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# Phylogeny-Based Classification of Human Rhinoviruses Detected in Hospitalized Children With Acute Lower Respiratory Infection in Paraguay, 2010–2011

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Human rhinovirus (HRV), a single-stranded, positive-sense RNA virus, is associated with mild upper respiratory tract infections in children. The aim of this study was to carry out a molecular characterization and phylogeny-based classification of the circulating genotypes of HRV in hospitalized children with clinical manifestations of acute lower respiratory infection in Paraguay. Nasopharyngeal aspirates were collected from 101 children under 5 years of age, hospitalized with symptoms of acute lower respiratory infection, between May 2010 and December 2011, at the largest public pediatric hospital in the Central Department of Paraguay. Detection was performed by a real-time polymerase chain reaction, followed by conventional amplification of the VP4/VP2 genomic region, sequencing, and phylogenetic analysis. Rhinovirus was detected in 33.7% of the samples. Amplification of 18 samples showed the presence of all three species (HRV-A, -B, and -C). Different genotypes were found for each species: 11 for HRV-A (-9, -12, -22, -30, -36, -43, -59, -61, -68, -88, and -89), one for HRV-B (-4), and four for HRV-C (-C2, -C3, -C6, and -C9). In South America, information about HRV diversity is scarce. This is the first report on HRV genotype diversity in South America. ***J. Med. Virol. 85:1645–1651, 2013.*** © 2013 Wiley Periodicals, Inc.

**KEY WORDS:** rhinovirus; genotypes; respiratory disease

## INTRODUCTION

Human rhinovirus (HRV) is the most frequent cause of respiratory infection across all age groups of the population, especially in children [Denny, 1995]. This virus can replicate in cells from both the upper and lower respiratory tract, and the clinical manifestation can range from a common cold to more severe complications [Papadopoulos et al., 2000; Kaiser et al., 2006]. HRVs are single-stranded positive-sense RNA viruses of ~7,200 bp, which belong to the *Picornaviridae* family and are closely related to members of human *Enterovirus*, another genus of the same family. The genome organization of *Picornaviridae* is conserved among the family, with a long 5′-untranslated region (UTR), a single open reading frame (ORF) encoding a polyprotein, a short 3′ UTR, and a poly(A) tail [Kitamura et al., 1981].

HRV strains have been classified into 99 serotypes (HRV-1 to -99) based on the ability of a given serum to neutralize virus growth of a given strain in cell culture. According to nucleotide sequence comparison of all serotypes in the VP1 [Ledford et al., 2004; Laine et al., 2006] and VP4–VP2 capsid protein-coding regions [Savolainen et al., 2002], the 99 serotypes segregate in two different groups: 74 belong to the HRV-A species and 25 to the HRV-B species. Recently, a third species (HRV-C), which shows a degree of diversity similar to that of species A and B [Simmonds et al., 2010], has been discovered in many countries [Arden et al., 2006; Lamson et al., 2006; Lau et al., 2007; Lee et al., 2007; McErlean et al., 2007; Kistler et al., 2007a]. This species has been

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classified into 33 genotypes (C1–C33) [Simmonds et al., 2010].

In South America, there are only few descriptions of HRVs circulation, and all of them are at species rather than at genotype level [Moreira et al., 2011; Marcone et al., 2012].

The aim of this study was to carry out a molecular characterization and phylogeny-based classification of the circulating genotypes of HRV in hospitalized children with severe acute respiratory infection in Paraguay.

## MATERIALS AND METHODS

### Samples

Nasopharyngeal aspirates were obtained from children (n = 101) under 5 years of age (mean age, 8 months; range, 1–52 months), hospitalized with symptoms of severe acute lower respiratory infection, between May 2010 and December 2011, at the Hospital Pediátrico Niños de Acosta Ñu, the largest a public pediatric hospital in Asunción, Paraguay. This Hospital provides medical care mainly to low-income families residing in the Central Department, which has a population of around 2 million (~25% of the Paraguayan population), including approximately 210,000 children less than 5 years of age, and a density of 800 inhabitants/km$^2$.

### Ethics Statement

Written informed consent was obtained from parents or guardians prior to study participation. This study was approved by the Ethics Committee at Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción (IICS-UNA) and the Hospital Pediátrico Niños de Acosta Ñu.

