Rotavirus strain distribution in Ghana pre- and post- rotavirus vaccine introduction

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Abstract

Background: Ghana introduced the monovalent rotavirus vaccine (Rotarix) into its national paediatric vaccination programme in May 2012. Vaccine introduction was initiated nationwide and achieved >85% coverage within a few months. Rotavirus strain distribution pre- and post-RV vaccine introduction is reported.

Methods: Stool samples were collected from diarrhoeic children <5 years of age hospitalized between 2009 and 2016 at sentinel sites across Ghana and analyzed for the presence of group A rotavirus by enzyme immunoassay. Rotavirus strains were characterized by RT-PCR and sequencing.

Results: A total of 1363 rotavirus EIA-positive samples were subjected to molecular characterization. These were made up of 823 (60.4%) and 540 (39.6%) samples from the pre- and post-vaccine periods respectively. Rotavirus VP7 genotypes G1, G2 and G3, and VP4 genotypes P[6] and P[8] constituted more than 65% of circulating G and P types in the pre–vaccine period. The common strains detected were G1P[8] (20%), G3P[6] (9.2%) and G2P[6] (4.9%).

During the post-vaccine period, G12, G1 and G10 genotypes, constituted more than 65% of the VP7 genotypes whilst P[6] and P[8] made up more than 75% of the VP4 genotypes. The predominant circulating strains were G12P[8] (26%), G10P[6] (10%) G3P[6] (8.1%) and G1P[8] (8.0%). We also observed the emergence of the unusual rotavirus strain G9P[4] during this period.

Conclusion: Rotavirus G1P[8], the major strain in circulation during the pre-vaccination era, was replaced by G12P[8] as the most predominant strain after vaccine introduction. This strain replacement could be temporary and unrelated to vaccine introduction since an increase in G12 was observed in countries yet to introduce the rotavirus vaccine in West Africa. A continuous surveillance programme in the post-vaccine era is necessary for the monitoring of circulating rotavirus strains and the detection of unusual/emerging genotypes.

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1. Background

Diarrhoea is the fourth leading cause of childhood mortality worldwide, responsible for an estimated 550,000 deaths annually among children below the age of five years, representing 8.9% of all deaths within this age group [1]. Group A rotaviruses (RVAs) are the most important etiologic agent of acute gastroenteritis in children <5 years worldwide, accounting for about 200,000 deaths per annum, with a greater percentage of mortality occurring in developing countries [2]. In Ghana, rotaviruses account for up to 28% of diarrhoeal disease hospitalizations [3,4]. To reduce the high

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morbidity and mortality due to rotavirus infection, the World Health Organization (WHO) recommended the introduction of rotavirus vaccines into national immunization programmes in 2009.

Rotavirus gastroenteritis in humans is associated with mainly six genotype combinations; G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8], causing majority of infections [5]. Although the distribution of these six globally important rotavirus genotypes can change dramatically in regions from year to year, the G1P[8] rotavirus strain has remained the most prevalent strain worldwide [6–8]. However, significant diversity of rotavirus genotypes continues to be observed worldwide with several novel combinations due to accumulation of point mutations, genome re-arrangements, and/or zoonotic transmission to human host resulting in the introduction of new antigenic variants across regions [9,10].

Presently, there are two rotavirus vaccines; RotaTeq, (Merck Vaccines, Whitehouse Station, New Jersey) and Rotarix (GlaxoSmithKline Biologicals, Rixensart, Belgium) that have been licensed and recommended by the WHO for inclusion in the immunization programmes in developing countries, especially those with a high burden of childhood diarrhoea disease as part of its strategy to control RV-associated diarrhoeal diseases [11,12]. The WHO has also recommended surveillance programmes at sentinel sites across Africa to monitor the burden of rotavirus disease and circulating strains before and after vaccine introduction as one of the crucial tools in measuring the impact of rotavirus vaccines [11]. Ghana has actively participated in the WHO surveillance programme and introduced the mono-valent rotavirus vaccine (Rotarix, GSK) in May 2012. The introduction of rotavirus vaccines into national immunization programmes has led to the decline in the burden of childhood acute gastroenteritis (AGE) in many vaccine-introducing programmes has led to the decline in the burden of severe childhood diarrhoea disease [13,14]. This report describes temporal trends in RV strain distribution pre- and post-vaccine introduction in Ghana.

