Full Length Research Paper

Variability in the genotypes of rotavirus detected in Côte d'Ivoire from 2010-2016

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**Group A rotaviruses** are the major viral agent of acute gastroenteritis and severe diarrhea in children <5 years old. The World Health Organization (WHO) recommends surveillance of circulating strains before and after introduction of vaccination in countries. However, the diversity of circulating strains in developing countries is a major challenge to the vaccination programs. This study, carried out in furtherance of the sentinel surveillance, aims to identify the different genotypes circulating before the introduction of the Rotavirus vaccine. All children with acute gastroenteritis aged 0 to 5 years, admitted in one of the sentinel surveillance collection sites were included in the study. The study period was from January 2010 to December 2016. Rotavirus was detected in stool specimens by enzyme-linked immunosorbent assay (ELISA). Rotavirus G and P types were determined by real-time polymerase chain reaction (RT-PCR). A total of 1472 stool samples were collected during this period. 31.8% of the stools were rotavirus positive by ELISA test. G1 was predominant with 39.6% followed by G12 (27%). P [8] was 50.4%. The predominant genotype combinations were G1P [8] with 26.1%; G12P [8], 15%; G1P [6], 11.3% and G12P [6], 10.8%. Genotyping of circulating rotavirus strains is important in monitoring strains before and after the introduction of the vaccine. With previous observations, these findings will contribute to baseline data to further monitor the impact of rotavirus immunization in Côte d'Ivoire.

**Key words:** Rotavirus, Côte d'Ivoire, diarrhea, vaccination, acute gastroenteritis.

**INTRODUCTION**

Group A rotaviruses are the major viral agent of acute gastroenteritis and severe diarrhea in children <5 years old (Gasparinho et al., 2017). Globally, this virus is responsible for about 40% of cases of severe diarrhea with hospitalization and 5% of deaths in children under five years (Eteme et al., 2015). The number of

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deaths is estimated to be 215,000/year in children <5 years with 80% cases in sub-Saharan Africa and South Asia (Tate et al., 2016). The envelope of rotavirus is made of VP7 and VP4 proteins which form the outer part of the capsid (15 genotypes G and 27 genotypes P) (Lorrot et al., 2012). The circulating genotypes of rotavirus are of different types all over the world; several studies in Africa have identified the G1P [8] genotype as the most common in pre-vaccination areas. The G1P [6], G8P [6], G6P [6], G8P [8], G12P [6] and mixed G and mixed P genotypes are also found in a lower rate in sub-Saharan Africa (Hokoro et al., 2014). The World Health Organization (WHO) recommends surveillance of circulating strains before and after introduction of vaccination programs in various countries (Damanka et al., 2016). However, the diversity of circulating strains in developing countries proves a real challenge to the vaccination programs (Todd et al. 2010). Currently, WHO recommends two types of oral vaccines: the Rotarix® vaccine (GlaxoSmithKline, Rixensart, Belgium) and Rotatetq® vaccine (Merck & Co., Whitehouse Station, NJ). Rotatetq® vaccine contains (RV5) five human-bovine reassortant viruses [W179-9 (G1P [5], SC-2 (G2P [5]), W178-8 (G3P [5]), Br-B-9 (G4P [5]) and W179-4 (G6P [8])]. Rotarix® vaccine (RV1) is a human G1P [8] virus RIX4414 derived from a serial passage in the cell culture of a virus recovered from the stool of an infected child (Agutu et al., 2017). Both recommended vaccines require multiple dose administration (two doses for Rotarix and three for RotaTeq); the first to be administered between 6 and 15 weeks of age to raise homo- and heterotypic immune response against RVA different strains. The two vaccines have been proven to be effective worldwide, but lower efficacy was observed in low-income countries from Africa and Southern Asia. Among the several hypotheses used to explain the differences in the immune response and consequent efficacy of these vaccines in low- versus high-income countries, RVA strains diversity, host genetic factors, malnutrition, host co-infection, deficient micronutrient ingestion, and interfering gut flora have been put forward (Gasparinho et al., 2017). Studies conducted in Côte d’Ivoire prior to the sentinel surveillance which started in 2010 showed a predominance of G1 followed by G2. A small number of G3, G8 and G9 variants were identified in this study (Akran et al., 2010). In view of the wide variety of circulating strains, it is therefore important to maintain continuous monitoring of the prevalence of rotavirus in order to understand the distribution of G and P genotypes in the country. Accurate information in respect of different types of circulating genotypes of rotavirus is essential to monitor the impact and effectiveness of the vaccine.

This study aimed to identify the different genotypes circulating in the country before the introduction of the Rotavirus vaccine. It proposes to describe the proportion of the different genotypes G and P circulating from 2010 to 2016 in the pre-vaccination area. Genotypic combinations were also determined.