### RNA Extraction for Real-Time RT-PCR

Nucleic acids were extracted from 200 μl of nasopharyngeal aspirate, using the silica powder method [Boom et al., 1990], and eluted in 60 μl of nuclease-free water.

### Real-time RT-PCR Analysis

The reaction contained 5 μl of RNA, 1.5 μl of generic primers and TaqMan probe (Fast-Track Respiratory Pathogens Plus Kit, FTD, Luxembourg), 1 μl of one-step enzymes (AgPath-ID One-Step RT-PCR Kit, Ambion), and 12.5 μl of 2× buffer in a final volume of 25 μl. A 7,500 Real-Time PCR System (Applied Biosystems, Foster City, CA) was used. The mixture was incubated at 50°C for 15 min, followed by incubation at 95°C for 10 min, and 40 cycles each consisting of incubations at 95°C for 8 sec, and 60°C for 34 sec, respectively.

### RNA Extraction for Sequencing

Viral RNA was extracted from 200 μl of sample using the AxyPrep Body Fluid Viral DNA/RNA Miniprep Kit (Axygen Biosciences, Union City, CA), following the manufacturer's instructions, and eluted in 60 μl of TE buffer.

### cDNA Synthesis

The reaction of cDNA synthesis contained 10 μl of RNA, 200 ng of random primers (Invitrogen, Paisley, UK), 0.25 mM dNTPs mix (Invitrogen), 80 U of RNAseOUT (Invitrogen), 200 U of M-MLV Reverse Transcriptase (Promega, Madison, WI), and 8 μl 5× buffer (250 mM Tris–HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT), in a final volume of 40 μl. The mixture was incubated at 25°C for 10 min, followed by incubation at 37°C for 4 hr, and a final incubation of 5 min at 85°C. Finally, the cDNA was stored at −20°C until use.

### PCR Analysis

A PCR directed to the VP4/VP2 genomic region was carried out using the primers 9895-F (5′ GGGACCAACTACTTTGGGTGTCCGTGT) and 9565-R (5′ GCATCIGGYARYTTCCACCACCANCC), as described elsewhere [Savolainen et al., 2002]. Briefly, the amplification reaction contained 5 μl of cDNA, 0.15 μM of each primer, 0.2 mM dNTPs mix (Invitrogen), 1.5 U of DFS-Taq DNA polymerase (Bioron, Ludwigshafen, Germany), and 5 μl 10× buffer II (500 mM KCl, 100 mM Tris–HCl pH 8.8, 0.1% Tween-20, 15 mM $MgCl_2$), in a final volume of 50 μl. The cycling conditions were as follows: denaturation at 95°C for 2 min, and 35 cycles each consisting of denaturation at 95°C for 30 sec, primer annealing at 48°C for 1 min, and primer extension at 72°C for 1 min; the final extension was at 72°C for 7 min, and 4°C ad infinitum. The PCR products (~530-bp) were separated in 1.8% agarose gels, stained with ethidium bromide, and visualized under UV light.

### Sequencing and Phylogenetic Analysis

The PCR products were purified from 1.8% agarose gels, using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences), and directly sequenced (both strands, 1× coverage each) in an ABI PRISM 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were manually edited with BioEdit v.7.0.5 [Hall, 1999], and aligned with CLUSTAL W. Primer sequences were discarded, and the remaining ~420-bp coding sequence from the VP4/VP2 region from field isolates was compared with a dataset of prototypes by phylogenetic analysis. Phylogenetic relationships were reconstructed by the neighbor-joining method with Kimura's two-parameter as the model of nucleotide substitution and bootstrap analysis of 1,000 replicates, as incorporated in MEGA v5 [Tamura et al., 2011]. Genotypes were assigned according to the clustering with known prototype sequences. Mutations were analyzed by comparison between isolates and circulating strains