This study was reviewed and approved by the Institutional Review Boards of the Centers for Disease Control and Prevention (Atlanta, Georgia), and the Noguchi Memorial Institute for Medical Research, University of Ghana (Accra).

2. Methods

2.1. Study population

The surveillance studies were carried out in rotavirus sentinel surveillance sites in health institutions across the southern, middle and northern belts of Ghana. The surveillance sites were Southern belt (Korle-Bu Teaching Hospital and Princess Marie Louise Children’s Hospital in the Greater Accra Region); Middle Belt (Agogo Presbyterian Hospital and Komfo Anokye Teaching Hospital in the Ashanti Region); Northern Belt (Navrongo War Memorial Hospital, Paga Health Centre and Kassena East Health Centre in the Upper East region). Diarrhoea disease surveillance and epidemiological studies have been on-going at these sites since 2009. Fecal samples were collected from children less than 5 years of age admitted with a primary diagnosis of AGE from participating hospitals and health Centers within 48 h of hospitalization and tested for the presence of rotavirus antigen using enzyme immunoassay (EIA) (ProSpect™, Oxoid Cambridge, United Kingdom). A total of 1363 rotavirus (RV) EIA-positive stool samples sent to the Regional Rotavirus Reference Laboratory (RRL) located at the Noguchi Memorial Institute for Medical Research, University of Ghana, were characterization. Demographic and clinical information of patients were also provided for each sample.

3. Laboratory analysis

3.1. Polyacrylamide gel Electrophoresis

All RV EIA-positive stool specimens were subjected to Polyacrylamide Gel Electrophoresis (PAGE) to ascertain the integrity of the RNA genome. All EIA-negative samples were also subjected to PAGE to screen for any non-group A rotavirus. Briefly, viral RNA was extracted from 10% faecal suspensions by the Bender method [15] with slight modification for PAGE analysis [16]. The extracted double-stranded RNA (dsRNA) was electrophoresed on a 10% polyacrylamide slab gel for 18–20 h at 100 V using the discontinuous buffer system as described by Laemmli [17]. A 3% stacking gel was employed to enhance the resolution of the segmented genes. Bands were visualized by silver-staining technique [18].

3.2. RT-PCR

RVA dsRNA was extracted from 10% fecal suspensions of EIA-positive and EIA negative PAGE-positive samples by the phenol/chloroform method as described by Steele and Alexander and purified with an RNaid Kit (Bio 101, Carlsbad, USA) [18]. RT-PCR was carried out using consensus primers Beg9/End9 and Con2/Con3 to amplify the VP7 and VP4 genes respectively [19–21]. Semi-nested multiplex PCR was done for G- and P-typing by using genotype-specific primers as described previously [20–22]. The amplified product was electrophoresed on a 2% agarose gel, and the genotypes determined by the sizes of the amplicons. Ten percent of all genotypes determined by PCR were further confirmed by sequencing. Briefly, the PCR amplicons were purified with the ExoSap-IT purification kit (USB products) following the manufacturer’s instructions and sequenced by the dideoxynucleotide chain termination method using the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit v3.1 (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences were read on an automated sequencer (ABI PRISM® 3130), and assembled contigs identified by querying the nucleotide database in GenBank using the Basic Local Alignment Search Tool [BLAST] (http://blast.ncbi.nlm.nih.gov/BLAST.cgi). The genotypes obtained were confirmed using the automated genotyping tool, RotaC v2.0 [23]. All demographic, clinical and laboratory data were entered into a database and analyzed using Stata version 13 (Stata Corp, College Station, TX, USA).

4. Results

A total of 1363 rotavirus-positive diarrhoeic stool samples from the southern (n = 876), middle (n = 173) and northern (n = 314) belts were included in the analysis.

Table 1a: Distribution of rotavirus strains detected in Ghana – Pre-vaccine era (January 2009–April 2012).

