INTRODUCTION

Group A rotavirus is the most important aetiologic agent of severe gastroenteritis in children, aged less than five years, worldwide (1). Incidence of morbidity due to rotavirus-associated disease in young children is similar in developed and developing countries (2,3). The disease is more often severe and fatal in children in developing countries (4). Hence, a rotavirus vaccine would be useful in both developed and particularly developing countries for the prevention of severe disease in young children and reduction in treatment costs (5).

Results of field trials on candidate rotavirus vaccines suggest the need to study the epidemiology of rotavirus strains to formulate an effective rotavirus vaccine, because the immunity induced by candidate rotavirus vaccines is serotype-specific (6). Although several studies have documented that G1-4 are the predominant serotypes causing human disease worldwide (7), G9 serotypes have been rapidly emerging over the past few years in many locations globally (8). These five VP7 (G1-4 and G9) serotypes are incorporated into most...
vaccines currently being formulated. These were already tested in Europe and the USA (5).

Surveys of VP4 genotypes in several countries found that P[8] genotype was the most predominant P type in circulation (7). P[8] was associated almost always with either G1, G3, or G4, and P[4] was almost always associated with G2 (6). In developing countries, unusual strains have been reported and were most prevalent in some locations, such as Brazil, where G5 serotype was most prevalent (9). In India also, unusual combinations and rare human strains predominate (8).

The Fifth Rotavirus Vaccine Workshop, held at the Centers for Disease Control and Prevention (CDC) in 1995, recommended gathering and sharing data on the incidence, seasonality, and identification of serotypes of circulating rotaviruses (10), aiming at identifying critical needs for a vaccine for these diverse environments.

Serotype epidemiology of rotavirus infection has been reported in limited studies in Nigeria. In one study in Ibadan, only G1 strains were found to be circulating by monoclonal antibody enzyme immunoassay (EIA) for rotavirus serotype (11,12). In a second study, which included specimens from the north and south regions of the country, only strains with VP7 genotype G3 or G1 or G1/G3 [mosaic] viruses were detected by a PCR-based assay (13). In addition, VP4 genotype was determined in rotavirus-positive specimens (14). Surprisingly, the most common VP4 genotype was reported to be P[6], followed by P[8] strains, which are globally more prevalent. In a study conducted in Zaria, northern Nigeria, human rotaviruses with G1 and with G3 of VP7 serotype-specificity circulated at similar levels, and ‘mosaic’ viruses, which reacted with both G1 and G3 of VP7 monoclonal antibodies, were detected (15).

This study was undertaken to characterize VP7 and VP4 types of Nigerian rotavirus strains in Lagos because of its dynamic nature to determine the most prevalent strains.

This study aimed at extending the knowledge of VP7 serotype epidemiology for Nigeria and encompassed a study in Lagos, Nigeria. The purpose was to know whether the currently-licensed vaccines and those on clinical trials could protect our children, since they may protect less against unusual strains circulating in any country.

MATERIALS AND METHODS

Selection of patients and screening of rotavirus

Faecal samples were collected from 287 children, aged less than five years, with acute diarrhoea attending the Gbaja Health Centre of Massey Street Children Hospital (n=106) and the Lagos University Teaching Hospital (n=181), Lagos State, Nigeria, during January 1996-December 1997. All children were seen initially as outpatients, and stool specimens were collected within 24 hours of presentation to the hospitals.

Ten percent suspensions of the sample in phosphate-buffered saline were tested for the presence of group A rotavirus antigen using a commercial enzyme immunoassay (Rotavirus IDEIA™, Dako, UK).

Polyacrylamide gel electrophoresis of viral RNA

Distribution and diversity of RNA electropherotypes were examined for 84 of 101 EIA rotavirus-positive samples, for which sufficient sample was available. Standard RNA extraction was performed using phenol-chloroform treatment and ethanol precipitation (16). Silver staining was used for identifying double-stranded RNA segments (16,17).

Depending on different RNA electrophoretic mobility patterns seen and results of VP7 serotype by ELISA, selected specimens were used for genotyping of VP4. These specimens included one or two strain(s) per RNA electropherotype (some RNA electropherotypes only occurred in one specimen) and included nine specimens which had an RNA pattern but were not typed by either VP7 method employed.

VP6 monoclonal antibody assays

VP6 subgroup-specificity of rotavirus strains was determined using VP6 monoclonal antibodies developed by Greenberg et al. (18). These monoclonal antibodies against subgroup I rotaviruses (255/60) and subgroup II rotaviruses (631/9) have been extensively used in studies worldwide. The methods for their use have been described elsewhere (18).

VP7 serotyping EIA for human rotavirus

Two panels of VP7 serotype-specific monoclonal antibodies were used in the survey. VP7 serotype-specific monoclonals specific for rotavirus serotype G1 (monoclonal KU-4), G2 (S2-G10), G3 (YO-1E2), and G4 (ST-2G7) were donated by Dr. Koki Taniguchi, Sapporo Medical College, Japan, 1987. In addition,
monoclonal antibodies specific for serotype G1 (5E8), G2 (1C10), and G3 (159) were supplied by Dr. Dennis Lang, National Institutes of Health, Bethesda, MD, USA, and used as described elsewhere (19).