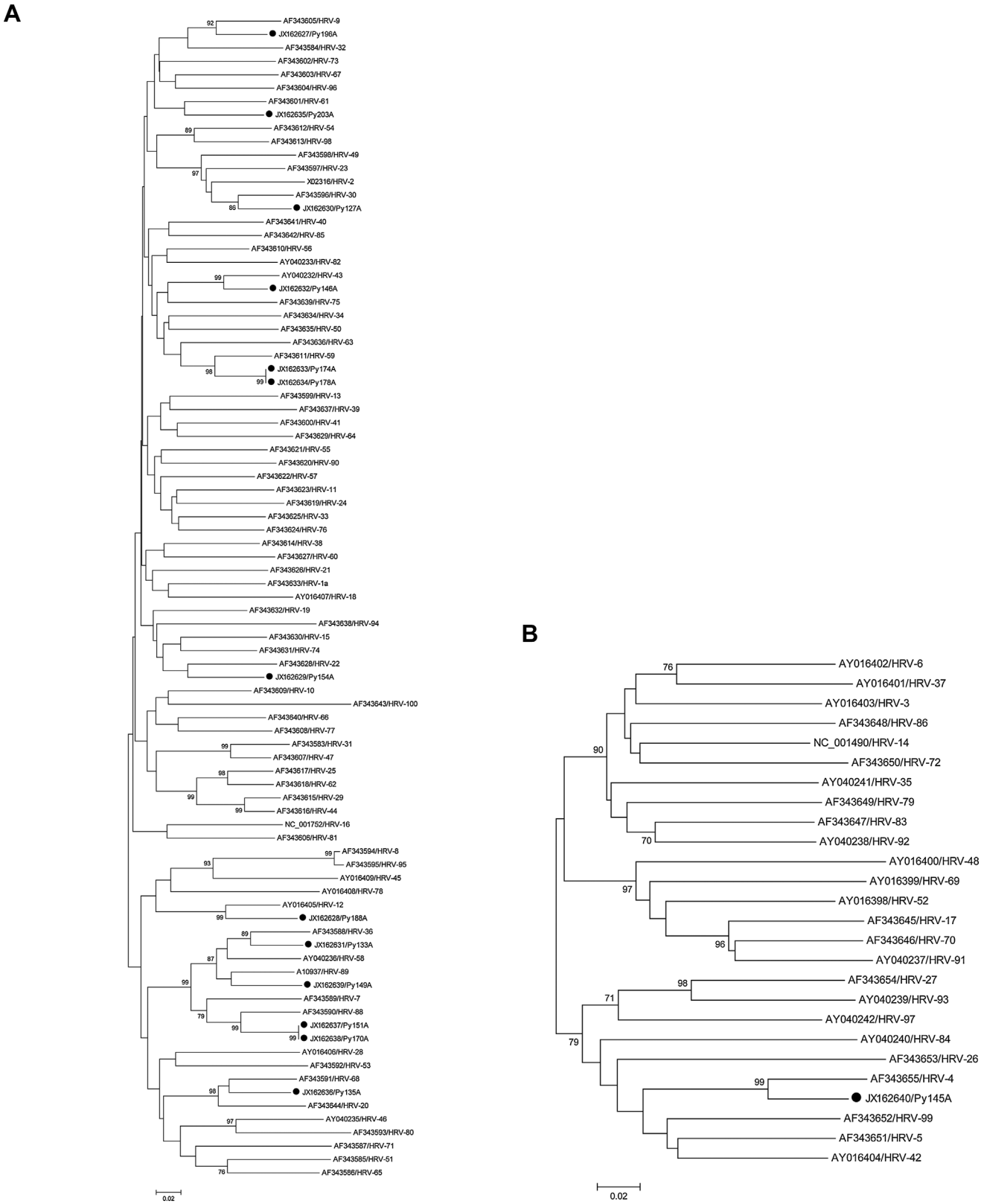


Fig. 1. Phylogenetic tree for members of HRV-A constructed with VP4/VP2 sequences (420-bp), comparing 76 prototype strains and 13 Paraguayan isolates (**A**). Phylogenetic tree corresponding to HRV-B using the same VP4/VP2 region as above, comparing 25 prototype strains and 1 Paraguayan isolate (**B**). Each genotype is indicated with its GenBank accession number and corresponding type. Bootstrap values greater than 70% are shown at branch nodes. Paraguayan isolates are shown with a filled circle. Branch distance is indicated by a scale bar at the bottom of the tree.

worldwide using the BLASTn algorithm [Altschul et al., 1997].

### Accession Numbers

Nucleotide sequences for the VP4/VP2 region of HRVs obtained in this study were deposited in the GenBank database, under the following accession numbers: HRV-A (JX162627–JX162639), HRV-B (JX162640), and HRV-C (JX162641–JX162644).