<table>
<thead>
<tr>
<th>VP7</th>
<th>VP4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[P4]</td>
<td>[P6]</td>
</tr>
<tr>
<td>G1</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>G10</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>G12</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>G2</td>
<td>17</td>
<td>40</td>
</tr>
<tr>
<td>G3</td>
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<td>76</td>
</tr>
<tr>
<td>G4</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>G6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>G8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G9</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>GMIX</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>GNT</td>
<td>4</td>
<td>43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th></th>
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<tbody>
<tr>
<td>41</td>
<td>253</td>
</tr>
<tr>
<td>285</td>
<td>172</td>
</tr>
<tr>
<td>72</td>
<td>823</td>
</tr>
</tbody>
</table>

MIX: mixed genotypes; NT: either G, P or both were non-typeable.
regions were successfully genotyped. Of these, 823 were from the pre-vaccine period (January 2009–April 2012) and 540 from the post-vaccination period (May 2012–December 2016). The most common G-genotypes detected during the entire period (2009 to 2016) were G1 (30.7%; 418/1363), G12 (13.8%; 188/1363), G3 (12.1%; 165/1363) and G2 (10.7%; 146/1363) whilst the most commonly detected P-genotypes were P[8] (37.8%; 515/1363), P[6] (31.6%; 431/1363), and P[4] (8.9%; 121/1363).

The circulation and detection of rotavirus VP7 and VP4 genotypes during the pre and post vaccine introduction periods were very similar. The most common VP7 and VP4 types detected during the pre vaccine introduction period were G1 (40.0%; 330/823) and P[8] (34.6%; 285/823) respectively as shown in Table 1a. Rotaviruses bearing the VP[6] genotypes were also commonly detected (30.7%, 253/823). The most prevalent G and P types detected were G1P[8] (20.0%, 165/823, G3P[6] (9.2%, 76/823) more than eighteen percent of all Vp7 could not be genotyped whilst the percentage of un-typable P types were less than 3%. More than 11% of all strains detected had mixed VP4 genotypes.

During the post vaccine period, the most common G and P types detected were G12 (32.6%; 176/540) and P[8] (42.6%; 230/540). The predominant rotavirus strains detected were G12P[8] (25.6%; 138/540), G10P[6] (10.0; 54/540) and G3P[6] (8.1%; 44/540) strains as shown in Table 1b. Strains bearing the common VP7 genotypes, G1 and G2, constituted 24.3% (131/540) of all rotavirus strains detected.

In addition to common strains, we identified several rare/uncommon genotype combinations in a low percentage of samples: G1P[4], G4P[4], G6P[6], G8P[8], G9P[4], G10P[4], G10P[6] and G12P[4]. The overall prevalence of these rare/uncommon strains is 10% (Tables 1a and 1b. We did not see significant differences between dominant strains within the three regions during the pre- or post-vaccine era.

Table 1b
Distribution of rotavirus strains detected in Ghana - Post-vaccine era (May 2012–December 2016).

<table>
<thead>
<tr>
<th>VP7</th>
<th>VP4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[P4]</td>
<td>[P6]</td>
</tr>
<tr>
<td>G1</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>G10</td>
<td>1</td>
<td>54</td>
</tr>
<tr>
<td>G12</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>G2</td>
<td>33</td>
<td>6</td>
</tr>
<tr>
<td>G3</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>G8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G9</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>GMIX</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>GNT</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>178</td>
</tr>
</tbody>
</table>

MIX: mixed; NT: either G, P or both were non-typeable.

