 0.05$). The prevalence of rotavirus infection in the study population was highest among children exclusively breast-fed (Table III).

Table I: Distribution of rotavirus infection in children 0-5 years old in four states in North-western Nigeria between 2002 and 2004

	Study Population		Diarrhoeic Population		Non-Diarrhoeic Population	
State	Total	Pos (%)	Total	Pos (%)	Total	Pos (%)
Kaduna	366	70 (19.1)	252	61 (24.2)	114	9 (7.9)
Kebbi	96	14 (14.6)	86	14 (16.3)	10	0 (0.0)
Sokoto	229	31 (13.5)	209	31 (14.8)	20	0 (0.0)
Zamfara	372	55 (14.8)	322	50 (15.5)	50	5 (10.0)
Total	1063	170 (16.0)	869	156 (18.0)	194	14 (7.2)

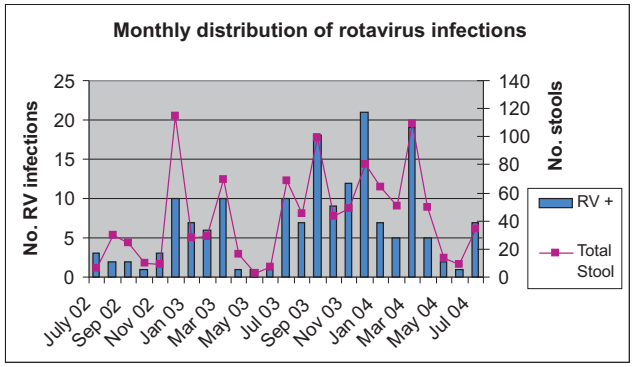

Figure I: Monthly distribution of children infected with rotavirus and of children presenting with diarrhoea in four states in North-western Nigeria between 2002 and 2004.

Table II: Age distribution of rotavirus infection in children 0-5 years old in four states in North-western Nigeria between 2002 and 2004

Age group (months)	Study population		Diarrhoeic population	
	No. Tested	RV +ve (%)	No. Tested	RV +ve (%)
0-6	155	40 (25.8)	130	39(30.0)
7-12	248	51(20.6)	203	47(23.1)
13-18	109	17 (15.6)	94	15(16.0)
19-24	200	25 (12.5)	168	24(14.3)
25-36	171	19 (11.1)	143	16(11.2)
37-48	91	10 (11.0)	68	8(11.8)
49-60	89	8 (9.0)	63	7(10.3)
0-60	1063	170 (16.0)	869	156 (18.0)

Table 3: Prevalence of rotavirus infection among children under different feeding regimens in four states in North-western Nigeria between 2002 and 2004

Food Type	Study Population			Diarrhoeic Population		
	Rotavirus			Rotavirus		
	Negative	Positive	Total	Negative	Positive	Total
BS	284 (77.8)	81 (22.2)	365 (34.4)	227 (75.0)	76 (25.0)	303 (34.9)
BW	36 (81.8)	8 (18.2)	44 (4.1)	28 (80.0)	7 (20.0)	35 (4.0)
EBF	34 (77.3)	10 (22.7)	44 (4.1)	28 (73.7)	10 (26.3)	38 (4.4)
Solid	539 (88.4)	71 (11.6)	610 (57.4)	430 (87.2)	63 (12.8)	493 (56.7)
Total	**893 (84.0)**	**170 (16.0)**	**1063 (100.0)**	**713 (82.0)**	**156 (18.0)**	**869 (100.0)**

EBF = Exclusive Breast Feeding BW = Breast and Water, BS = Breast and Solid, Figures in parentheses represent percent of total.

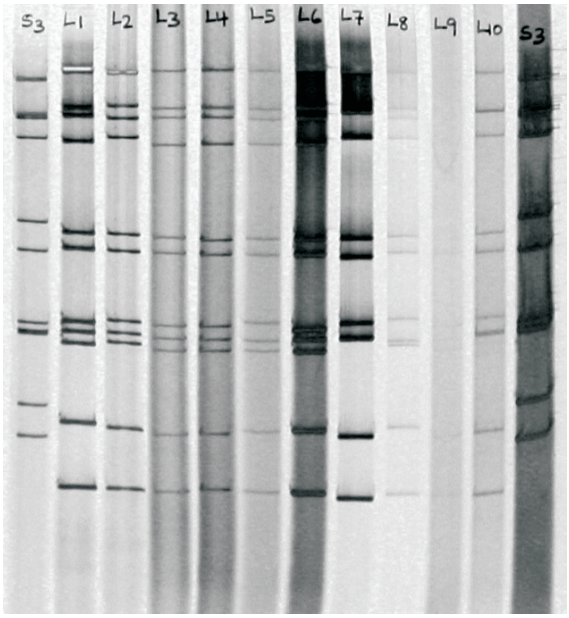

Figure 2: Electropherotypes detected in four states in North-western Nigeria between 2002 and 2004. L1-L10 represents RV dsRNA long electropherotypes flanked by a short (S3) electropherotype.