### Selection Test

The type of evolutionary selection acting on each HRV species was analyzed using the method described by Nei and Gojobori [1986]. Codon-aligned sequences for each VP4/VP2 dataset were analyzed separately using the Perl-based SNAP program (http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html) [Korber, 2000] in order to calculate the variability within each dataset. The selective pressure was measured by comparing the rate of nonsynonymous nucleotide substitutions per nonsynonymous site (dN) against that of synonymous substitutions per synonymous site (dS). The dN/dS ratio was used as an index to assess positive selection. dN/dS $>1$ means positive (diversifying) selection, dN/dS $= 1$ means neutral selection, and dN/dS $<1$ means negative (purifying) selection.

## RESULTS

A total of 34 out of 101 nasopharyngeal aspirates were positive for HRV by real-time RT-PCR (33.7%), and 18 of them were positive for VP4/VP2 genomic amplification. Sequence analysis showed the presence of 13 HRV-A, 1 HRV-B, and 4 HRV-C. The amplification of VP4/VP2 capsid-coding region was not achieved in the remaining samples (n = 16) possibly due to a low amount of genetic material, as indicated by the high CT values (>30, data not shown).

Sixteen genotypes, including 11 genotypes for HRV-A (-9, -12, -22, -30, -36, -43, -59, -61, -68, -88, and -89) (Fig. 1A), one for HRV-B (-4) (Fig. 1B), and four for HRV-C (-C2, -C3, -C6, and -C9) (Fig. 2), were found. HRV-A and HRV-B were detected in nasopharyngeal aspirates collected in 2011, whereas, HRV-C was detected in those collected in 2010. The percentages of identities between HRV isolates and prototype strains ranged from 89% to 96%, indicating their close relationships at nucleotide level. Half of the isolates (9/18) contained nonsynonymous substitutions (one to four mutations) that resulted in amino acid changes when compared to the prototype strains (Table I). Analysis of nucleotide positions that caused amino acid changes revealed that most (9/13) were situated at second codon positions; novel nonsynonymous mutations found in this study corresponded to genotypes HRV-22 (N26S, change A → G) and HRV-C3 (S107N, change G → A).