Identification of VP4 P and VP7 G genotypes by reverse transcription-polymerase chain reaction

Genomic VP4 types P[4], P[6], P[8], P[9], and P[10] were identified as described by Gentsch et al. (20). In total, 23 specimens were selected for typing based on variation of RNA electropherotype and VP7 serotype results available. Nine specimens were additionally selected from polyacrylamide gel electrophoresis (PAGE)-positive but ELISA-negative specimens for typing of VP4. Viral RNA was extracted by treatment with genetron and purified by RNAid (Bio101, California) before analysis using reverse transcription (RT) and PCR method with primers con2 and con3. The PCR products were then typed using a cocktail of primers (1T-1, 2T-1, 3T-1, 4T-1 and 5T-1 respectively) for different human VP4 genotypes (20).

VP7 genotypes were examined on all specimens, which were non-reactive in monoclonal EIA, using the RT-PCR typing method of Gouvea et al. (21). Purified RNA was reverse transcribed, and primers directed to terminal sequences were used for amplifying the entire gene (21). Cocktail of primers for typing assay included BT1, CT2, ET3, DT4, AT8, and FT9 for recognition and amplification of human VP7 serotypes G1-4, G8, and G9 respectively. These techniques have been described elsewhere, and similar conditions were used here (21).

RESULTS

Overall, 101 specimens (35%) were positive by enzyme immunoassay for rotavirus antigen. Of rotavirus-positive children, 83% were aged less than 12 months, and 6% were aged over two years. The rotavirus-positive cases were spread throughout the study period, indicating no seasonal distribution in this limited study. There was sufficient material available from 84 specimens for further characterization.

Polyacrylamide gel electrophoresis

Of the 84 rotavirus-positive samples for which there was sufficient material to examine the strains more thoroughly, all were examined by PAGE. There were eight variations of long RNA electropherotype occurring in 63 cases and three strains with two short patterns. Eighteen strains yielded no discernible RNA profile by PAGE.

Specificity of VP6 subgroup

Subgroup II viruses were detected far more commonly than subgroup I strains; 43 strains (51.0%) vs 3 strains (3.6%). Eleven strains reacted with group A-specific monoclonal antibody but were non-reactive with subgroup-specific monoclonals. Twenty-seven viruses had such low levels of antigen that there was no reactivity with any of the three monoclonal antibodies used.

Distribution of VP7 serotypes

Of the 84 samples screened for VP7 serotype by monoclonal antibody assay, G1 strains were the most predominant type occurring in 30 cases (35.7%), circulating in Lagos State during the study. Additional eight strains were typed as G1 by RT-PCR (Table 1). Serotype G3 viruses were shown in three cases (3.6%), and one extra was detected by the PCR method. G2 and G4 serotype viruses were not at all detected.

Viruses with mixed reactivity in ELISA were identified in 11 cases. Two of these viruses reacted with G1 and G2-specific monoclonal antibodies and are

<table>
<thead>
<tr>
<th>VP7 serotype</th>
<th>No. (%) detected by mab ELISA (n=84)</th>
<th>No. detected by RT-PCR* (n=40)</th>
<th>No. (%) detected by both methods (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>30 (35.7)</td>
<td>8</td>
<td>38 (45.2)</td>
</tr>
<tr>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G3</td>
<td>3 (3.6)</td>
<td>1</td>
<td>4 (4.8)</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed specificity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1 and G2</td>
<td>2 (2.4)</td>
<td>2</td>
<td>2 (2.4)</td>
</tr>
<tr>
<td>G1 and G3</td>
<td>4 (4.8)</td>
<td>6</td>
<td>6 (7.1)</td>
</tr>
<tr>
<td>All four G types</td>
<td>5 (6.0)</td>
<td>5</td>
<td>5 (6.0)</td>
</tr>
<tr>
<td>Not typed</td>
<td>40 (47.6)</td>
<td>29</td>
<td>29 (34.5)</td>
</tr>
</tbody>
</table>

* Only 40 specimens non-reactive to ELISA were analyzed by RT-PCR
reported for the first time in Nigeria. Four additional specimens reacted with G1 and G3 monoclonals. Five specimens contained a non-specific factor that reacted with all monoclonal antibodies to some degree. In addition, two more G1/G3 strains were detected by PCR genotyping (Table 1).

Many of 40 samples, non-reactive to monoclonal antibody ELISA, were negative by PAGE (n=18) and by VP6 subgroup assay (n=27), and probably reflect low levels of virus in stool. All the 40 strains were analyzed by the RT-PCR genotyping method for VP7 gene. No first-round product was observed in 25 cases. Additional eight G1 types, one G3 strain, and two G1/G3 strains were identified by PCR genotyping.