MATERIALS AND METHODS

Study population

Children with acute gastroenteritis aged 0 to 5 years, admitted in one of the sentinel surveillance sites were recruited. There are six collection sites altogether in five municipal localities in the city of Abidjan, the commercial capital of Côte d’Ivoire. Children either hospitalized or kept under observation for treatment of acute diarrhea (less than two weeks) were included in the study with stools that had no mucus or blood associated with fever from January 2010 to December 2016.

Stool samples

Stools samples were collected from each child after obtaining informed consent from the parents, in a sterile jar on the same day or the next day of admission. Participants were request to complete some forms containing details of the child’s socio-demographics and clinical information. The samples were kept in refrigerator at a temperature of between 0 and 4°C, and sent to the laboratory at the Yopougon University Teaching Hospital, Bacteriology-Virology unit, where they were stored at 4°C for a maximum of 30 days until ELISA test was performed. ELISA positive samples were then stored at -20°C before being taken to one of the WHO rotavirus reference laboratories, namely the Limpopo Regional Laboratory in South Africa or the West Africa Regional Laboratory in Ghana for genotyping.

Laboratory analysis

Detection of group A Rotavirus antigens

Samples were screened for the presence of rotavirus structural protein VP6 by the use of Rotaclone® a rapid EIA test kit following the manufacturers’ instructions. Samples with optical density >0.25 at 450 nm wavelength were considered positive.

Molecular characterization of Rotavirus strains

G- and P-genotyping assays

To determine the VP7 (G-) and VP4 (P-) genotypes, viral RNAs was extracted from the clarified supernatant of 20% stool suspensions using the QIAamp® Viral RNA Mini kit (QIAGEN®, Hilden, Germany) based on the manufacturer’s instructions. Reverse transcription (RT)-PCR was performed using both forward and reverse consensus primers Beg9/End9 and Con3/Con2 to amplify a 1,069bp and 835bp fragments of the VP7 and VP4 genes respectively. Multiplex PCR was carried out for G- and P-typing with genotype specific primers as previously described (Gouvea, 1990; Gentsch, 1992; Iturriza-Gomara, 2004). PCR amplicons were electrophoresed on a 2% agarose gel in Trisborate- EDTA buffer together with a 100-bp DNA ladder.

Data analysis

All statistical analysis was performed with the EPI-Info version 3.5.4 software (CDC Atlanta, USA). All categorical variables were summarized as proportions, and significance of their difference in distribution with the outcome was assessed using Pearson’s Chi-square and Fisher test at 5% risk.
Table 1. Sex distribution and ELISA test results, 2010-2016.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2010 N (%)</th>
<th>2011 N (%)</th>
<th>2012 N (%)</th>
<th>2013 N (%)</th>
<th>2014 N (%)</th>
<th>2015 N (%)</th>
<th>2016 N (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>41 (43.2)</td>
<td>26 (50)</td>
<td>83 (36.4)</td>
<td>131 (41.8)</td>
<td>89 (40.1)</td>
<td>153 (47)</td>
<td>104 (43.9)</td>
<td>627 (42.6)</td>
</tr>
<tr>
<td>Male</td>
<td>54 (56.8)</td>
<td>26 (50)</td>
<td>145 (63.6)</td>
<td>182 (58.2)</td>
<td>133 (59.9)</td>
<td>172 (53)</td>
<td>133 (56.1)</td>
<td>845 (57.4)</td>
</tr>
<tr>
<td>Total</td>
<td>95 (100)</td>
<td>52 (100)</td>
<td>228 (100)</td>
<td>313 (100)</td>
<td>222 (100)</td>
<td>325 (100)</td>
<td>237 (100)</td>
<td>1472 (100)</td>
</tr>
</tbody>
</table>

ELISA test result

<table>
<thead>
<tr>
<th></th>
<th>2010 N (%)</th>
<th>2011 N (%)</th>
<th>2012 N (%)</th>
<th>2013 N (%)</th>
<th>2014 N (%)</th>
<th>2015 N (%)</th>
<th>2016 N (%)</th>
<th>Total N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>95 (100)</td>
<td>52 (100)</td>
<td>228 (100)</td>
<td>313 (100)</td>
<td>222 (100)</td>
<td>325 (100)</td>
<td>237 (100)</td>
<td>1472 (100)</td>
</tr>
<tr>
<td>Negative</td>
<td>76 (80)</td>
<td>34 (65.4)</td>
<td>169 (74.1)</td>
<td>219 (70)</td>
<td>152 (68.4)</td>
<td>206 (63.4)</td>
<td>148 (62.4)</td>
<td>1004 (68.2)</td>
</tr>
<tr>
<td>Total</td>
<td>95 (100)</td>
<td>52 (100)</td>
<td>228 (100)</td>
<td>313 (100)</td>
<td>222 (100)</td>
<td>325 (100)</td>
<td>237 (100)</td>
<td>1472 (100)</td>
</tr>
</tbody>
</table>

