Human rotavirus genotypes causing acute watery diarrhea among under-five children in Benin City, Nigeria

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Abstract

Background: Diarrhea is a major cause of childhood morbidity and mortality in the developing countries. Rotavirus is a major cause of acute watery diarrhea.

Aim: This study aims at characterizing the prevalent rotavirus G-genotypes among under-five children presenting with acute watery diarrhea in Benin City, Nigeria.

Methodology: A total of 470 children <5 years presenting with diarrhea of <2 weeks duration, were over a period of 1 year consecutively recruited for the study. Stool samples were collected for rotavirus antigen detection using Enzyme-Linked Immunosorbent Assay (ELISA) and further analyzed with reverse transcriptase polymerase chain reaction (RT-PCR) for VP7 genotyping.

Results: Comparing the ability of the two methods to detect rotavirus in stool samples, 65 (13.8%) and 90 (19.2%) of the stools tested positive for rotavirus using ELISA and RT-PCR, respectively. Using VP7 primers, genotypes G1 were detected in 49 out of 90 stool samples (54.4%), G2 in 26 out of 90 stool samples (28.9%), G3 in 19 out of 90 stool samples (21.1%), G4 in 34 out of 90 stool samples (37.8%) and G9 in 8 of the 90 stool samples (8.9%). Some strains were observed to be reactive with 2 or more of the primers yielding dual or triple VP7 genotype reactivity.

Conclusion: Rotavirus of varying genotypes as shown cause acute watery diarrhea among under-five children and vaccine with strains peculiar to this environment should be introduced. Techniques such as RT-PCR rather than ELISA, where affordable, should be used in stool rotavirus screening.

Key words: Acute watery diarrhea; Benin City, characterization, human rotavirus genotypes, under-five children

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Introduction

Rotavirus infection is the leading cause of severe acute diarrhea among young children worldwide[1-3] and has been reported to be responsible for endemic viral diarrhea in children in Nigeria.[2] Rotaviruses constitute a genus in the family *Reoviridae*. Mature rotavirus particles are approximately 75 nm (750 A) in diameter with triple-layered icosahedral protein capsid made up of an outer layer, an intermediate layer and an inner core layer. The rotavirus genome contains double-stranded (ds) RNA in 11 segments, and the pattern of migration of these dsRNA segments in polyacrylamide gel electrophoresis is used to distinguish rotavirus in serogroups A to G.[4] Two proteins VP4 (P protein-4) and VP7 (G protein) form the rotavirus outer capsid. Based on VP4 and VP7 neutralizing epitopes, group A rotaviruses are classified using dual serotype P and serotype G designations. According to the nucleotide sequence of VP4 and VP7 genes, P and G genotypes are also distinguished.[5] So far 19 G genotypes and 27 different P genotypes have been identified, with

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strains belonging to 11 G types (G1–G6, G8–G12) and *P* types have been isolated from humans.[6] Electrophoretic and genotypic studies have demonstrated that several serotypes/genotypes may co-exist within a community, but these patterns do not necessarily correlate with serologic classifications. Several serotypes may also co-exist within a community, but each season is usually dominated by a single serotype that may vary from year to year.[7,8] It is known that seasonal outbreak of rotavirus induced diarrhea usually consist of only one or two serotypes. Furthermore in a community, a given serotype may persist from year to year, but the strains appear to be constantly shifting with a given electrophoretype rarely persisting for more than a season or two.[9] In 1973, Bishop first identified rotavirus as a human pathogen, when it was detected by electron microscopy in duodenal biopsy specimens of children with acute nonbacterial gastroenteritis.[9] Since then, several other workers have detected rotavirus in fecal specimens using electron microscopy.[10] Severe rotavirus infection occurs more frequently in young children aged 6–12 months in developing countries compared with developed countries where the disease is most frequent in 12–18 months age group.[10] Infants in the first 2–3 months of life seem to be relatively protected from severe rotavirus disease probably because of residual maternal immunity mediated by transplacental antibodies and breast milk, which contain lactadherin.[11-13] Repeated rotavirus infections are necessary to induce effective immunity. With additional rotavirus infection the symptoms decrease in severity and immunity increases (IgA, IgG, and memory cells). This could explain the asymptomatic infection in older children and adults. Two licensed rotavirus vaccines have shown efficacy of 85–98% against severe rotavirus diarrhea in trials conducted in the Americas and Europe[14,15] and have been introduced into routine immunization programs in many countries in these regions and in Australia as well as in low-income countries of Asia and Africa. It is expected that the prevalent and circulating rotavirus genotypes will be covered when modifying rotavirus vaccines that will be introduced into routine immunization program in Nigeria.