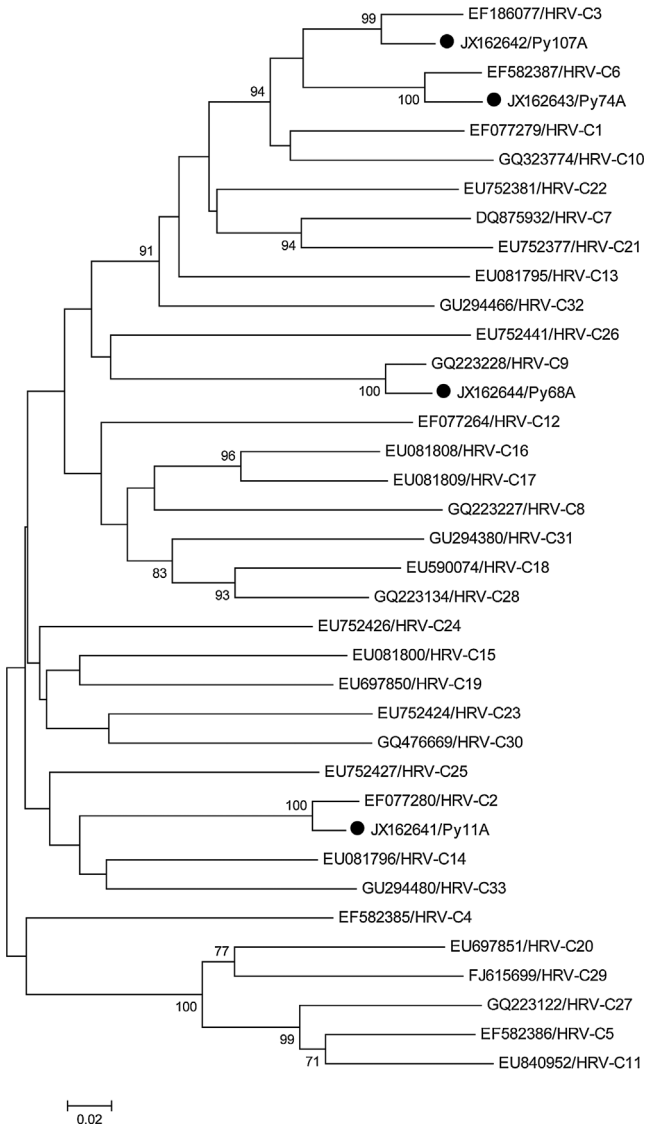


Fig. 2. Phylogenetic tree for members of HRV-C constructed with VP4/VP2 sequences (414-bp), comparing 33 prototype strains and four Paraguayan isolates. Each genotype is indicated with its GenBank accession number and corresponding type. Bootstrap values greater than 70% are shown at branch nodes. Paraguayan isolates are shown with a filled circle. Branch distance is indicated by a scale bar at the bottom of the tree.

Calculation of average dN/dS rates of evolution of the VP4/VP2 genomic region from all three HRV species (including Paraguayan isolates) showed that they have evolved through negative (purifying) selection (Table II), with dN/dS values ranging from 0.0235 to 0.0498. A similar result was obtained by analysis of VP1, another important capsid protein (data not shown).

## DISCUSSION

The diversity of HRV genotypes circulating in children in a defined area (and in a short period of

TABLE I. Percentage of Nucleotide Identities, Number of Mutations and Non-synonymous Changes for HRV Genotypes (VP4/VP2 Region) Reported in This Study.

| Species | Genotype | % Identity | No. of mutations in isolate | Nonsynonymous change (gene) |
|---|---|---|---|---|
| A | 9 | 91 | 37 | T20S (VP4) |
| A | 12 | 91 | 38 | N21G (VP4) |
| A | 22 | 89 | 46 | N26S (VP4), F27Y (VP4), E114Q (VP2), I139T (VP2) |
| A | 30 | 92 | 34 | I139T (VP2) |
| A | 36 | 92 | 34 | L53F (VP4) |
| A | 43 | 92 | 33 | T18M (VP4), D130N (VP2) |
| A | 59[a] | 92 | 32 | — |
| A | 61 | 89 | 45 | — |
| A | 68 | 90 | 40 | — |
| A | 88[a] | 91 | 37 | — |
| A | 89 | 90 | 42 | T97I (VP2) |
| B | 4 | 92 | 33 | — |
| C | C2 | 96 | 15 | M137T (VP2) |
| C | C3 | 95 | 22 | S107N (VP2) |
| C | C6 | 95 | 18 | — |
| C | C9 | 96 | 13 | — |

[a]This genotypes was detected in two isolates.

time) reflects the diversity of HRV genomes that can be generated in a given population. Thus, existence of hot spots for mutations throughout the rhinovirus genome was reported (with both synonymous and nonsynonymous changes) [Kistler et al., 2007b], and even the appearance of different genetic variants throughout the course of respiratory infection [Tapparel et al., 2011]. Based on the published capsid protein structures of HRV-16 [Hadfield et al., 1997], which have at least 90% and 62% of amino acid identities with Paraguayan HRV species A/B and C, respectively, the most nonsynonymous mutations were situated on loops of the 3D protein structures of both VP4 and VP2; thus, the variability observed in the loops could be explained by the presence of low structural constraints in these secondary structures that allow the free interchange of amino acids without compromising the integrity of protein structure. However, three Paraguayan HRV samples contained nonsynonymous mutations situated on beta-sheet secondary structures. These were sample Py154A (HRV-22), which contained mutations N26S and F27Y on VP4, and I139T on VP2; sample Py127A (HRV-30), which had mutation I139T on VP2; and sample Py11A (HRV-C2), which had the same corresponding mutation M137T on VP2. Only one (N26S) of these mutations represented a conserved substitution of amino acid with the same chemical characteristic (asparagine to serine, both with polar side-chains); the remaining mutations represented drastic changes from a nonpolar to polar side-chains (i.e., F27Y, phenylalanine to tyrosine; I139T, isoleucine to threonine; and M137T, methionine to threonine). Among these patients bearing nonsynonymous substitutions on HRV VP4 or VP2 beta-sheets, the one bearing HRV Py154A died; thus, the effect of nonsynonymous mutations on the stability of secondary structures of HRV VP4 and VP2 should be studied more carefully.