4.1. Temporal strain distribution pre- and post- vaccine era

G1P[8] was the predominant rotavirus strain (>20%) during the pre-vaccine era (Fig. 1). Other common strains detected were G3P[6] (9.2%), G1P[6] (4.1%), G10P[6] (3.8%) and G2P[6] (4.9%). The first three rotavirus seasons after vaccine introduction (2012–2015) saw the emergence and dominance of G12P[8] (138/540; 26%) and G10P[6] (54/540; 10%) strains as shown in Table 1b. However, this observed phenomenon was short lived and there was a return of G1P[8] as one of the dominant strains in the fourth year post-vaccine introduction (2015–2016 rotavirus season) (Fig. 1). The 2015–2016 rotavirus season also saw the emergence of the unusual G9P[4] strains (16/540; 3.0%), most of which were detected in the middle belt. This unusual strain was first detected in Ghana in the early 2000s. Successive detections of G9P[4] strain were in 2013 (2/144, 1.4%), 2015 (1/53, 1.3%), and the spike in 2016...
Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, et al. Global, regional, and national Armah G, Pringle K, Enweronu-Laryea CC, Ansong D, Mwenda JM, Diamenu SK, Enweronu-Laryea CC, Boamah I, Sifah E, Diamenu SK, Armah G. Decline in Matthijnssens J, Van Ranst. Genotype constellation and evolution of group A revealed these G9 genotypes to be of human origin (unpublished diversity in circulating strains [34] and it is quite strain introduction may not be associated with vaccine introduction and may represent natural secular variation in rotavirus strains. Earlier reports indicate that rotavirus G9s were usually in combination with the P[8] genotypes and to a lesser extent, P[6] [24]. The emergence of the unusual G9[P4] strain more than ten years after its first detection in a child with diarrhea in northern Ghana [25] was intriguing. Outbreaks of rotavirus G9[P4] strains have also been reported over the last decade in Asia (India, Bangladesh and Japan) and Latin America (Brazil, Mexico, Guatemala and Honduras) [26–33]. Though several of these countries reporting the increased detection of G9[P4] rotavirus strains had previously introduced the monovalent rotavirus vaccine, it remains unclear whether the observed increased detection of this unusual strain is due to vaccine introduction. Earlier reports from Ghana have shown increased diversity in circulating strains [34] and it is quite likely that the observed increase of these unusual strains may be due to increased re-assortment processes in the community. It is important to note that studies in Mexico showed that the monovalent rotavirus vaccine (Rotarix) provided protection against the fully heterotypic G9[P4] strains [33]. However, this study was limited by its small sample size and the observational nature of the evaluation. While it has not yet been established whether the Ghanaian G9[P4] strains are identical to the Mexican strains, it remains unclear whether they will become established or transient, and what the implications will be for vaccine efficacy in Ghana. The observed increase in detection of rotavirus strains with mixed G and P types in circulation after Rotarix vaccine introduction has also been reported in other countries in India, Bangladesh, South Africa and Malawi [35–38] where a substantial proportion of mixed rotavirus circulating strains were also detected post-vaccination. Whilst these findings may indicate naturally occurring variations in circulating rotavirus strains, it could also be due to selective pressure from the introduction of rotavirus vaccination. A continuous rotavirus surveillance programme is thus necessary and important for the monitoring of circulating strains in the post-vaccine era to fully understand the effect of vaccine introduction on strain distribution and the emergence of new strains.

5. Discussion

Rotavirus infection was monitored as part of the ongoing World Health Organization sponsored rotavirus surveillance study in Ghana. A total of 1363 RVA positive samples were subjected to G and P genotyping as described earlier [20–22]. Genotype data before vaccine introduction showed that strains carrying the G1 genotype specificity were responsible for 40% of all RV cases recorded during the pre-vaccine period (2009–2012) and this was followed by G3 (13.9%) and G2 (12.5%). Most of the common genotypes identified (G1, G2) were usually found in combinations with P[8] and P[4] VP4 genotypes (Tables 1a and 1b). The prevalence of the common G3[P8] strain remained below 2% during the study period. In addition to common strains, we identified several rare/uncommon genotype combinations in a few samples: G1P [4] (1.5%; 21/1363); G4P[4] (<1%; 10/1363), G9P[4] (1.2%; 16/1363) and G10P[6] (6.2%; 85/1363). The sudden appearance of G12P[8] rotavirus strains soon after the introduction of rotavirus vaccines in 2012 and its drop to less than 6% of detected strains by 2016 is quite interesting. Concurrent genotyping of diarrhoeal stool samples submitted to the Rotavirus Regional Reference Laboratory (RRL) in Ghana (data not shown) from countries in the WHO sponsored African Rotavirus surveillance programme showed the emergence of G12 strains as the dominant strain in Nigeria and Senegal, countries that had not yet introduced rotavirus vaccines in their immunization programme. Therefore, the observed emergence of G12 strains post-vaccine introduction may not be associated with vaccine introduction and may represent natural secular variation in rotavirus strains.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC) or the World Health Organization (WHO).

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Potential conflicts of interest

The authors have declared no competing conflict of interests.

References