There was no statistically significant relationship between mothers' level of education and viral infection ($p > 0.05$), even though, there were differences in the prevalence rates. The highest rate of infection in children in the study population was 19.1% (36/188) among children from mothers with a secondary school education, closely followed by 18.4% (52/283) in children from illiterate mothers and then 16.4% (34/207), 13.4% (41/305) and 8.75% (7/80) in children from mothers with an Islamic, primary and tertiary school education respectively. In the diarrhoeic population, the highest rate (20.7%: 49/237) of infection was recorded for children among illiterate mothers closely followed by children from mothers with a secondary school education (20.0%: 32/160). In children from mothers with an Islamic, primary and tertiary school education, the rates were 18.5% (33/178), 15.3% (36/235) and 10.2% (6/59) respectively.

There was no relationship between socio-economic background and viral infection ($p > 0.05$). The highest prevalence (17.5% and 18.6% for study and diarrhoeic population respectively) was observed among children from low socio-economic background while the lowest (13.0% and 16.7% for study and diarrhoeic population respectively) was observed among those from high socio-economic background.

Electropherotypes could be obtained from 54% (92/170) of samples analyzed. Long RNA migration patterns predominated (80.4%: 74/92) and 10 distinct electrophoretic variants were noted (Figure 2). In addition, six distinct short electropherotypes (n=18) and a small proportion of mixed patterns (2.2%) were detected. The predominant long and short patterns detected over the course of study were L5 and S2 respectively. There was a significant variation in the electropherotypes within the states ($p < 0.01$). The long pattern predominated in Sokoto State (92.3%: 12/13), while the short profile was more common in Zamfara State (34.6%: 9/26). The long pattern predominated in the hospitalized children ($p < 0.01$).

Subgroup specificity (SG) could be assigned to 146/170 samples, with 22 samples not reacting to any of the antibodies used and a further two samples having insufficient stool for testing. Subgroup II specificity was found in 41/170 samples, SGI in 37/170 samples and SGnon-I/non-II in 16/170 samples. Surprisingly, SGI+II specificity was detected in 52/170 samples, by far the predominant subgroup in samples from north-western Nigeria. Three strains exhibited the unusual combination of VP6 SGI specificity with a long (L) electropherotype.

Discussion

Nigeria has recently been ranked second among six countries with the greatest number of rotavirus disease-associated deaths per year in children less than 5 years old.[24] The highest prevalence of rotavirus was recorded in Kaduna State. This is probably due to the cooler, less dry climate of the state compared to other states. Detection of rotaviruses throughout the study period is not unexpected and similar seasonality trends have previously been reported in Africa. [5, 9] The higher prevalence of RV infection in the dry season may be attributable to geographical and environmental factors with low relative humidity being the most important environmental factor. [25]

Rotavirus infection predominated in children <6 months old. This implies greatest burden in the youngest and most vulnerable unlike developed countries where rotavirus infections are more common in children 9-15 months old.[1, 3] This age group might be the appropriate age for vaccine intervention in the study area. Furthermore, rotaviruses were still detected in roughly 10% diarrhoeal episodes in children older than 24 months and this probably represent secondary or tertiary infections in these settings rather than the first exposure event. Gender was not a determinant of rotavirus infection in this study, as both sexes were

equally infected. Although the rate was higher in males than females it was not statistically significant (P>0.05). This is because at 0-5 years, there is generally no difference in life style between the boy and girl child.

As previously reported in Nigeria [17, 26, 35], the prevalence of rotavirus infection was highest among children exclusively breast fed. This result maybe a reflection of the presence of many children on this regimen that are within the rotavirus infection peak age group or, may be highly probable that some children fell into this group as a result of misunderstanding of the question of feeding. Hendricks and Badruddin [27] reported in their study that, when asked what a child eats, most mothers do not mention food given as snacks because they equate the term 'eat' with mealtime foods. An occasional observation was made on the contrary to what the mothers were claiming. For example, some mothers were seen with feeding bottles containing water or even milk but still claimed their children were fed on only breast milk. This claim is attributable to fear of being reprimanded by medical personnel who lecture mothers on exclusive breast-feeding. It has also been reported that sometimes mothers' statements do not tally with researchers observations. [28] The lower prevalence observed among children fed on solid food maybe as a result of decrease in rotavirus infection with age since children fed on solid food are much older. Primary symptomatic rotavirus infection occurs much early in life [29] thus, children are protected against subsequent symptomatic re-infection. [30]

