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Diversity of Human Rotavirus G and P Genotypes in Panama, Costa Rica, and the Dominican Republic

Lurys Bourdett-Stanziola,* Carlos Jiménez, and Eduardo Ortega-Barria

Instituto de Investigaciones y Servicios de Alta Tecnología (INDICASAT), Panamá; Programa de Investigación en Enfermedades Tropicales (PIET), Escuela de Medicina Veterinaria, Universidad Nacional (UNA), Heredia, Costa Rica

Abstract. In this study 2,089 fecal samples from patients with gastroenteritis were analyzed from different hospitals in Panama, Costa Rica, and the Dominican Republic during the period comprised between December 2002 and July 2003. One hundred samples per country from the positives to the enzyme-linked immunosorbent assay (ELISA) kit were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) to determine the G and P genotypes: in Panama, Costa Rica, and Dominican Republic the combinations G and P have a great diversity and unusual genotypes. These results highlight an unexpected diversity among rotavirus strains in these countries and emphasize the need for further serologic and genetic surveys on more rotavirus strains in Central America and the Caribbean. In this context, the next generation of rotavirus vaccines will need to provide adequate protection against diseases caused by unusual genotypes. These results represent the second report of rotavirus genotypes in Costa Rica and first-time reports of rotavirus genotypes in Panama and the Dominican Republic.

INTRODUCTION

Gastrointestinal illnesses resulting from rotavirus infections among young children contribute greatly to morbidity and mortality rates in many countries in Latin America and other parts of the world. Human rotaviruses are one of the major contributors of severe infantile diarrhea, responsible for more than 600,000 deaths annually.[1]

Furthermore, rotavirus infections are an important cause of hospitalization, causing considerable economic impact on poor countries.[1,2] As a result, the development of a successful vaccine against these agents has been an international goal in the last decades. These efforts have been hampered by the large genetic diversity characterizing these viruses. To counteract the burden of this disease, several vaccines against rotavirus are under development for its control. However, the continuing genetic drifts of strains and, as a consequence, the changing and diverse rotavirus antigenic may complicate a rational design of vaccines.[2,3] Within rotavirus A, there are different strains called serotypes. The glycoprotein VP7 defines G-types, and the protease-sensitive protein VP4 defines P-types; along with VP7, it is involved in immunity to infection. The P-type is indicated by a number for the P-serotype and by a number in square brackets for the corresponding P-genotype. G-serotypes are similarly numbered, but the G-genotype number is the same as the G-serotype.[2,3] Group A rotaviruses have been classified into 15 G serotypes and 26 P genotypes.[4,5] The combinations of G1P8, G2P4, G3P8, and G4P8 are the most frequently found genotypes in humans.[5]

Recent studies have revealed unusual combinations of G y P in various countries where they believe these strange combinations have possibly emerged because of reassortment; among these genotypes they have detected G1P6,[6,7] G2P8,[6,8] G1P4,[9–11] and G3P6.[12]

Furthermore, genotypes thought to be rare, such as G9 in combination with P6, P8, or P4, represent important emergent strains.[9,13–19] This challenge will have to be addressed in future vaccine trials. Before an introduction of a rotavirus vaccine, the World Health Organization (WHO) recommended surveillance in hospitalized children with gastroenteritis to determine the disease burden, to establish the current strain's characteristics, and to learn about subtypes on a molecular level for future comparisons of vaccine effectiveness and properties of the vaccine strain.[20] In the present study, we report the diversity of rotavirus strains observed in Panama, Costa Rica, and the Dominican Republic from December 2002 to July 2003 to provide relevant epidemiologic information for the evaluation of potential benefits from a rotavirus vaccination program in Central America and the Caribbean. The diversity of rotaviruses in these countries implies that rotavirus vaccines in development will need to be able to protect against a wider panel of serotypes than originally envisioned.

MATERIALS AND METHODS

Study group and sample analyses. Samples from patients with gastroenteritis under three years of age were collected at several hospitals in Panama City, Panama and the city of David in the Chiriqui province; in Costa Rica, Central Valle, and Guanacaste; and in the National Children's Hospital in Santo Domingo, Dominican Republic, during the period comprised between December 2002 and July 2003. There were a total of 1,007 samples in Panama, 680 in Costa Rica, and 402 samples in the Dominican Republic. Samples were taken in ice to the virology laboratory at the Instituto de Investigaciones Científicas (INDICASAT) in Panama, where they were diluted 1:5 in phosphate-buffer-salt-solution (PBS) and stored at −70°C for further analysis.

Detection of rotavirus by enzyme-linked immunosorbent assay (ELISA) kit. The 2,089 samples were examined for group A rotavirus by an ELISA assay (Rota IDEA; DakoCytomation Ltd., United Kingdom) to detect the VP6 antigen, according to the manufactureŕs instructions.

