



Potential Bat-like Rotavirus in Hospitalized Children with Diarrhea from the Dominican Republic

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2021/v8i130225

Editor(s):

(1) Dr. Hetal Pandya, SBKS Medical Institute & Research Center- Sumandeep Vidyapeeth, India.

Reviewers:

(1) Shreyans Singhvi, Smt NHL Municipal Medical College and SVP Hospital, India.

(2) Pon.Arun, King Institute Of Preventive Medicine & Research, India.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/72847>

Short Research Article

Received 20 June 2021
Accepted 25 August 2021
Published 31 August 2021

ABSTRACT

Interspecies transmission is an important aspect of rotavirus evolution and is enhanced by the close contact between humans and animals. The role of bats as a reservoir or intermediary host for viruses associated with human gastroenteritis is poorly understood, but there are reports of rotaviruses detected in humans that contain genes from bat rotavirus. In this study, a total of 15 rotavirus positive samples from children hospitalized for gastroenteritis in 2007 in the Dominican Republic were investigated by sequencing of the capsid VP4, VP7 and VP6 genes to identify genetic variants. The most common genotypes were G1-P[8]-I1, G3-P[6]-I2 and G12-P[8]-I1. Interestingly, 3 of the 15 sequenced strains had VP7 encoding genes highly similar ($\geq 97\%$) to those of bat rotaviruses of the G3 genotype detected in Bulgaria in 2008. These VP7 sequences were more distantly related ($\leq 92\%$) to other G3 rotavirus found in bat, human, rabbit, pigs, rat and monkeys. Only 1 VP4 sequence was available from the bat-like rotavirus yielding genotype P[6].

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This and other identified P [6] sequences were more related to human than to porcine derived P[6] sequences. Furthermore, 4 of 10 available VP6 sequences, including the 3 from the G3 bat-like strains, showed high nucleotide identity (>97%) with VP6 of I2 genotype from bat rotavirus detected in Kenya in 2015. A novel observation was the finding of 4 children of ≥ 1 year of age hospitalized with gastroenteritis and infected with bat-like rotavirus. This study extends previous knowledge on rotavirus interspecies transmission and warrants future rotavirus studies on bats and children from Dominican.

Keywords: Rotavirus; bat; children; VP7; VP4 and VP6.

1. INTRODUCTION

Rotavirus are segmented double stranded RNA viruses that can be classified into nine different genogroups (A-I) based on the major capsid protein VP6, with group A rotavirus being the most important cause of severe gastroenteritis illness in young children worldwide, rotavirus is an endemic pathogen in many regions of the world [1]. It has been considered one of the causes of death in children under five years of age; worldwide it produced more than 500,000.00 deaths [1]. In the Dominican Republic the population of children ≤ 5 years of age experienced due rotavirus mortality rate of 9.1 (per 100,000 children) [2]. Group A rotaviruses are highly diverse genetically and are further classified based on their outer capsid proteins in G-genotypes (VP7) and P-genotypes (VP4), with at least 36 G-genotypes and 51 P-genotypes described to infect humans and animals [3]. However, only six G-P genotypes account for >90% of globally circulating genotypes in humans: G1P[8], G2P[4], G3P[8], G4P[8], G9P[8] and G12P[8] (3). Interspecies transmission is an important aspect of rotavirus evolution and is enhanced by the close contact between humans and animals; with pigs and cattle considered major group A rotavirus reservoirs [4]. Rotaviruses have the capacity to naturally interchange genes during host co-infection with rotavirus of the same or different species; such ability has been exploited in the design of human vaccines [5, 6]. Reassortant rotavirus strains have been described in humans in Central America and the Caribbean Basin (G1P[6], G2P[8], G3P[6], G9P[4]) and a triple reassortment have been described from the Dominican Republic [7,8].

Bats are important reservoirs for zoonotic viral diseases, hosting several viruses pathogenic for humans. Group A rotavirus are also known to infect bats where the most commonly found genotype is G3P[3] [9, 10]. The role of bats as reservoir or intermediary host for viruses

associated with human gastroenteritis is poorly understood, but there are reports of rotavirus detected in humans that has genes from bat rotavirus [10, 11].

