Molecular Characterization and Genotyping of Human Rotavirus Strains in Abidjan, Cote d’Ivoire

Veronique Akran, I. Peenze, C. Akoua-Koffi, H. Kotte, M. C. de Beer, M. Dosso, and A. D. Steele

Laboratoire de Bacteriologie/Virologie, Institut Pasteur, Abidjan, Cote d’Ivoire; Medical Research Council Diarrhoeal Pathogens Research Unit, University of Limpopo, Medunsa Campus, Pretoria, South Africa; Vaccines and Immunizations, PATH, Seattle, Washington.

In this study, we characterized human rotavirus strains recovered from infants and young children with acute diarrhea in Abidjan, Cote d’Ivoire, during 2000–2004. In total, 719 fecal specimens were collected from children aged 1–60 months with acute infantile gastroenteritis. Examination with a commercial enzyme-linked immunosorbent assay showed the presence of group A rotavirus antigen in 208 diarrheal specimens (28.9%). Polyacrylamide gel electrophoresis of the RNA extracted from rotavirus-positive stools yielded a variety of “long” and “short” RNA electropherotypes, which were used to help select strains for VP4 and VP7 genotyping. VP7 genotype G1 strains were circulating most commonly during the study period (53%), followed by G2 (22%) and G3 (5%) strains. Strains with multiple VP7 genotype reactivity were observed in 7.6% of specimens, and a similar number (8%) could not be typed at all. VP4 P[6] and P[8] strains circulated at similar levels (33%). Strains demonstrating multiple VP4 types were quite common (9%); however, 20% of the strains were untypeable by the methods used. Rotavirus strain diversity in Cote d’Ivoire was similar to that observed in other West African countries.

Rotaviruses are important agents of acute infantile gastroenteritis in infants worldwide [1]. In addition, rotaviruses have been reported to be associated with a high degree of morbidity and mortality in developing countries and are associated with more than half a million deaths per year in Africa and Asia [2]. Rotavirus is well described as an important etiological agent for severe dehydrating diarrhea in young children <1 year of age in West Africa [3–7].

In Cote d’Ivoire, the epidemiology of rotavirus infection has been reported in several small studies in Abidjan [8–13], and in the most recent studies was associated with 20%–28% of the diarrheal episodes investigated [8, 12, 13]. However, further characterization of the rotavirus strains was attempted in only 3 of these studies. In the first study, the VP6 subgroup specificity was determined using monoclonal antibodies, and subgroup II rotaviruses predominated [9]. In the second study, the RNA profile was determined in 17 of 33 rotavirus-positive specimens, and great strain diversity was observed, as represented by RNA electropherotype diversity [11]. In the third study, we reported rotavirus strain characterization using RNA polyacrylamide gel electrophoresis (PAGE), monoclonal antibody determination of the VP6 genotype, and genotypic characterization of the VP4 genotypes of human rotaviruses circulating in Abidjan, Cote d’Ivoire, between 1997 and 1999 [13]. The VP7 genotypes have not been reported elsewhere, and this study extends the observations made earlier.

Global rotavirus surveillance activities are ongoing.
to evaluate the burden of disease and epidemiology of the infection in developing countries [14], and in many countries the molecular characterization of the 2 important neutralizing antigens is performed by reverse-transcriptase polymerase chain reaction (RT-PCR) techniques. The VP7 types are considered important for vaccine development and have been the target of surveillance efforts to determine the circulating types globally [1].

In West Africa, the VP4 and/or VP7 types of rotavirus strains have been characterized only in a limited number of studies in a few countries, including Ghana, Guinea-Bissau, and Nigeria [15–19]. In an early study in The Gambia, VP7 serotype G1 and G2 viruses—detected by monoclonal antibodies—circulated at similar levels, and G3 strains were also identified in this small study [7]. In Ghana, the VP6 subgroup and VP7 serotype were also determined by monoclonal antibody enzyme immunoassay [15]. VP6 subgroup II strains predominated, and VP7 serotype G1 strains were most often detected, followed by G4 strains; G2 and G3 strains were present at lower levels.