Selective pressure analysis of the VP4/VP2 genomic region from all three HRV species (including Paraguayan isolates) showed that they have evolved through negative (purifying) selection, indicating that this DNA region coding for capsid proteins has apparently reached genomic stability, and would resist the appearance of deleterious mutations. It is known that HRVs evolve rapidly due, in part, to their error-prone RNA polymerase, which can generate a high diversity of mutants with different genomic modifications [Drake, 1999]. Furthermore, it has been observed that experimental human infection with HRV starting with a low-frequency mutation population generates a high diversity of hypervariable mutants with modifications on genes coding for both capsid and non-structural proteins [Cordey et al., 2010]. Thus, HRV transmission will be dependent on the generation of hypervariable and stable mutants, and not on a bottleneck effect.

Concerning the epidemiology of HRV in South America, other groups in Brazil and Argentina have reported similar results about the circulation of HRV in children (27–30%) [Moreira et al., 2011; Marcone

TABLE II. Nucleotide Variation on HRV Species (VP4/VP2 Region), Including Paraguayan Isolates.

| Species | Average dS | Average dN | dN/dS | Evolutionary selection |
|---|---|---|---|---|
| A | 2.2537 | 0.0529 | 0.0235 | Negative |
| B | 2.2487 | 0.0576 | 0.0256 | Negative |
| C | 2.3412 | 0.1165 | 0.0498 | Negative |

et al., 2012]. As stated above, in South America few descriptions of HRVs circulation have been reported until now, and the viruses have been characterized only at species level, not genotype level. Only two countries (Brazil and Argentina) have previously reported the known HRV species; these reports include studies with outpatients and hospitalized children as well as adults with respiratory infection [Watanabe et al., 2010; de Almeida et al., 2010; Moreira et al., 2011; Marcone et al., 2012; da Silva et al., 2013]. Thus, future efforts should be made in South America to provide a more detailed genetic characterization of HRVs isolated from patients, in order to clarify the potential severity of different clinical manifestations caused by specific HRV genotypes. In conclusion, this study describes for the first time the diversity of HRV genotypes infecting children with severe lower acute respiratory infection in Paraguay, as well as in South America.

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## REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res 25:3389–3402.

Arden KE, McErlean P, Nissen MD, Sloots TP, Mackay IM. 2006. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. J Med Virol 78:1232–1240.

Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. 1990. Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503.

Cordey S, Junier T, Gerlach D, Gobbini F, Farinelli L, Zdobnov EM, Winther B, Tapparel C, Kaiser L. 2010. Rhinovirus genome evolution during experimental human infection. PLoS ONE 5: e10588.

da Silva ER, Watanabe A, Carraro E, Granato C, Bellei N. 2013. Rhinovirus genetic diversity among immunosuppressed and immunocompetent patients presenting with a severe respiratory infection. J Clin Virol 56:82–83.

de Almeida MB, Zerbinati RM, Tateno AF, Oliveira CM, Romao RM, Rodrigues JC, Pannuti CS, da Silva Filho LV. 2010. Rhinovirus C and respiratory exacerbations in children with cystic fibrosis. Emerg Infect Dis 16:996–999.

Denny FW Jr. 1995. The clinical impact of human respiratory virus infections. Am J Respir Crit Care Med 152:S4–S12.

Drake JW. 1999. The distribution of rates of spontaneous mutation over viruses, prokaryotes, and eukaryotes. Ann NY Acad Sci 870:100–107.

Hadfield AT, Lee W, Zhao R, Oliveira MA, Minor I, Rueckert RR, Rossmann MG. 1997. The refined structure of human rhinovirus 16 at 2.15 A resolution: Implications for the viral life cycle. Structure 5:427–441.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Kaiser L, Aubert JD, Pache JC, Deffernez C, Rochat T, Garbino J, Wunderli W, Meylan P, Yerly S, Perrin L, Letovanec I, Nicod L, Tapparel C, Soccal PM. 2006. Chronic rhinoviral infection in lung transplant recipients. Am J Respir Crit Care Med 174:1392–1399.

Kistler A, Avila PC, Rouskin S, Wang D, Ward T, Yagi S, Schnurr D, Ganem D, DeRisi JL, Boushey HA. 2007a. Pan-viral screening of respiratory tract infections in adults with and without asthma reveals unexpected human coronavirus and human rhinovirus diversity. J Infect Dis 196:817–825.