Identification of G and P genotypes by reverse transcription-PCR. A subset of 300 samples (100 of each country) scored positive by the ELISA assay, selected per month (12 to 13 up to a total 100 samples), was randomly chosen and analyzed by RT-PCR to determine G (VP7) and P (VP4) genotypes.

* Address correspondence to Lurys Bourdett-Stanziola, Laboratorio de Virología, Instituto de Investigaciones y Servicios de Alta Tecnología (INDICASAT). E-mails: lbourdett@aupsa.gob.pa or lurysb@yahoo.com

RNA extraction. The stool samples were diluted in phosphate-buffered saline (PBS) pH 7.2. The viral double-stranded RNA was extracted from Trizol (Molecular Research Center, Cincinnati, OH), according to the manufacturer's protocol. The purified RNA was used as RT-PCR.

Multiplex RT-PCR. To determine G and P genotypes, the specimens were analyzed by a nested multiplex RT-PCR assays to determine the most common G genotypes (G1–G4, G8, and G9) and P4, P6, P8, and P9 performed following published methods by Gentsch and others,[21] Gouvea and others,[22] and Iturriza-Gómara and others.[23]

RESULTS

Panama, Costa Rica, and the Dominican Republic ELISA. A total of 1,007 samples from Panama were analyzed by ELISA, 473 (47%) were positive; of the 680 samples from Costa Rica, 360 (53%) were positive by ELISA, and of the 402 samples from the Dominican Republic analyzed by the ELISA, 249 were positive (62%).

Panama, Costa Rica, and the Dominican Republic single types. A total of 300 stool specimens from children with diarrhea were analyzed by RT-PCR. The most prevalent single G types detected between December 2002 and July 2003 in Panama, Costa Rica, and the Dominican Republic were the G1, as the most frequent in Panama and the Dominican Republic, whereas the G3 was the most frequent in Costa Rica (Table 1).

The P types' distribution in specimens collected in Panama, Costa Rica, and the Dominican Republic showed predominately P8 as the most common P type, followed by P4 and P6 (Table 2).

Combinations G (VP7) and P (VP4) genotypes were obtained in Panama, Costa Rica, and the Dominican Republic. The most frequent in Panama and the Dominican Republic was G1P8 (61%) and (53%), respectively, and in Costa Rica G3P8 (54%), following other combinations of the G and P (Table 2).

We found uncommon combinations and rather strange strains in Panama, the Dominican Republic, and Costa Rica; for example, G1P6 (7%), (4%), and (4%), respectively, and other uncommon combination strains, the detection of G9P8 in Costa Rica and Dominican Republic, and for the first time in Central America and the Caribbean (Table 2).

According to the results, there exists an association between the genotypes and the place of origin, for the Pr:

TABLE 1

Distribution of G genotypes detected by reverse transcription-polymerase chain reaction (RT-PCR) of rotavirus positives samples from Panama, Costa Rica, and Dominican Republic, 2002–2003

Genotype	Panama no. (%)	Costa Rica no. (%)	Dominican Republic no. (%)
G1	88	33	68
G2	3	ND	2
G3	9	63	29
G4	ND	3	ND
G9	ND	1	1
Total	100	100	100

ND = not detected.

TABLE 2

Distribution of G and P genotypes detected by reverse transcription-polymerase chain reaction (RT-PCR) of rotavirus positives samples from Panama, Costa Rica, and Dominican Republic, 2002–2003

Genotypes (G and P)	Panama no. (%)	Costa Rica no. (%)	Dominican Republic no. (%)
G1P8	61	28	53
G2P4	1	ND	2
G3P8	7	54	23
G4P8	ND	2	ND
G9P8	ND	1	1
Combinations unusual			
G1P4	3	ND	3
G1P6	7	4	4
G2P8	2	ND	ND
G3P6	ND	ND	4
Untyped genotype (P)	19	11	10
Total	100	100	100

ND = not detected.

$5.72334 \times 10^{-11} < 0.05$. The differences of the proportional indicate that the probability of 0.063 is greater than 0.05, which concludes that the proportions were considered statistically different, a level of significance of 5%.

DISCUSSION

It is necessary to ascertain the rotavirus types circulating in different countries over the course of a number of years. Globally, different surveys indicate that G1P8, G2P4, G3P8, and G4P8 are the most common G and P types. However, since the introduction and wider use of molecular biology-based typing methods over the last 10 years, other rotavirus types have increasingly been reported in different parts of the world, such as G9.[5,24,25]

In this report, we describe the results of a survey conducted in Panama, Costa Rica, and the Dominican Republic during December 2002 and July 2003 to determine the circulating genotypes.