Rotavirus infection circulated at similar level in children whose mothers had a secondary school education and in children whose mothers were illiterate and was lowest among children whose mothers had a tertiary school education. The reason for this similar prevalence is not clear. However, it is highly probable that many of these mothers delegate childcare to housemaids who are usually illiterate elderly women or young girls. Ojeh *et al*. [31] has earlier reported illiteracy as one of the factors that enhances the outbreak and spread of rotaviruses. Rotavirus infection predominated among children from low socio-economic background. This result is not unexpected and has been previously reported in Nigeria [15, 31, and 32]

As expected, RV strains with long electropherotypes predominated in this study. Extensive genomic diversity observed in the region as indicated by the eighteen RNA electrophoretypic variants identified; signifies the potential for active genetic re-assortment between viral strains. Mixed patterns noted for the first time in Nigeria may represent possible means of emergence of genetic re-assortant RV strains. These mixed strains might have originated from animals; because the study area is predominantly inhabited by nomadic pastoral farmers who live in close association with their animals and share common sources of drinking water. Furthermore, the continuous viral infection in older children may facilitate antigenic shift.

Interestingly, 31% of samples were of SGI+II specificity indicating the possibility of mixed infections. This has been reported previously in very low level. [33] The unusual combination of a VP6 SGI specificity with a 'long' electropherotype noted in this study may be a consequence of re-assortment process occurring between samples from different genogroups. SGI/long pattern samples have been described in different regions of the world [21, 34-36]

This pioneer study in Kebbi, Sokoto and Zamfara States showed rotaviruses to be important cause of diarrhoea in children 0-5 years in North-western Nigeria. Other than vaccination, there is no effective way to completely eliminate rotavirus infection or its spread. The introduction of an effective vaccine is therefore crucial. However, an effective strategy for immunization must take cognisance of the peak of infection reported to occur among children 0-6 months old in the study area. An effective vaccine would therefore need to be administered at birth for children in the study area. In addition, there is the need for additional studies in this region to provide data required to hasten the introduction of effective rotavirus vaccines to children, who would clearly benefit from these interventions.

Acknowledgements

This study was conducted with the grant awarded to Dr. Maryam Aminu by UNESCO-L'ORÉAL Fellowship for Women in Life Sciences. The World Health Organisation also supported the research.

References

1. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003; 9: 565-72
2. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhoea. Emerg Infect Dis 2006; 12: 304-6
3. Glass RI, Bresee JS, Turcios R, Fischer TK, Parashar UD and Steele, AD. Rotavirus vaccines: targeting the developing world. The J Infect Dis 2005; 192: S160-S66