2. MATERIALS AND METHODS

Stool samples from 15 children with diarrhea of ≤ 3 years of age from Santo Domingo, the Dominican Republic were randomly selected from a subset of 47 rotavirus-positive from a surveillance study (unpublished) carried out between February and April 2007. The rotavirus VP7, VP4 and VP6 encoding genes from these samples were sequenced as described elsewhere [12]. Sequence alignments were performed with the Clustal W algorithm from BioEdit Sequence Alignment Editor, version 7.1.3.0 [13]. Phylogenetic analysis of the aligned sequences was performed with the MEGAX 10.0.5.1 [14], using a distance-based neighbour-joining method. Phylogenetic distances were measured using the Kimura 2-parameter model. The statistical significances of the phylogenetic trees constructed were supported by bootstrap values calculated from 1,000 replicates.

3. RESULTS

3.1 Rotavirus Genotypes

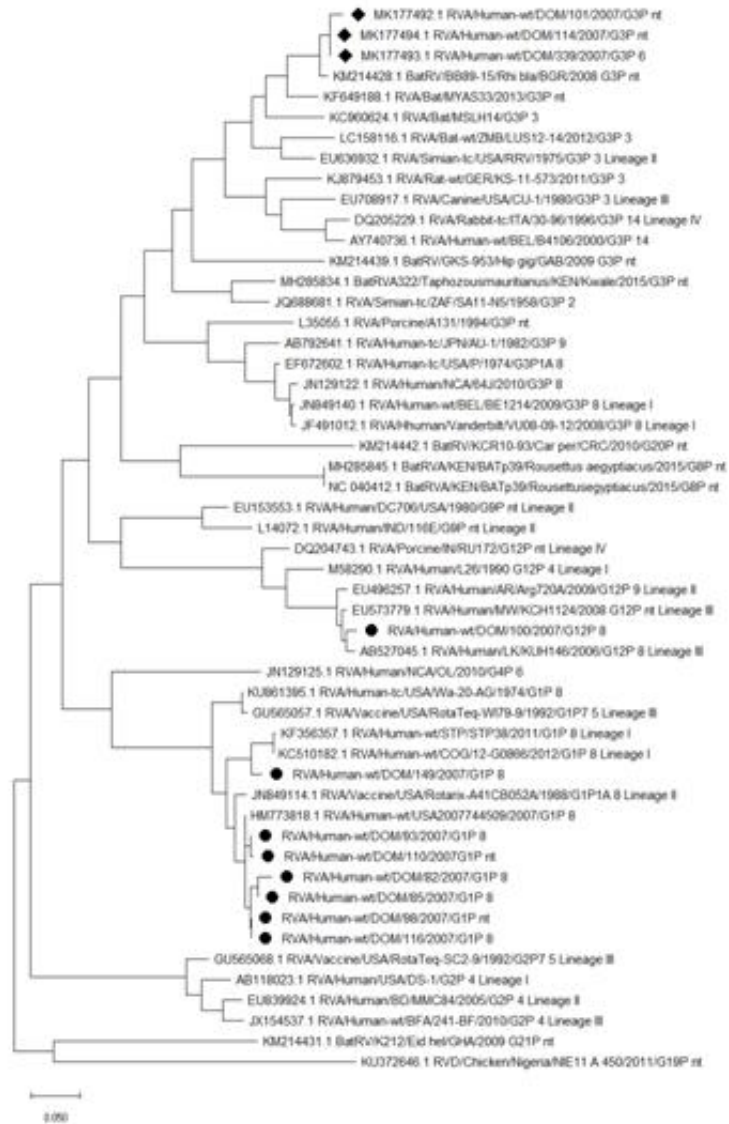
Among the 15 sequenced samples, the most common G-type was G1 (n=7), followed by G3 (n=3) and G12 (n=1), G genotype was not determined in 4 samples due to poor sequence quality. The most common P-types were P[8] (n=6) and P[6] (n=3) with P genotype not determined in 6 samples. Most of the VP6 genotypes were I1 (n = 6) followed by I2 (n = 4), in 5 samples VP6 genotype was not determined. The most common combinations were G1-P[8]-I1 (n=3), G3-P[6]-I2 (n=1) and G12-P[8]-I1 (n=1), besides, partially typed G1-P[8]-Int (n = 2), G1-P[nt]-I1 (n = 1), G1-P[nt]-Int (n = 1), G3-P[nt]-I2 (n = 2), Gnt-P[6]-Int (n = 2), Gnt-P[nt]-I1 (n = 1), Gnt-P[nt]-I2 (n = 1).