Identification of VP4 genotypes by PCR

The commonest VP4 genotype was P[6], detected in seven (30%) of the 23 selected specimens, and P[8] was found in six (26%) of the 23 specimens. A single P[4] genotype was identified. There was a relatively high rate of mixed genotypes with P[6+8] occurring in additional seven specimens (30%). Two samples could not be typed.

Table 2 shows the distribution of VP4 genotypes (P) and VP7 serotypes (G) identified in the same specimen. There was a higher rate of combinations of mixed VP4 genotypes and VP7 serotypes than recognized specific combinations, i.e. G1P[8].

### Table 2. Combinations of rotavirus G and P types in children from Lagos

<table>
<thead>
<tr>
<th>Type</th>
<th>G1 (n=6)</th>
<th>G3 (n=2)</th>
<th>G1+2 (n=2)</th>
<th>G1+3 (n=4)</th>
<th>Not typed (n=9)</th>
<th>Total (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[6]</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>P[8]</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>P[4]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P[6+8]</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Not typed</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In previous studies on epidemiology of rotavirus VP7 serotype, G1 strains were most commonly identified (22-25). Isolation of G1 strains has also been reported from Nigeria (11,12,15), although G3 strains were more prevalent in northern Nigeria (13). This suggests that there is a diversity of prevalence of strains in different parts of Nigeria. Interestingly, in none of these studies employing both monoclonal antibody-based ELISAs and RT-PCR genotyping, G2 or G4 strains have been identified.

In our study, G1 strains were commonly found, with G3 and mixed viruses also circulating. The common ‘mosaic’ rotavirus strain, which has been described previously in Nigeria and which was confirmed in this study, is a G1/G3 dual reactivity (13,15). These strains are real manifestations of a dual VP7 serotype specificity, as they have been shown to react antigenically with monoclonal antibodies (15) and genetically by PCR-based techniques (13). The G1/G2 ‘mosaic’ strain, which reacted with both G1 and G2 monoclonal antibodies, is reported for the first time in this study. In none of these cases, the RNA electrophoretic profiles observed determine a mixed infection with two strains.

G2 and G4 rotaviruses have not been detected previously in Nigeria, although these are amongst the four common serotypes worldwide (26). Inability to type 48% of rotavirus-positive specimens by ELISA in the present study may be due to the absence of epitopes recognizable by particular monoclonal antibodies used. This has been shown before (26,27), although the panel of rotavirus monoclonal antibodies used consisted of two G2 monoclonals. Only one G4 monoclonal was available. An alternative reason may be problems relating to storage and transport of stool specimens to Pretoria for typing and which might have resulted in deterioration of integrity of viral particles.

However, the PCR techniques should have been able to easily detect VP7 genotype of these strains non-reactive to ELISA, which was not the case in our study. Of the 40 samples, for which VP7 genotyping was performed, only 11 yielded a result. This may be either due to strain sequence variation as was shown previously in Nigeria (28), or due to presence of inhibitory factors in stool specimens (29).

In this study, we observed that some strains reacted unusually with all the serotype-specific monoclonal antibodies of four serotypes. The reason for this unusual
Diversity of rotavirus strains in Nigeria

The G1P[6] strain, which is usually regarded as an unusual combination, was the most predominant strain in this study, followed by G1P[8] and G3P[8] which are more commonly seen worldwide (7). G1P[6] strain, which was initially reported as a classical asymptomatic strain, was found in symptomatic children which is similar to the reports in India, South Africa, and Brazil (8,29,32). However, P[6] strains have been reported before in Nigeria (14). In this study, P[6] strains were the most commonly-detected VP4 genotype, although almost one-third were reported from ‘symptomatic’ neonates. This has also been reported previously in South Africa (33). The predominant strain seen by Adah and colleagues was a G3P[6] strain (14), whereas G1P[6] strains identified in the present study highlight the dynamic nature of rotavirus diversity in the field.

The P[4] genotype, which is usually associated with rotaviruses bearing VP7 serotype G2 specificity (29), had an untypeable G serotype which may support the notion that G2 monoclonal antibody used is not specific for these strains.

A large number of specimens in this region have multiple G and/or P types (14, and also found in this study). This was also reported by Timenesky et al. in Brazil (32). This is consistent with infections with more than one rotavirus strain. One potential explanation of the presence of multiple G and/or P types in specimens is either a high degree of viral reassortment during natural infection with two viruses, or mixed infections (13,32). It is important to analyze further by sequence analysis specimens with dual reactivity of VP7 and those specimens with apparently dual reactivity on VP4 gene to understand the natural diversity of rotaviruses in the developing world.

ACKNOWLEDGMENTS

This study was funded by the World Health Organization. In addition, the Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria, and the South African Medical Research Council, Tygerberg, granted support in part.

REFERENCES

14. Adah MI, Rohwedder A, Olaleye OD, Durojaie