Kistler AL, Webster DR, Rouskin S, Magrini V, Credle JJ, Schnurr DP, Boushey HA, Mardis ER, Li H, DeRisi JL. 2007b. Genome-wide diversity and selective pressure in the human rhinovirus. Virol J 4:40.

Kitamura N, Semler BL, Rothberg PG, Larsen GR, Adler CJ, Dorner AJ, Emini EA, Hanecak R, Lee JJ, van der Werf S, Anderson CW, Wimmer E. 1981. Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature 291:547–553.

Korber B. 2000. HIV signature and sequence variation analysis. Computational analysis of HIV molecular sequences. Dordrecht, Netherlands: Kluwer Academic Publishers.

Laine P, Blomqvist S, Savolainen C, Andries K, Hovi T. 2006. Alignment of capsid protein VP1 sequences of all human rhinovirus prototype strains: Conserved motifs and functional domains. J Gen Virol 87:129–138.

Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St George K, Briese T, Lipkin WI. 2006. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York state during 2004–2005. J Infect Dis 194:1398–1402.

Lau SK, Yip CC, Tsoi HW, Lee RA, So LY, Lau YL, Chan KH, Woo PC, Yuen KY. 2007. Clinical features and complete genome characterization of a distinct human rhinovirus (HRV) genetic cluster, probably representing a previously undetected HRV species, HRV-C, associated with acute respiratory illness in children. J Clin Microbiol 45:3655–3664.

Ledford RM, Patel NR, Demenczuk TM, Watanyar A, Herbertz T, Collett MS, Pevear DC. 2004. VP1 sequencing of all human rhinovirus serotypes: Insights into genus phylogeny and susceptibility to antiviral capsid-binding compounds. J Virol 78:3663–3674.

Lee WM, Kiesner C, Pappas T, Lee I, Grindle K, Jartti T, Jakiela B, Lemanske RF Jr, Shult PA, Gern JE. 2007. A diverse group of previously unrecognized human rhinoviruses are common causes of respiratory illnesses in infants. PLoS ONE 2:e966.

Marcone DN, Videla C, Ricarte C, Carballal G, Vidaurreta S, Echavarria M. 2012. Rhinovirus detection by real-time RT-PCR in children with acute respiratory infection in Buenos Aires, Argentina. Rev Argent Microbiol 44:259–265.

McErlean P, Shackelton LA, Lambert SB, Nissen MD, Sloots TP, Mackay IM. 2007. Characterisation of a newly identified human rhinovirus, HRV-QPM, discovered in infants with bronchiolitis. J Clin Virol 39:67–75.

Moreira LP, Kamikawa J, Watanabe AS, Carraro E, Leal E, Arruda E, Granato CF, Bellei NC. 2011. Frequency of human rhinovirus species in outpatient children with acute respiratory infections at primary care level in Brazil. Pediatr Infect Dis J 30:612–614.

Nei M, Gojobori T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol 3:418–426.

Papadopoulos NG, Bates PJ, Bardin PG, Papi A, Leir SH, Fraenkel DJ, Meyer J, Lackie PM, Sanderson G, Holgate ST, Johnston SL. 2000. Rhinoviruses infect the lower airways. J Infect Dis 181:1875–1884.

Savolainen C, Blomqvist S, Mulders MN, Hovi T. 2002. Genetic clustering of all 102 human rhinovirus prototype strains: Serotype 87 is close to human enterovirus 70. J Gen Virol 83:333–340.

Simmonds P, McIntyre C, Savolainen-Kopra C, Tapparel C, Mackay IM, Hovi T. 2010. Proposals for the classification of human rhinovirus species C into genotypically assigned types. J Gen Virol 91:2409–2419.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739.

Tapparel C, Cordey S, Junier T, Farinelli L, Van Belle S, Soccal PM, Aubert JD, Zdobnov E, Kaiser L. 2011. Rhinovirus genome variation during chronic upper and lower respiratory tract infections. PLoS ONE 6:e21163.

Watanabe A, Carraro E, Kamikawa J, Leal E, Granato C, Bellei N. 2010. Rhinovirus species and their clinical presentation among different risk groups of nonhospitalized patients. J Med Virol 82:2110–2115.