Investigators in the other West African studies, using molecular techniques similar to those described in this study, have reported the wide and unusual diversity of rotavirus strains circulating in the region. In Nigeria and Guinea-Bissau, for instance, G1 and G3 strains were detected by similar RT-PCR methods for the VP7 genotype determination [16–18], and circulation of so-called “mosaic” G1/G3 viruses was observed. Furthermore, many “untypeable” VP7 and unusual rotavirus strains are observed commonly in this region [16–20]. The VP4 P[6] genotype has been reported to be widely circulating in West Africa [16–19] and, although unusual in other regions, appears to occur commonly in virulent strains associated with disease in young children [20].

Currently, rotavirus vaccine strategies are based on the need for a polyvalent vaccine candidate encompassing the epidemiologically important group A rotavirus VP7 serotypes [21]. At present, this has included the 4 most commonly occurring VP7 rotavirus serotypes (G1–G4), which are considered to be critical for inclusion in a vaccine candidate. Both currently licensed international vaccines also contain the most prevalent VP4 type found in human rotaviruses (ie, P[8]). A recent review highlighted the development of these 2 rotavirus vaccines and their antigenic composition [21]. Both vaccines are being used in other regions and have demonstrated good safety, efficacy, and effectiveness.Neither rotavirus vaccine is being used in Africa currently, although both have been evaluated in phase III efficacy trials in 5 African countries.

Little is known about the distribution and diversity of the rotavirus VP7 genotypes and VP4 genotypes in West Africa, and there have been no reports of the VP7 types in Cote d’Ivoire. In this study, we investigated the VP4 and VP7 genotype of human rotaviruses in Abidjan, Cote d’Ivoire.

**MATERIALS AND METHODS**

**Patients.** Single diarrheal specimens were collected from infants and young children in the Yopougan district in Abidjan, Cote d’Ivoire, during the period February 2000 to December 2004 (n = 719). The specimens were collected from infants and young children aged 1–60 months, who presented with acute diarrheal illness to the local hospital. Rotavirus detection was performed on 10%–20% suspensions of the fecal specimens, using a commercially available enzyme-linked immunosorbent assay (ELISA) (Rotavirus Pathfinder; Kallestad). The test was performed as specified by the manufacturer, at the Institut Pasteur Laboratories in Abidjan.

The stool specimens were frozen at −20°C at the Laboratoire de Bacteriologie/Virologie, Institut Pasteur, Abidjan, and then transported at 2°C–8°C to the Medical Research Council Diarrhoeal Pathogens Research Unit, University of Limpopo, Pretoria, at different times. Specimens were typed by the lead author (V.A.) using reagents and techniques available in South Africa.

**PAGE analysis.** All fecal specimens were analyzed by PAGE to identify the presence of rotavirus double-stranded RNA (dsRNA) in the stool specimens. In brief, the stool specimens were prepared as 10%–20% fecal suspensions in phosphate-buffered solution in the ELISA kit. Once in South Africa, the fecal samples were vortexed; a working volume was stored at 2°C–8°C while the laboratory evaluation was performed, and the remainder was stored at −20°C. Initially, 450 µL of fecal sample was mixed with an equal volume of 1 mol/L sodium acetate (pH 5) and 1% sodium dodecyl sulfate to disrupt the proteinaceous material in the fecal specimen. This was followed by incubation with an equal volume of phenol-chloroform to deproteinize the viral particles and release the dsRNA. The mixture was centrifuged, the aqueous phase containing the dsRNA was transferred to a clean Eppendorf tube, and the dsRNA was precipitated in 3 volumes of absolute ethanol overnight at −20°C. After centrifugation at 12,000 g for 10 min, the pellet was resuspended in Tris–ethylenediaminetetraacetic acid buffer for the PAGE [22].

Standard PAGE for rotavirus was performed, as described from this laboratory elsewhere [22]. In brief, electrophoresis of the extracted dsRNA was performed in 10% polyacrylamide slab gels using the discontinuous buffer system. A 5% stacking gel was used to enhance the resolution of the RNA segments. Approximately 20 µL of each sample was loaded onto the gels, and electrophoresis was conducted at 100 V for 16–18 h at room temperature. The gels were stained using a modification of the silver staining method. The PAGE profiles were used to identify the number of strains circulating and to select the samples for typing.

