Rotavirus Genotypes in Costa Rica, Nicaragua, Honduras and the Dominican Republic

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Abstract
In this study, 574 stool samples from children with gastroenteritis were obtained from different hospitals in Costa Rica, Honduras, Nicaragua and the Dominican Republic during 2005–2006. Diarrhea stool samples were analyzed for rotavirus (RV) by ELISA and typed by the RT-PCR-based method. Unusual strains were detected: G1P6, G2P8, G3P6, G9P4, and mixed infections. Recent studies have indicated that unusual human RV strains are emerging as global strains, which has important implications for effective vaccine development. In this context, the next generation of RV vaccines will need to provide adequate protection against diseases caused not only by mixed infections, but also by unusual G/P combinations.

Gastrointestinal illnesses due to rotavirus (RV) infections among young children contribute greatly to morbidity and mortality rates in many Latin American countries and in other parts of the world. According to previous research, the RV is considered responsible, on a global scale, for nearly 611,000 deaths per year in children [1].

The experience and data of previous studies carried out in Central America have demonstrated that gastrointestinal infections are one of the most prominent health problems that contribute to high morbidity and mortality [2–4].

RVs belong to the Reoviridae family and possess a trilaminar capsid enclosing 11 segments of double-stranded RNA. RVs are classified into G and P types according to the antigenic or genetic diversity of the capsid proteins, VP7 (G-types) and VP4 (P-types) [5]. Up to now, at least 19 G genotypes and 27 P genotypes have been described [6]. Theoretically, many G/P combinations are possible within the binary system utilized to classify RV genotypes; however, the G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] combinations are the most commonly identified genotypes worldwide [7]. The proteins VP7 and VP4 are important for vaccine development, as they are the major antigenic targets for virus neutralization [8]. Due to the prominent use of the RV vaccine Rotarix\textsuperscript{®} in South America, Central America and the Caribbean, research...
efforts in this field are urgent in order to understand the epidemiology, standard diagnostics, and characterization of viral strains [9].

Taking into account that RV disease is a global problem and that its epidemiology is relevant to public health, efforts are underway to consolidate a regional network in Central America for the detection and characterization of the most prevalent RV strains circling the region. This effort is headed by the ‘NeTropica Net’ (Network for Research and Training of Tropical Diseases in Central America).

We report in this study the circulation of the G and P genotypes of RV strains in some Central American countries and the Dominican Republic during 2005–2006.

In this study, 574 stool samples from children with gastroenteritis were obtained from different hospitals. Of the 574 samples, a total of 166 samples tested positive by ELISA for the RV and were submitted for G and P genotype characterization.

The RV samples from the Dominican Republic were collected from Santo Domingo, between February and June 2005. Samples were stored on ice and taken to the virology laboratory at the Instituto de Investigaciones Científicas (INDICASAT) in Panama where they were diluted 1:4 in PBS and stored at –70° for further analysis.

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The RV samples from Costa Rica were collected from children with gastroenteritis who were admitted at the National Children’s Hospital, in San José, between August and October 2005. A total of 129 samples were analyzed by ELISA, of which 39 were positive. From May to June 2006, an additional 25 RV-positive samples (as determined by ELISA) were obtained. All ELISA-positive samples were analyzed by RT-PCR to determine the G and P genotypes in the Laboratory of Virology of the University of Veterinary, Heredia, Costa Rica.

In Nicaragua, 20 RV-positive samples were obtained in May to June 2006 from hospitalized children at the Hospital School of León. G and P genotyping were analyzed by RT-PCR for the all the samples in the Department of Microbiology, University of León, Nicaragua.

In Honduras, a subset of 45 out of 300 RV-positive samples were collected in May to June 2006 at the Pediatric Service and the Pediatric Infectology ward at Hospital Regional Santa Teresa in Comayagua, Hospital San Francisco in Juticalpa, and a private clinic in Tegucigalpa. 22 samples from May and 23 from June were tested for RV by genotyped for VP7 (G-type) and VP4 (P-type) gene by RT and multiplex PCR, at the Department of Microbiology in National University, Tegucigalpa, Honduras.

In Nicaragua and Honduras, the primary detection method for fecal RV was performed by a RV immunochromatographic assay test (Rota-Strip BioConcept, Brussels, Belgium). In Costa Rica and the Dominican Republic, screening was performed by Rota IDEA (Dako-Cytomation Ltd, UK) to detect the VP6 antigen according to the manufacturer’s instructions.

To determine the G and P genotypes, the specimens were analyzed by nested multiplex RT-PCR assays to determine the most common G genotypes (G1–G4, G5 and G8) and P genotypes (P[4], P[6], P[8] and P[9], P[10]). Primers and procedures were performed as previously described [12]. This method is a modification of the original methods described by Gouvea et al. [10] for G typing and Gentsch et al. [11] for P typing. This methodology has been adopted by the Central American laboratories participating in the net [12].

In agreement with previous studies performed in the region [2–4], this study shows the presence of the most predominant RV types that have been encountered globally. However, uncommon genotype combinations and rare genotypes were present as well. The detected uncommon RV genotype combinations G1P[6], G2P[8] and G3P[6] have been reported before in 2002–2003 [4]. Additionally, uncommon G9P[4] RVs were detected. The G1P[8] type was the most frequent RV type in the Dominican Republic and Costa Rica during 2005, which agrees with the first reported outbreak in the Dominican Republic during 2003 [4].