4. Bresee JS, Glass RI, Ivanoff B, Gentsch JR. Current status and future priorities for rotavirus vaccine development, evaluation and implementation in developing countries. Vaccine 1999; 17: 2207-22
5. Cunliffe NA, Kilgore PE, Bresee JS, et al. Epidemiology of rotavirus diarrhoea in Africa: A review of studies to anticipate rotavirus immunization. Bull Wld Hlth Org 1998; 76: 525-37
6. Desselberger U, Iturriza-Gomara M and Gray J. Rotavirus epidemiology and surveillance. Novartis Found Symp 2001; 238: 125-147.
7. Cunliffe NA, Bresee JS, Gentsch JR, Glass RI, Hart, CA. The expanding diversity of rotaviruses. Lancet 2002; 359: 640-642.
8. Steele AD, Ivanoff B and the African Rotavirus Network. Rotavirus strains circulating in Africa during 1996-1999: emergence of G9 strains and P [6] strains. Vaccine 2003; 21: 361-367.
9. Adah MI, Rohwedder A, Olaleye OD, Durojaiye OA and Werchau H. Serotype of Nigerian rotavirus strains. Trop Med Int Hlth 1997; 2: 363-370
10. Adah MI, Wade A and Taniguchi. Molecular epidemiology of rotaviruses in Nigeria: Detection of unusual strains with G2P [6] and G8P [1] specificities. J Clin Microbiol 2001; 39: 3969-3975
11. HoshinoY and Kapikian AZ. Rotavirus serotypes: classification and importance in epidemiology, immunity, and vaccine development. J Hlth Popul Nutr 2000; 18: 5-14
12. Abiodun PO, Ihongbe JC, Ogibimi A. Asymptomatic rotavirus infection in Nigerian day-care centres. Ann Trop Pediatr 1985; 5: 1663-5
13. Abiodun PO, Omoigberale A. Prevalence of nosocomial rotavirus infection in hospitalized children in Benin City, Nigeria. Ann Trop Paediatr 1994; 14: 85-88.
14. Audu R, Omilabu SA, de Beer M, Peenze I, Steele AD Diversity of human rotavirus VP6, VP7, and VP4 in Lagos State, Nigeria. J Hlth Popul Nutr 2002; 20: 59-64
15. Olusanya O, Taiwo O. Rotavirus as an etiological agent of acute children diarrhoea in Ile-Ife, Nigeria. E Afri Med J 1989; 66: 100-4
16. Paul MO, Erinle EA. Rotavirus infection in Nigerian infants and young children with gastroenteritis. Am J Trop Med Hyg 1982; 31: 374-5
17. Gomwalk NE, Gosham LT, Umoh JU. Rotavirus gastroenteritis in pediatric diarrhoea in Jos, Nigeria. J Trop Pediatr 1990; 36: 52-5
18. Nimzing L, Geyer A, Sebata T, de Beer M, Angyo I, Gomwalk NE, Steele AD. Epidemiology of adenoviruses and rotaviruses identified in young children in Jos, Nigeria. S Afri J Epid Infect 2000; 15: 40-2
19. Steele AD, Alexander JJ. Molecular epidemiology of rotavirus in black infants in South Africa. J Clin Microbiol 1987; 25: 2384-7
20. Herring AJ, Inglis NF, Ojeh CK, Snodgrass DR, Menzies JD. Rapid diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained polyacrylamide gels. J Clin Microbiol 1982; 16: 473-7
21. Steele AD, Alexander JJ. The relative frequency of subgroup I and II rotaviruses in black infants in South Africa. J Med Virol 1988; 24: 321-7
22. Beards GM, Campbell AD, Cottrell R, et al. Enzyme-linked immunosorbent assay based on polyclonal and monoclonal antibodies for rotavirus detection. J Clin Microbiol 1984; 19: 248-54
23. Greenberg HB, McAuliffe V, Valdesuso J, et al. Serological analysis of the subgroup protein of rotavirus using monoclonal antibodies. Infect Immun 1983; 39: 91-9
24. Burton, T. Global Immunization News 25 May 2007; available at http://www.who.int/immunization_monitoring/burden/rotavirus_estimates/en/index.html
25. Paul, MO. and Erinle, EA. Influence of humidity on rotavirus prevalence among Nigerian infants and young children with gastroenteritis. J Clin Microbiol 1982; 15: 212-215.
26. Omotade OO, Olaleye OD, Oyejide CO, Avery RM, Pawley A and Shelton AP. Rotavirus serotypes and subgroups in gastroenteritis. Nig J Paediatr 1995; 22: 11-17.
27. Hendricks KM and Badruddin SH. Weaning and diarrhoea disease. J Diarr Dis Res 1994; 12: 4-13.
28. Molbak K, Wested N, Hojlyng N. The etiology of early childhood diarrhoea: a community study from Guinea Bissau. The J Inf Dis 1994; 168: 581-587.
29. Steele AD. (2002). Rotavirus in Africa. In: African rotavirus network Proceedings of the Third African Rotavirus Symposium (Ed. Armah, G.) Noguchi Memorial Institute for Medical Research, Legon, Ghana. 15-17 September 2002, pp 9.
30. Bishop RF. Natural history of human rotavirus infection. Arch Virol 1996; 2: 119.
31. Ojeh CK, Atti DJI and Omotade OO. Comparison of the genome dsRNA of human rotavirus strains shed in parts of Ibadan, Nigeria. Afri J Med Sci 1995; 24: 359-63.
32. Fagbami AH, Oyejide CO and Enahoro F. Neonatal rotavirus infection in urban and rural communities in Nigeria. Trop Geog Med 1987; 39: 341-344.
33. Steele AD, Nimzing L, Peenze I, et al. Circulation of the novel G9 and G9 rotavirus strains in Nigeria in 1998/1999. J Med Virol 2002; 67: 608-612.
34. Kobayashi N, Lintag IC, Urasawa T, Tanigushi K, Saniel MC, Uraswaw S. Unusual human rotavirus strains having subgroup I specificity and 'long' RNA electrropherotype. Arch Virol 1989; 109: 11-23.
35. Pennap GRI. Epidemiology of group A rotavirus infection among 0-5 years old children with diarrhoea in and around Zaria, Kaduna State, Nigeria. Unpublished PhD Thesis. 2002. Ahmadu Bello University Zaria, Nigeria.
36. Esona MD, Armah GE and Steele AD. Molecular epidemiology of rotavirus infection in Western Cameroon. J Trop Paediatr 2003; 49: 160-163.