3.2 VP7 and VP6 Encoding Genes were Related to bat Rotavirus Strains

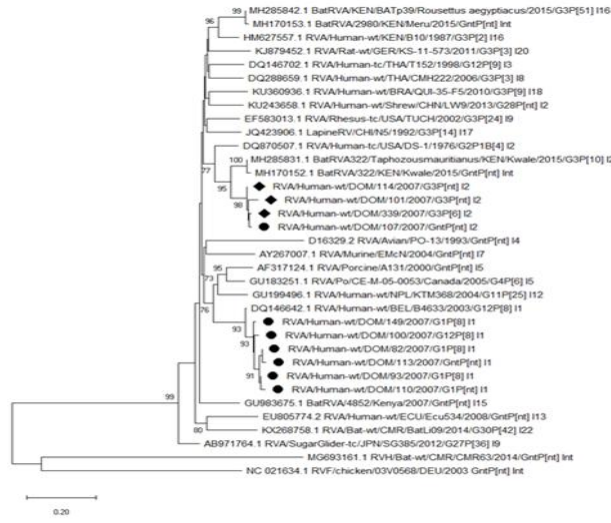
A total of 3 of 11 VP7 available sequences were highly similar to those of bat rotaviruses of the G3 genotype (Fig 1A). Pairwise nucleotide identities of these VP7 genes were highest ($\geq 97\%$) with VP7 sequences of bat rotavirus from Bulgaria (BatRV/BB89-15/Rhi_bla/BGR/2008). These sequences were more distantly related ($\leq 92\%$) to other G3 rotavirus from bat, human, rabbit, pigs, rat and monkeys (Fig. 1A). A total of 4 VP6 sequences, including the Dominican Republic G3 strain, showed high nucleotide

identity ($>97\%$) with VP6 of I2 genotype derived from bat rotavirus from Kenya (Fig 1B). The remaining VP6 sequences of the other strains clustered together with the human VP6 of I1 genotype (Fig. 1B). Of the Dominican Republic G3 strains, only 1 VP4 sequence was available yielding the genotype P[6]. This and the other 2 identified P[6] sequences were more related to human than to porcine derived P[6] sequences (Fig. 1C). The remaining P[8] sequences from the current study clustered with human P[8] sequences of rotavirus circulating in either USA (United States of America) or COD (Democratic Republic of the Congo) in 2007.

VP7 (A)



VP6 (B)



VP4 (C).

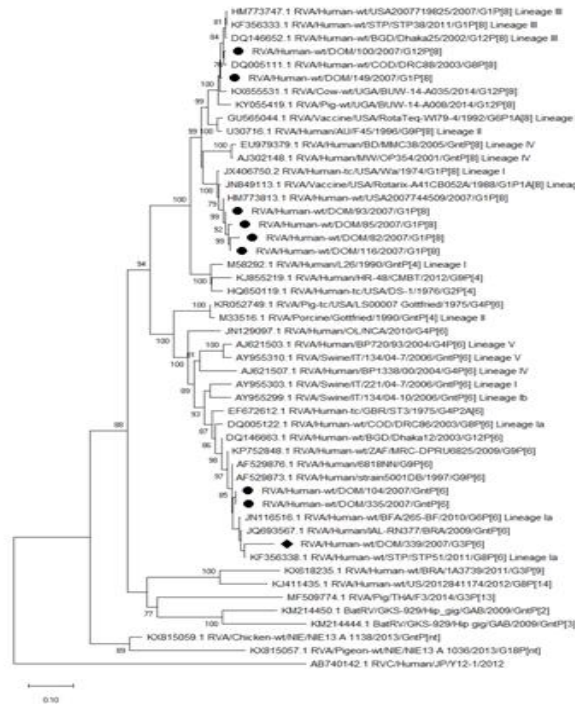


Fig. 1. Phylogenetic analysis of partial VP7 (A), VP6 (B) and VP4 (C) encoding genes from rotavirus strains circulating in the Dominica Republic in 2007. Trees were constructed with VP7, VP6 and VP4 sequences segments of 776, 790 and 330 base pairs, respectively by using the Neighbor-Joining method, with Kimura 2-parameter model, with 1,000 bootstrap re-samplings (bootstrap values ≥ 70 are indicated at each node). References for Lineage within each genotype were also included in each VP7 and VP4 trees. The black rhombus represents the bat-like rotavirus and black circle represents others variants observed in this study. The name of each sequence is given according to recommendations from the international committee for rotavirus strains classification [19]. [nt] stands for Not Typed due to poor sequence quality