In the Dominican Republic, the most prevalent strain (2005) was G1P[8] (29%), followed by G2P[4] (22%), G3P[8] (13%), G1P[6] (10%) and G3P[6] (5%). In addition, two mixed infections, one sample with G1+G3 and one sample with G1+G2, were detected. In both, the associated P genotype(s) could not be determined (table 1).

In Costa Rica, the most prevalent strain during 2005 was G1P[8] (69%), G2P[8] (3%) and G1P[6] (10%) (table 1). In 2006, G9P[8] was the most prevalent strain (60%), followed by G9P[4] (24%) (table 1). In Nicaragua, the genotype G4P[8] (60%) was the most prevalent type, followed by G9P[8] (35%). In Honduras, infection by G2P[4] (42%) appeared to be the most frequent cause of the RV outbreak, occurring primarily in children under 1 year of age. Other genotypes encountered were G4P[8] (16%) and G9P[8] (13%).

In Nicaragua and Honduras, the genotype G4 P[8] was detected in 60 and 16%, respectively, in stool samples.
from children with gastroenteritis. This genotype was detected in Costa Rica in the study of 2002–2003 [4]. The G4P[8] was responsible for the outbreak of gastroenteritis at the beginning of 2005, in Nicaragua. It was most commonly identified in those regions where fatal cases in children occurred [2]. In addition, this strain has been reported in other countries of the world [13]. Furthermore, in one study, the G4P[8] genotype was considered the re-emergent strain in Ireland [14].

In Honduras, at least 3 genotypes were observed to be simultaneously circulating during the research period; however, it appears that the genotype G2P[4] (42%) contributed to the majority of cases in Honduras and was also detected in the Dominican Republic in 2005. This genotype is considered, along with G1P[8], to be the most frequent combination in humans [7]. The remarkable re-emergence of G2P[4] during the last few years seems to reflect a continental phenomenon [7].

During 2006 in Costa Rica, the combinations G9P[8] and G9P[4] were detected; however, in Nicaragua and Honduras only the G9P[8] combination was found.

Worldwide, the G9 genotype has recently been considered as emergent [15] and has been found in different P-type combinations in Brazil [16]. Even though the quantity of the analyzed samples is small, it is important to note that G9 genotypes reappeared in Costa Rica in 2006 where it had been already detected in 2002–2003 [4].

As the Central American countries are considered attractive by Asian and European populations, the high frequency of immigration and travelling constitutes a potential source for introduction of new genotypes into the local population.

In response to current reports of unusual RV strains in Central American countries that are also being reported around the globe, we believe that vigilance programs in the post-vaccination era must be set into action in an attempt to respond to pending queries regarding the potential of re-assortments and the emergence of new genotypes. Studies made on the phylogenetics of RV have demonstrated differences in the lineage and sublineage of genotypes, highlighting the great genetic variability of RV [17]. In this context, the immunogenicity and efficacy of RV vaccines may be challenged by the evolution of the RV viral genome.

As mixed infections were detected in the Dominican Republic, a prerequisite for re-assortment events, co-surveillance of animal and human RV strains will be vital in better understanding the relationships between co-circulating viruses, as well as assessing any relevant vaccination programs. Thus, it is imperative to undertake this investigation in Central America, where socioeconomic situations are unsatisfactory and in some countries of the region, such as the Dominican Republic, farm animals and humans co-exist in the same house.

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**Table 1. Distribution of G and P types of RV strains isolated during gastroenteritis in Costa Rica, Nicaragua, Honduras, and the Dominican Republic, 2005–2006**

<table>
<thead>
<tr>
<th>Genotypes and P</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Costa Rica</td>
<td>Dominican Republic</td>
</tr>
<tr>
<td>G1P[6]</td>
<td>4 (10%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>G1P[8]</td>
<td>27 (69%)</td>
<td>11 (30%)</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>8 (22%)</td>
<td></td>
</tr>
<tr>
<td>G2P[8]</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>G3P[6]</td>
<td>2 (5%)</td>
<td></td>
</tr>
<tr>
<td>G3P[8]</td>
<td>5 (13%)</td>
<td></td>
</tr>
<tr>
<td>G4P[8]</td>
<td></td>
<td>12 (60%)</td>
</tr>
<tr>
<td>G9P[4]</td>
<td>6 (24%)</td>
<td></td>
</tr>
<tr>
<td>G9P[8]</td>
<td>15 (60%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>GP[8]</td>
<td>1 (4%)</td>
<td></td>
</tr>
<tr>
<td>G1+G2P[?]</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>G1+G3P[?]</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Untyped G</td>
<td>7 (18%)</td>
<td>5 (14%)</td>
</tr>
<tr>
<td>Untyped P</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>37</td>
</tr>
</tbody>
</table>

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For RV characterization, the use of multiplex PCR in the identification of the VP7 and VP4 genes should be handled with caution. A low percentage of samples were untypeable for G and P; the possibility of unspecific primer binding has to be taken into consideration, mainly due to the accumulation of point mutations and to the diversification on specific lineages, has been reported [18]. We also believe that for VP4, it is possible that genotypes are unusual because we used common primers for detection of group P.

In light of these considerations, additional experiments are necessary to confirm the results obtained during G and P typing. In particular, the confirmation of the high frequency of unusual G/P combinations found by sequence analysis is recommended. With the aid of ‘NeTropica Net’, we are establishing a vigilance program on the RV strains in Central American countries, with the aim of not only keeping a monitoring record on the circulating strains, but also with the intention of unifying the protocols in all investigatory laboratories in Central American countries.

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