Table 2. Rotavirus strain distribution between the period January 2010 and December 2016.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>3</td>
<td>45</td>
<td>104</td>
<td>1</td>
<td>5</td>
<td>158 (39.6)</td>
</tr>
<tr>
<td>G2</td>
<td>24</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>31 (7.8)</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20 (5)</td>
</tr>
<tr>
<td>G6</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4 (1)</td>
</tr>
<tr>
<td>G8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>G9</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>13 (3.2)</td>
</tr>
<tr>
<td>G10</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7 (1.7)</td>
</tr>
<tr>
<td>G12</td>
<td>1</td>
<td>43</td>
<td>60</td>
<td>3</td>
<td>108</td>
<td>27 (27)</td>
</tr>
<tr>
<td>GMix</td>
<td>0</td>
<td>7</td>
<td>15</td>
<td>3</td>
<td>32</td>
<td>8 (8)</td>
</tr>
<tr>
<td>GNT</td>
<td>2</td>
<td>4</td>
<td>14</td>
<td>5</td>
<td>25</td>
<td>6.3 (25)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>34 (8.5)</td>
<td>126 (31.6)</td>
<td>201 (50.4)</td>
<td>15 (3.7)</td>
<td>23 (5.8)</td>
<td>399 (100)</td>
</tr>
</tbody>
</table>

GMix/P[Mix]: Multiple genotypes detected for either G, P or both; GNT/P[NT]: either G, P or both were nontypable

RESULTS

A total of 1472 <5 year old children were recruited in this study and 1472 stool samples were collected. Of the recruited children, the male sex was predominant about 57.4% (845/1472). The sex ratio was 1.36. ELISA positive stool specimens were 31.8% (468/1472) (Table 1). RT-PCR and genotyping were performed on 85.2% (399/468) of the positive samples. 93.7% (376/399) of the tested samples were positive to VP7 with 6.3% (25/399) non-typable strains (Table 2) and 94.2% (376/399) were positive to VP4 and 5.8% (23/399) non-typable strains (Table 2). Regarding the VP 7 genotype, the G1 was predominant with 39.6% (158/399) followed by G12 with 27% (108/399) and Mix G 8% (32/399) (Table 2). Concerning the VP4, the predominant genotype was P [8] which was found in 50.4% (108/399) followed by P [6] 31.6% (32/399) (Table 2). Throughout the study period, rotavirus genotype G12 was most prevalent in 2012, 2013 and 2014, except the year 2010 when G9 was the most predominant genotype (Figure 1a). Genotypes G1, G2 and G3 were detected throughout the study period at varying frequencies. On the other hand, there was no change in the predominant P-type as P [8] remained dominant over the study period (Figure 1b). The predominant genotype combinations were G1P [8] with 26.1% (104/399); G12P [8], 15% (60/399); G1P [6], 11.3% (45/399) and G12P [6], 10.8% (43/399). The genotypes G3P [6] and G9P [6] were found at lower rate with 4.7 (19/399) and 1.2% (5/399) (Table 2).

DISCUSSION

WHO recommends that all countries introduce rotavirus vaccines into their national expanded program on immunization. This study was conducted as part of the sentinel surveillance of rotavirus diarrhea prior to the introduction of the vaccine in Côte d'Ivoire. The <5 year old children are more vulnerable to severe gastroenteritis with more serious consequences probably due to the fact that after 5 years they develop immunity due to natural rotavirus infections (Steel et al., 2016).