The aim of this study was to characterize the circulating genotypes of rotavirus among children of <5 years presenting with acute diarrhea in Benin City, Nigeria.

Methodology

Sampling

A total of 470 patients with diarrhea in the preceding 2 weeks were recruited from the tertiary, secondary, primary health care centers, and twelve randomly selected private hospitals in Benin City. Benin City is the capital (2006 estimated population 1,147,188) of Edo state, southern Nigeria. Information such as age, sex, home address, onset of diarrhea, and sources of drinking water were obtained through a structured questionnaire. The clinico-demographic features and stool samples of the studied population were analyzed. Subjects, above 5 years, with chronic diarrhea, or with obvious underlying causes of diarrhea, for example, HIV/AIDS, malignancy, prolonged steroids and so on. were excluded from the study. Informed verbal consent from the parents/guardians and approval by Ethics and Research Committee of University of Benin Teaching Hospital (UBTH) were obtained after due explanation of this noninvasive study.

Specimen

Fresh stools were collected in clean specimen bottles by the care-givers or the hospital staff from each child with instructions on proper method of collection. The stool specimens were kept in − 70°C deep freezer until the total number was collected.

Antigen detection using Enzyme-Linked Immunosorbent Assay

The stools were processed for rotavirus antigen detection using Enzyme-Linked Immunosorbent Assay (ELISA) kits from Diagnostic Automation Incorporation (DAI), USA. This was carried out according to the manufacturer's instruction.

Rotavirus G genotyping

The samples were further characterized using reverse transcriptase polymerase chain reaction (RT-PCR) and the positive ones genotyped using the method as described by Gouvea *et al*.[16] Briefly, the VP7 gene was reverse transcribed and amplified using plus-sense primer sBeg9 (nucleotides 1-21, 5′-GGCTTTAAAAGAGAGAATTTC-3′) and minus-sense primer End9 (nucleotides 1062-1036, 5′-GTCACATCATACAATTCTAATCTAAG-3′), followed by G genotyping using a cocktail of primers specific to the six human rotavirus serotypes (G1–G4, G8, and G9). A 1.5% agarose gel was prepared by weighing 0.75 g of agarose into 50 ml of 1 × Tris acetate ethylene-diamine-tetraacetic acid buffer. About 2 μl ethidium bromide was added and poured into slab to set. A total volume of 10 μl of amplified cRNA was mixed with 2 μl of the bromophenol blue tracking dye. This was then loaded into the gel alongside with a gene marker (fermenter) with a molecular weight of 100–1500 bp. The agarose gel was subjected to electrophoresis at 120 V for 45 min. The results were read under ultraviolet transilluminator and photodocumentation was obtained. Confirmation of the G type results was conducted using additional primer sets and a positive sample for G types 1, 2, 3, 4, and 9 is the sample whose PCR band co-migrates with the molecular markers for the G genotypes.

Results

A total of 470 children with diarrhea were recruited for the study, out of which 65 (13.8%) stool samples tested positive

Table 1: Rotavirus genotypes and their base pair ranges

Genotypes	Base pair range	Number	Percentage
G1	158	49	54.4
G2	244	26	28.9
G3	466	19	21.1
G4	403	34	37.8
G9	110	8	8.9

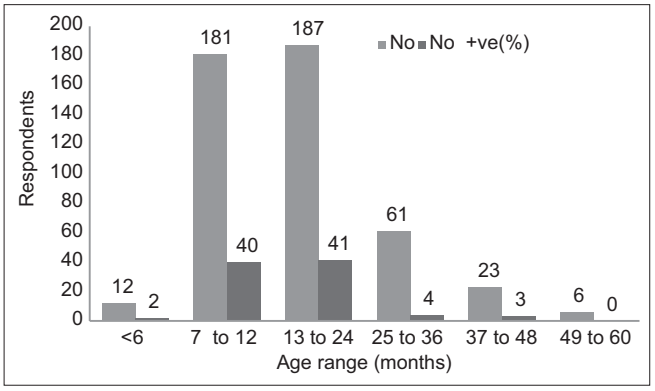

Figure 1: Age distribution of subjects and rotavirus detection rate using reverse transcriptase polymerase chain reaction

for rotavirus antigen using ELISA. Ninety samples (19.2%) tested positive with RT-PCR. VP7 genotyping showed G1–G4 and G9 with base pair ranges as presented in Table 1. Mixed infections were demonstrated as dual and triple reactivity to VP7 primers.