**RT-PCR determination of VP7 genotypes.** A total of 157 rotavirus-positive specimens were selected for further charac-
terization using the RT-PCR method described by Gouvea et al. [23]. All rotavirus-positive specimens from 2000 and 2002 were included in the analysis for genotyping; for the remaining years, however, ~60% of the specimens were selected for characterization. This was based on the RNA electropherotype observed, to obtain a selection of strains, and the availability of the stool material (Table 1).

The RNA of selected rotavirus-positive strains was extracted using TRIzol (Life Technologies), according to the manufacturers’ instructions. The RNA extracted with TRIzol was resuspended in 15 μL of RNase-free water. The G-specific primers used in this analysis (G1–G4, G8, and G9) have been described by Gouvea et al. [23], Das et al. [24], and Cunliffe et al. [25]. Briefly, the VP7 gene was reverse transcribed and amplifed using plus-sense primer sBeg9 (nucleotides 1–21, 5′-GGCTTT-AAAAGAGAGAATTTC-3′) and minus-sense primer End9 (nucleotides 1062–1036, 5′-GGTCACATCATACAATTCTAATCTCTAAAG-3′), followed by G genotyping using a cocktail of primers specific to the 6 human rotavirus serotypes (G1–G4, G8, and G9) [23]. Confirmation of the G type results was conducted using additional primer sets described elsewhere [24, 25].

**Determination of VP4 genotypes.** The VP4 genotypes were analyzed in the same 157 specimens using the RT-PCR method and VP4-specific primers (P[4], P[6], P[8], P[9], and P[10]), described by Gentsch et al. [26] and Iturriza-Gómarra et al. [27]. In brief, the VP4 gene was reverse transcribed and amplified using plus-sense primer sBeg9 (nucleotides 1–21, 5′-GGCTTT-AAAAGAGAGAATTTC-3′) and minus-sense primer End9 (nucleotides 1062–1036, 5′-GGTCACATCATACAATTCTAATCTCTAAAG-3′), followed by G genotyping using a cocktail of primers specific to the 6 human rotavirus serotypes (G1–G4, G8, and G9) [23]. Confirmation of the G type results was conducted using additional primer sets described elsewhere [24, 25].

**RESULTS**

**Rotavirus-positive specimens.** Of the 719 diarrheal specimens collected from infants and young children in Abidjan, 208 (28.9%) were positive for rotavirus antigen as determined by the Pathfinder ELISA (Table 1). All of the rotavirus-positive children were <2 years of age, and the majority (162 [77.9%]) were <12 months of age.

The duration of this study period, coupled with our observations in previous years, allowed an examination of the monthly variation in rotavirus infection. The peak occurrence of rotavirus infection in young children presenting to the hospital was in the cooler and drier months between October and February (data not shown).

**PAGE results.** PAGE was performed on all the rotavirus-positive specimens to examine the genomic diversity of the dsRNA of the strains. In total, 82% of the rotavirus specimens yielded an RNA electropherotype by PAGE, with a variety of both long and short RNA profiles.

**VP7 genotype analysis.** The details of the VP7 genotype analysis are presented in Table 2. G1 strains predominated during the course of the surveillance (52.9% of isolates), followed by G2 strain (22.3%). Small numbers of G3, G8, and G9 strains circulated, and no G4 strains were identified. Twelve strains were observed to be reactive with 2 of the primers, yielding dual VP7 genotype reactivity; in the first 3 years of the study period, the most common dual type was G1/G3, which has been reported elsewhere from this region. Just over 8% of the strains could not be typed with the multiple primer sets used, despite the fact that the dsRNA was observed by PAGE.

**VP4 genotype studies.** The VP4 genotype analysis is shown in Table 3. Interestingly, strains bearing the VP4 P[8] and P[6] genotype circulated in similar numbers (33.1%), and 13 additional strains had a dual reactivity for P[6] and P[8], indicating the high circulation of this VP4 genotype in the community. Almost 20% of the strains (31) could not be characterized for the VP4 genotype.