The prevalence of rotavirus diarrhea in this age group
is high, particularly in the developing countries (Boula et al., 2014). Children under 12 months are the most affected by this virus (Todd et al., 2010). The peak of rotavirus gastroenteritis was found in some countries such as Kenya between 6 and 24 months (Agutu et al., 2017). In Cameroon, a prevalence of 44.7% was found in children under 24 months (Ndze et al., 2013). Hospitalization rates of 50.6% were observed in children aged 6-8 months in Ghana (Damaka et al., 2016). In this study, higher detection rates were observed than those found in previous studies in Côte d’Ivoire (Akoua-Koffi et al., 2007, 2014; Akran et al., 2010). The administration of the vaccine is therefore recommended in early infancy in sub-Saharan Africa as the infection is acquired early there than in Western countries (WHO, 2009). WHO recommends that the first dose of either RotaTeq or Rotarix be administered at age 6-15 weeks; the maximum age for administering the last dose of either vaccine should be 32 weeks (WHO, 2009). A predominance of male gender was observed in our study. This has also been observed in other studies (Selvarajan et al., 2017) but there was no statistical difference. Studies showed male susceptibility to rotavirus infection (Junaid et al., 2011). However, several studies have suggested the absence of gender-related occurrence (Saluja et al., 2014). Rotavirus is the primary cause of diarrhea in children under five years of age with a prevalence rate of 41% (Selvarajan et al., 2017) in area where vaccination is not yet introduced. The rate of 45% found in our study is similar to that found in other African countries before the introduction of the vaccine (Bwogi et al., 2016). However, in neighboring countries to Ivory Coast, where the vaccine has been introduced, rotavirus remains the leading viral cause of diarrhoea in children under five years of age, with high prevalence. In Burkina Faso, Ouedraogo found Rotavirus (63.5%), adenovirus (31.2%) and genogroup II norovirus (18.2%) in a study conducted (Ouedraogo et al., 2016). In Ghana, similar results have been found, but with lower prevalence rate of rotaviruses (27.9%), astroviruses (7.5%), noroviruses (6.8%) and adenoviruses (5.4%) (Akkufo et al., 2017). The outer layer protein, VP4 and VP7 of the group A rotavirus induce the production of neutralizing antibodies. The attachment protein VP 4 determines the type P. This has a more conservative specificity than type G determined by the glycoprotein VP 7. Ten G genotypes and 8 P genotypes have been detected in humans (Wylie et al., 2015). Two rotavirus vaccines are currently

Figure 1. Temporal rotavirus genotype distribution in Côte d’Ivoire. a). Rotavirus G-type distribution, January 2010 to December 2016; b). Rotavirus P-type distribution, January 2010 to December 2013. MIX: multiple genotypes detected for either G, P or both; NT: either G, P or both were nontypable.
licensed by WHO. RotaTeq® (RV5) (Merck & Co) consists of a mixture of 5 bovine viruses that contain VP7 and VP4 genes from human G1, G2, G3, and G4 and P viruses. Rotarix® (RV1) (GlaxoSmithKline Biologicals) consists of an attenuated virus derived from a human G1P [8] strain (Wylie et al., 2015). The two vaccines offer comparable protection against commonly circulating rotavirus serotypes G1-4 [30]. The P [4], P [6] and P [8] genotypes are the most frequently found throughout the world (Abdel-Haq et al., 2003). The VP4 genotypes found in this study are similar to those circulating in the years 2000 to 2008 in Côte d'Ivoire. The P [4], P [6], and P [8] genotypes have been identified with similar rates in previous studies (Damanka et al., 2016; Kirkwood et al., 2014) with a predominance of P (Boula et al., 2014).

In the sub region there is a correlation of circulating VP4 genotypes with similar rates (Damanka et al., 2016; Enweronu-Laryea et al., 2013). Concerning the G-genotype, the G1 prevalence rate found in our study correlates with the results observed in other countries in the West African sub-region, particularly in Ghana (43%) (Laryea, 2013) and 46% in Cameroon (Enweronu-Laryea et al., 2013; Etene et al., 2015). The G2 and G3 genotypes were found at rates >10% in the sub-region; in other African countries (Eteme et al., 2015; Damanka et al., 2016; Ngum Ndze et al., 2012) they were found at lower rates of 7.8% for G2 and 5% for G3, respectively. The G8 genotype present in the year 2000 in Côte d'Ivoire (Akoua-Koffi et al., 2007, 2014) virtually disappeared.

G12 genotype is a non-common strain with resurgence at 27%. This strain is not included in the target strains of the two vaccines recommended by WHO. Its emergence has been observed globally in several studies. In Thailand, Maneekarn et al. (2014) observed the predominance of the G12 from 2007 to 2009 only. In Australia, Kirkwood et al. (2014) reported incidence of G12P [8] (23%) in 2012, and Wylie (2014, 2015) reported an emergence of this strain in the same year in the Saint Louis, United States. This emergence was also observed in Africa, particularly in Cameroon in the same period (Ngum Ndze et al., 2012). Its significance is yet to be evaluated in the post vaccine era. Concerning genotypic combinations, the G1P [8], G12P [8], G1P [6] and G12P [6] genotypes are predominant in our study. The association of the genotype G12 with the different genotypes P suggests a high capacity of adaptation of this unusual strain. In this study, a lower rate was observed for non-tybable and mixed strain.

Conclusion

Continuous monitoring of circulating strains is important as vaccine pressure may lead to the emergence of new epidemic strains in the post vaccine era. The determination of the different genotypes of rotavirus strains before the introduction of vaccination is fundamental to better understand the mechanisms leading to the emergence of new strains. Countries are encouraged to monitor strains in circulation before and after the introduction of the vaccine to determine the impact of the vaccine on circulating strains which might potentially escape protection covered by the currently recommended vaccines.

CONFLICT OF INTERESTS

The authors declared that there is no conflict of interest.

ACKNOWLEDGMENTS

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