Of the 470 children, 170 were males and 300 females with a male to female ratio of 0.6:1. Thirty (17.6%) of the 170 samples collected from males tested positive for rotavirus using RT-PCR, while 60 (20%) of the samples collected from females tested positive.

The age distribution shows that 12 of the total respondents were <6 months old, of which 2 (i.e. 2.2%) samples tested positive. One hundred and eighty-one respondents were ages 7–12 months from which 40 (44.4%) tested positive. One hundred and eighty-seven respondents were between 13 and 24 months of age with 41 (45.6%) testing positive. Thus, a total of 368 respondents (i.e. 78.3% of the respondents) were within 7 and 24 months of age and also has the highest rotavirus excretion (90%) [Figure 1].

Discussion

The prevalence of rotavirus among children of <5 years in Benin City in this study using RT-PCR is 19.2% (90 out of 470 were positive) and 13.8% (65 out of 470 were positive) using ELISA. This is low compared with other studies that have been conducted in this environment. Abiodun reported a prevalence rate of 59% in 1989 and 32.4% in 1994 in two hospital-based studies.[17,18] The low prevalence obtained in this current study could be due to year to year variation and the fact that samples were collected for a limited period of 1 year.

Very few studies have been carried out in Nigeria to detect distinct antigenic species. Avery reported 8/13 strains belonging to G1, while 5/13 (38.5%) were nontypeable.[19] Although the G1 type has been the predominant serotype in most studies, some unusual serotypes have recently been reported. Adah *et al*. had reported that besides the common G1, G2, and G3 types, an unusual G8 type was also frequently detected in 31/112 (27%) samples.[20]

A study of 150 children with diarrhea in Lagos, southern Nigeria confirmed detection of this unusual strain, identifying G8P (6) in 38% of 21 strains.[21] In a report by Pennap *et al*. from Zaria, northern Nigeria, a case-controlled study using VP7 primers, G1 and G2 were detected in 29% and 25%, respectively, while G3 and G4 were not detected.[22]

These virological studies from the different parts of Nigeria, especially from the north, middle-belt and south-western Nigeria, though few, confirm the predominance of the G1 strain using VP7. However, the studies also confirm a high rate of mixed infection, an unexpected diversity of rotavirus strains and unusual strains in the country.

Mixed infections have often been associated with malnutrition and prolonged diarrhea. A study of children in Benin City with protein-energy malnutrition showed that 37.5% had diarrhea caused by rotavirus, while a high prevalence of asymptomatic rotavirus infection was found among them.[18] It is quite conceivable that even an asymptomatic rotavirus infection of the malnourished infant could compromise intestinal food absorption and worsen the malnutrition. However, this study was unable to relate the multiple genotype infection with nutritional status and could be an area for further study.

The RT-PCR analysis appears to be more sensitive than ELISA in detecting rotavirus in stool, 90 in this study as against 65. However, the high cost of RT-PCR method may not make this readily available for routine diagnosis of rotavirus in our laboratory.

Reverse transcriptase polymerase chain reaction analysis using primers for VP7 shows the regular genotypes of G1–G4 and a few of G9, which are not so different from what has been reported in previous studies in other parts of Nigeria.[21] Primers for VP4 could not be assessed for P-genotyping in this study. These common VP7 genotypes are already taken care of in most available rotavirus vaccines. The unusual G9 type also needs to be covered, and further characterization with more primers is required in this regard. The genetic relatedness among various genotypes has been studied, using radio-labeled single-stranded RNA transcripts, which are

hybridized to the dsRNA of other rotavirus. Throughout the world, four rotavirus strains have been described: G1P[8], G2[P4], G3P[8] and G4[P] 8, and are frequently recovered from children with diarrhea.[22,23] There is a need for regular strain surveillance to determine the effect of vaccine pressure on the circulating strains, so as to detect any strain replacement.

Conclusion

Rotavirus remains an important cause of acute diarrhea in under five children in Benin City, Nigeria. The prevalent genotypes are the usual G1, G2, G3, and G4, with few unusual G9 strains. Mixed infections were also demonstrated. Molecular tools such as RT-PCR are indispensible in the study of this important pathogen. There is a need to introduce rotavirus vaccine, as part of a routine immunization in Nigeria so as to protect children aged <5 years from rotavirus induced diarrhea. The prevalent genotypes should be taken into consideration in developing rotavirus vaccine for this environment.

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