**Strain characterization of VP4 and VP7 strains.** The G1 strains were identified in combination with the VP4 P[8] (61%) and P[6] (24%). Surprisingly, the G2 strains were seen almost exclusively in combination with the VP4 P[6] allele (83% of G2 strains); the remainder carried the classic P[4]. The few G3 and G8 strains were seen in combinations of G3P[8], G8P[4], and G8P[6]. One G3P[4] strain was identified, and a single G3P[9] strain was also observed. The most commonly identified strains were G1P[8]; G2P[6] and G1P[6] strains were identified at similar frequency.

**DISCUSSION**

In this study, we report the identification and molecular characterization of group A rotaviruses in Abidjan, Cote d’Ivoire, West Africa. First, rotaviruses were detected in the diarrheal stools of 28.9% of young children presenting to the hospital with diarrheal disease during a 4-year period, representing the longest-term surveillance for rotavirus in this country. Although the prevalence of rotavirus infection varied during the 4-year period, from a low of 24% in 2000 to a high of 34% in 2002, this proportion of diarrheal hospitalized patients is similar to the range of 22%–28% reported elsewhere in smaller studies in Cote d’Ivoire [8–13]. The prevalence of rotavirus

<table>
<thead>
<tr>
<th>Year</th>
<th>Overall No. (%) of samples</th>
<th>Positive for rotavirus Children with diarrhea</th>
<th>Children with rotavirus Patient age in months, range or median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>130 (40.1)</td>
<td>32 (24.6)</td>
<td>1–36</td>
</tr>
<tr>
<td>2001</td>
<td>340 (26.7)</td>
<td>101 (29.7)</td>
<td>1–64</td>
</tr>
<tr>
<td>2002</td>
<td>76 (26)</td>
<td>26 (34.2)</td>
<td>1–29</td>
</tr>
<tr>
<td>2004</td>
<td>173 (49.3)</td>
<td>49 (28.3)</td>
<td>1–21</td>
</tr>
</tbody>
</table>
Table 2. VP7 Genotypes of Rotavirus Strains Recovered from Young Children in Abidjan, Cote d’Ivoire, 1999–2004

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G8</th>
<th>G9</th>
<th>Mixed</th>
<th>UTa</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1 (G1/G3)</td>
<td>3</td>
</tr>
<tr>
<td>2001</td>
<td>63</td>
<td>33</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7 (3 G1/G3, 3 G1/G2, 1 G1/G9)</td>
<td>7</td>
</tr>
<tr>
<td>2002</td>
<td>26</td>
<td>13</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1 (G1/G3)</td>
<td>3</td>
</tr>
<tr>
<td>2004</td>
<td>36</td>
<td>21</td>
<td>6</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3 (2 G1/G8, 1 G1/G9)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>83</td>
<td>35</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>12 (7.6)</td>
<td>13 (8.3)</td>
</tr>
</tbody>
</table>

a UT, untypeable with the primers used.

Table 3. VP4 Genotypes of Rotavirus Strains Recovered from Young Children in Abidjan, Cote d’Ivoire, 1999–2004

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>32</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>...</td>
<td>10</td>
</tr>
<tr>
<td>2001</td>
<td>63</td>
<td>12</td>
<td>27</td>
<td>...</td>
<td>...</td>
<td>11 (10 P[8]/P[6], 1 P[8]/P[9])</td>
<td>13</td>
</tr>
<tr>
<td>2002</td>
<td>26</td>
<td>6</td>
<td>11</td>
<td>2</td>
<td>...</td>
<td>...</td>
<td>7</td>
</tr>
<tr>
<td>2004</td>
<td>36b</td>
<td>17</td>
<td>12</td>
<td>2</td>
<td>...</td>
<td>3 (P[8]/P[6])</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>157</td>
<td>52 (33.1)</td>
<td>52 (33.1)</td>
<td>6 (3.8)</td>
<td>1 (0.6)</td>
<td>14 (8.9)</td>
<td>31 (19.7)</td>
</tr>
</tbody>
</table>

a UT, untypeable with the primers used.
b One sample was not analyzed owing to lack of sufficient material.

infection in this study is also similar to that reported in other countries in West Africa, such as Ghana [3] and Nigeria [4, 6]. In these reports, rotavirus infection was associated with acute infantile gastroenteritis in 14%–28% of cases.