In Panama, Costa Rica, and the Dominican Republic, rotavirus infections are not subject to specific surveillance. The rotavirus genotype in these countries has shown two characteristic features: 1) a great diversity of G and P genotypes circulating simultaneously, and 2) a frequent occurrence of unusual G-P combinations.

In Panama and the Dominican Republic, the genotype G1 was the most frequent, whereas the most frequent in Costa Rica was G3. The genotype P8 was the most frequent in the three countries followed by P4 and P6, which coincides with the research of Martella and others[4] and Santos and others.[5] For some researchers, it is necessary to examine the role of P6 and to include it in future vaccines for the rotavirus[26] recommendation we support, because in this research we discovered some considerable and unusual strains that have this genotype.

The combination G1P8 type was the most frequent rotavirus type in Panama and the Dominican Republic during the December 2002 through July 2003 period, following the combination G3P8 with 7% in Panama and 23% in the Dominican Republic, which agrees with other reported studies in other

countries where they clearly observed that the genotype G1P8 is the most frequent. In a recently published study, it was reported that in the United States, Europe, and Australia this strain G1P8 represents 70% of the infections for rotavirus, 30% in South America and 23% in Africa.[5]

However, in Costa Rica in 2003 the most frequent genotype was G3P8 (54%) followed by G1P8 (28%), which contrasts with the first study undertaken in the years 2001–2002, in which it was observed that the genotype G1P8 was the most common in this country (L. Bourdett-Stanziola, unpublished data). In Panama, G3P8 was found in David, Chiriqui, which is on the border with Costa Rica. We consider that was possibly how this genotype was introduced from Costa Rica into Panama during the year 2003. The genotype G4P8 (2%) was also detected in Costa Rica, which has been reported in other countries, and it is considered as the common strain.[5,6] This genotype in Nicaragua was responsible for the recent gastroenteritis outbreak, which occurred in the beginning of 2005.[27] The present study adds information and further confirms the emergence of these unusual strains in these three countries, and also present in other countries.[7,11,12,28–37]

According to Banerjee and others,[38] the natural reassortment strain may be emerging. Other research, such as Abdel-Haq and others,[9] considered that this has something to do with the ethnic diversity and these genotypes are a product of re-assortment that could have arisen through the introduction of genotypes in other parts of the world, which could be an acceptable conclusion for the Panama and Dominican Republic cases. These are considered to be countries where a great ethnic diversity exists.[9,38]

Recent studies have indicated that unusual human rotavirus strains are emerging as global strains, which has important implications for effective vaccine development.[8,19,26,32] The detection of unusual G-P types, such as these, may be the result of possible re-assortments during natural infections. The results from the molecular characterization of these strains will help us in discussing further the issue of possible re-assortments.

This study reports the first detection of the G9 in human fecal samples in the Dominican Republic, and the first discovery in Central America of the combination G9P8, considered a strain of epidemic emergency in the last few years in several countries in children with diarrhea.[13,14,16,17,39,40] The increase in reports of G9 from developed and developing countries shows the need for continued surveillance to identify the persistence of G9. Whether new available rotavirus vaccines are effective against genotype G9 and to what extent type–specific immunity plays a role effectively is still under discussion.

Even though there is a reduction of the number of samples, the tendency of the genotypes that circulate in those countries has been clearly observed. There should be new epidemiologic studies in Central America and the Caribbean to observe the seasonal variation of the strains and to determine the possible outbreak with emergent strains.

Molecular characterization of rotavirus in different geographic areas, at different times, need to be continually evaluated to know the current distribution and the genomic variation over time.

This study extends our understanding of the tremendous diversity of rotavirus strains in these countries, and the implications for the formulation of effective rotavirus vaccines.

It is important to maintain surveillance of rotavirus strains, particularly in developing countries. Such events may lead to the appearance of new strains or new variants that could escape immune protection induced by an outdated vaccine. It would suggest that the vaccine strains did not evoke sufficient heterotypic protection against novel strains not present in the vaccine, and would indicate the need to incorporate these specificities in additional re-assortants to increase vaccine efficacy.

This study is the first to report the P (VP7) genotype and G (VP4) genotype of circulating rotavirus strains in Panama and the Dominican Republic and the second report in Costa Rica.

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Authors' addresses: Lurys Bourdett-Stanziola, Carlos Jiménez, and Eduardo Ortega-Barría, Laboratorio de Virología, Escuela de Medicina Veterinaria, Heredia, Costa Rica, Tel: 507-517-0089, Fax: 507-317-0219, E-mails: lbourdett@aupsa.gob.pa or lurysb@yahoo.com.

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