4. DISCUSSION

This study extends previous knowledge on rotavirus interspecies transmission and genetic reassortment. A novel observation was the finding of a bat-like rotavirus with the genotype G3P[6]I2, which is reported for the first time in hospitalized children with diarrhea. Furthermore, the VP4 encoding gene of this sample (RVA/Human-wt/DOM/339/G4P[6]) was more similar to VP4 of P[6] genotypes circulating in human than to porcine P[6] genes (Fig. 1C). Data on direct rotavirus transmission from bats to humans is poorly understood [10], but, some studies suggest multiple intermediary hosts [15]. For instance, Donato and coworkers [11], reported a G3P[14] rotavirus strain causing gastroenteritis in a 12 year old Australian child which contains genes (VP7, VP1, VP2 and NSP1) highly homologous to a Chinese bat rotavirus (MSLH14) and others genes homologous to bovine, canine and feline rotavirus strains. Similarly, Dong and coworkers [16] reported the genomic characterization of a G3P[10] rotavirus (MYAS33) from a child with gastroenteritis from Guangxi, China, which had genes (VP7, VP1, VP2, NSP2 and NSP3) highly homologous to bat rotavirus circulating in the same region, but also other genes (VP4, VP3, NSP1, NSP4 and NSP5) highly homologous to simian rotavirus. Studies of rotavirus infecting children from Surinam and Ecuador have independently reported G20 P [28] strains harboring genes (VP7, VP4, VP6) highly similar to bat rotavirus [17, 18]. The finding in this study of G3P [6] I2 rotavirus with VP7 and VP6 genes highly similar to those of bat rotavirus, with the VP4 gene similar to humans rotavirus is suggestive that one or several intermediary hosts may have played a role in transmission to humans. The observation that G3 rotavirus are commonly found in humans, canine and feline species, but P[6] genotype is commonly detected in human patients all around the world, and it was also previously detected in the Dominican Republic and Costa Rica (7). Sequences from the entire rotavirus genome from bat-like rotavirus reported in the current study would have been helpful in elucidating the evolutionary origin of this rotavirus, but the remaining stools from the previous RT-PCR analysis were not sufficient to perform more analysis. The current and previous studies by Bourdett-Stanziola and coworkers (7), reporting common detection of G3P[6] rotavirus in Dominican Republic is interesting and warrants future rotavirus studies in bat and children from this country.

It is worth noting that the VP4 (ligand protein) is important for attachment and entry in the host cells, and is likely a major determinant of species susceptibility, with the globally dominant human genotypes P[8], P[6] and P[4] recognizing different histo-blood group antigens common in human populations (19). The fact that the VP4 gene, genotype P[6] of the Dominican Republic bat-like rotavirus was more similar to humans than to porcine genes thus suggests possibility for these and similar strains to establish in the human population.

Phylogenetic studies carried out worldwide for the rotavirus VP7 and VP4 protein demonstrate differences in the lineage and sub-lineage of genotypes that affect humans and also reveal a great genetic variability of rotavirus genotypes [15]. To date, research carried out in Central America and the Caribbean shows the appearance of unusual strains [7] and genetic rearrangements that reveal a possible zoonotic transmission [18]. It is important to highlight that in the Dominican Republic there are reports of genetic rearrangements of rotavirus strains excreted in children with diarrhea of origin, equines, bovines and pigs [20, 21]. Although, interspecies transmission has not been documented to occur directly, an increase of the number of reports of atypical rotavirus strains has been reported in the world. However, studies on rotavirus zoonosis have a limitation because there are not full study reports in Latin America, on the sequencing of the rotavirus genotypes in animals, where, there is an information bank of circulating strains that allows epidemiologically correlate among strains detected in humans and animals. Therefore, the study of the zoonotic event of a certain strain of rotavirus is confirmed only on the basis of phylogenetic evidence [22].

5. CONCLUSION

In response to the surveillance reports of unusual strains with zoonotic potential carried out in the Dominican Republic, little information is evidenced. Therefore, we consider urgent, the need to maintain molecular surveillance of rotavirus strains in this country; the results of the molecular characterization of these strains would contribute in the future new knowledge about the possible genetic rearrangements of rotavirus. We consider that the detection of new unusual strains with emerging zoonotic potential of rotavirus in the Dominican Republic, raises interesting questions about the evolution of rotavirus in the Latin American region; with the

intention of evaluating the impact of vaccine in the future.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard, parental written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENTS

This study was supported with grants from NETROPICA (FB, No. 05-N-2010), The Swedish Research Council (FB&JN, No. 2016-05641), Glaxo Smithkline Panamá and ADA was supported by the National Secretariat of Science and Technology of Panama (SENACYT) through the Research National System (SNI).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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