Second, this study confirms that rotavirus infection in this region occurs in children <2 years of age, and predominantly in infants <12 months of age [8–10]. This also reflects the situation observed in sub-Saharan countries [3–7, 28]. Third, the duration of this study, coupled with our earlier observations of rotavirus infection [13], enabled an observation of the seasonality of rotavirus infection, which indicated that these infections peak in November to February during the cooler, drier months, similar to the seasonality observed in Ghana and Nigeria [3, 4]. All previous studies in Cote d’Ivoire were conducted within a single year or during a short period of a few months.

Studies on the distribution and epidemiology of the human rotavirus VP7 types in West Africa are limited in number, although some specific countries have generated extensive data. In Ghana and Nigeria, for instance, rotavirus strains have been characterized over several years. In both these countries, the diversity of rotavirus VP7 types is well documented, with the common human VP7 types (G1–G4) identified but a multitude of other “unusual” and “untypeable” strains observed [16, 18, 19]. In this study, we identified the widespread circulation of G1 and G2 strains, with limited circulation of G3, G8, and G9 types. Common themes in these 2 neighboring countries are the diversity and changing prevalence of rotavirus VP7 types, so the lack of G4 strains and the limited numbers of G3 strains in Cote d’Ivoire mirror what has happened in Nigeria and Ghana during the last decade, where a decline in G3 and G4 strains was observed [18, 19]. “Uncommon” rotavirus strains with the G8 and G9 genotype are widely dispersed in these 2 countries and in Guinea-Bissau, where a long-term surveillance study was conducted [5, 17]. The numbers of these strains seen in this study are too small to support any conclusions, except that they are circulating in the community.

Interestingly, the “mosaic” rotavirus strains, which show dual reactivity with >1 set of primers, were also observed in this study, and the most common was the G1/G3 type seen previously in Nigeria [29]. This dual reactivity is unlikely to be merely “antigenic drift” of the VP7 gene but rather probably represents a true genetic and antigenic dual reactivity, as identified in previous studies in Nigeria [29]. In addition, molecular evolution of the viral strains, with added complexity for the priming site on the RNA strand and the emergence of novel G8 and G9 strains, has been reported in Nigeria [30] and Guinea-Bissau [31], highlighting the evolving and diverse nature of rotavirus strains in the region. Finally, almost 10% of the strains could not be typed even with use of a series of primers; however, this proportion is better than that observed in other African studies, where >20% of strains could not be typed [20].

The VP4 genotypes of the strains recovered in Cote d’Ivoire also reflects what has been seen in other sub-Saharan African
studies. The VP4 P[6] genotype occurs commonly in many African countries [16,20] and occurs at higher rates here than in other developing countries. In this study there was an equal distribution of the P[8] and P[6] gene alleles in the circulating strains. This P[6] genotype was not restricted to G1 strains, as has been described elsewhere [20]. G2P[6] was the second most commonly seen strain in Cote d’Ivoire during the 4 years of the study; it has been reported before, but at much lower levels [16, 32]. Significantly, almost 20% of strains could not be typed at the VP4 level, which may mean that other “novel” VP4 types are occurring. Given the range of primers for human rotaviruses used in this study, these untypeable strains could represent animal rotavirus VP4 alleles, although this would need to be established with further laboratory analysis.

Further studies to determine the VP7 type of circulating strains of rotaviruses are needed in developing countries for a number of reasons. The recent development of effective rotavirus vaccines and their recommendation for use by the World Health Organization will benefit from ongoing strain surveillance. It is important to monitor the strains as vaccines are rolled out to determine whether there is vaccine pressure on circulating strains and whether there is any strain replacement. This study is the first to report the VP7 genotypes of circulating rotavirus strains in Abidjan, Cote d’Ivoire, and it adds to the growing body of evidence of rotavirus strain diversity in Africa.

References