Available online at www.sciencedirect.com
ScienceDirect
Virus Research 125 (2007) 219–225
Virus Research
www.elsevier.com/locate/virusres

Sequence analysis and evolution of group B rotaviruses

Mustafizur Rahman [a,b,*], Zahid M. Hassan [a], Hasan Zafrul [a], Farjana Saiada [a], Sukalyani Banik [a], Abu S.G. Faruque [a], Thomas Delbeke [b], Jelle Matthijnssens [b], Marc Van Ranst [b], Tasnim Azim [a]

[a] *Laboratory of Virology, ICDDR,B, GPO Box-128, Dhaka 1000, Bangladesh*
[b] *Laboratory of Clinical and Epidemiological Virology, Rega Institute for Medical Research, University of Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium.*

Received 29 July 2006; received in revised form 10 January 2007; accepted 12 January 2007
Available online 12 February 2007

Abstract

Human group B rotaviruses were isolated from hospitalized patients in Bangladesh between July 2003 and December 2004. Phylogenetic analyses of the gene segments encoding the hemagglutinin (VP4), glycoprotein (VP7) and RNA-binding protein (NSP2) of group B rotaviruses showed that Bangladeshi strains were more similar to the Indian strains than to the prototype Chinese strains. Moreover, all human strains were clustered together and were distantly related to the animal strains. With limited sequence data, the evolutionary rate of the glycoproteins (VP7) of human group B rotaviruses was estimated to be 1.57×10^{-3} nucleotide substitutions/(site year), which was comparable to other rapidly evolving RNA viruses. The most recent common ancestor (MRCA) of the extant human group B rotaviruses was calculated to date to around 1976.

Keywords: Group B rotavirus; VP4; VP7; NSP2; Evolution; Phylogeny

1. Introduction

Rotaviruses are a major causative agent of severe diarrhea in humans and animals. They are classified into seven antigenically distinct groups (A–G) on the basis of a common group antigen VP6 (Estes, 2001). Groups A–C infect humans and animals but groups D–G infect animals only (Kapikian et al., 2001). Group B rotaviruses are called adult diarrhea rotavirus (ADRV) because they mostly infect adults and older children (Hung et al., 1983; Mackow et al., 1993). The human group B rotaviruses were first detected in China in 1982 and since then they have been causing large waterborne epidemics infecting thousands of people there (Chen et al., 1985; Hung et al., 1984; Fang et al., 1989; Yang et al., 1998). For many years they were restricted to China, but recently they have been isolated sporadically in India (since 1998) and Bangladesh (since 2001) (Ahmed et al., 2001; Sanekata et al., 2003; Kelkar et al., 2004; Kobayashi et al., 2001). The animal group B rotaviruses were isolated from lambs (United Kingdom and the United States), pigs (United States and Japan), cows (the United States, Japan and India), goat (China) and rats (the United States) (Chasey and Banks, 1984; Theil et al., 1985, 1995; Parwani et al., 1996; Sanekata et al., 1996; Barman et al., 2004; Tsunemitsu et al., 1999; Eiden et al., 1991). Interestingly, like human group B strains, the animal group B rotaviruses were mostly isolated from adult animals. The scientific mystery surrounding the reason why they infect mainly adults remains to be addressed.

The importance of the viruses in causing diarrhea in humans is poorly understood because no diagnostic method for detecting group B rotaviruses is commercially available at this moment.

* Corresponding author at: Virology Laboratory, Laboratory Sciences Division, ICDDR,B, GPO Box-128, Dhaka 1000, Bangladesh.
Tel.: +880 2 8860523–32x2409; fax: +880 2 881 2529.
E-mail addresses: mustafizur.rahman@uz.kuleuven.ac.be (M. Rahman), zahidbd74@yahoo.com (Z.M. Hassan), jafrul05@yahoo.com (H. Zafrul), farsa7@gmail.com (F. Saiada), sukalyani_banik@yahoo.com (S. Banik), gfaruque@icddrb.org (A.S.G. Faruque), thomas.delbeke@student.kuleuven.be (T. Delbeke), jelle.matthijnssens@uz.kuleuven.ac.be (J. Matthijnssens), marc.vanranst@uz.kuleuven.ac.be (M. Van Ranst), tasnim@icddrb.org (T. Azim).

0168-1702/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2007.01.008

Nonetheless, few sero-prevalence studies in China, Hong Kong, Australia, the United States, Canada, Kenya, and the United Kingdom suggest that they are present in low proportion of the global population (Penaranda et al., 1989; Nakata et al., 1987; Krishnan et al., 1999; Mackow, 1995; Ushijima et al., 1992).

Group B rotaviruses belong to the Reoviridae and have a double stranded RNA genome made up of 11 independent gene segments. They can be differentiated from groups A and C rotaviruses by tracking the migration pattern of their segmented RNA (4-2-1-1-1-1-1) on polyacrylamide gel electrophoresis (PAGE) (Saif and Jiang, 1994). No serotyping/genotyping system has been described for group B rotaviruses due to the difficulties in adapting them in cell culture and availability of adequate amount of gene sequence data.

During July 2003–December 2004 we detected group B rotaviruses in diarrhea patients attended the Dhaka hospital of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). We partially sequenced the gene segments encoding VP4, VP7 and NSP2 proteins of Bangladeshi group B rotaviruses to investigate their genetic relationships with the cognate genes of other group B rotavirus strains circulating worldwide. The molecular dating and evolutionary rates were estimated only for the VP7 genes since adequate sequence data for other gene segments were unavailable.

2. Materials and methods

2.1. Study population and data collection

The Dhaka hospital of ICDDR,B treats over 100,000 diarrhea patients each year and includes every fiftieth (2%) patient in the hospital surveillance system to determine the presence of various common enteric pathogens. During July–December 2003, a total of 856 stool specimens which were included in the hospital surveillance system were tested for group A rotavirus antigen and 245 (28.6%) were positive. The negative specimens ($n = 611$) were tested for group B rotaviruses. In contrast, 2188 stool specimens from hospital surveillance system were tested for various enteric pathogens during January–December 2004, which included group A rotaviruses, *Salmonella typhi*, non-typhi Salmonella, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, *Vibrio cholerae O139*, and *Vibrio cholerae O1*. No pathogen was detected in 1050 samples from which 10% samples ($n = 100$) were tested for group B rotaviruses. The clinical data of the patients included in the study were collected from the hospital surveillance system.

2.2. Electropherotyping

One hundred microliters of 10% stool suspensions in phosphate buffered saline were treated with sodium acetate and extracted with an equal volume of phenol:chloroform: isoamylalcohol (25:24:1) mixture. The extracted RNA was tested for electropherotype (E-type) by polyacrylamide gel electrophoresis (PAGE) as described by Herring et al. (1982).

2.3. Reverse transcription (RT)-PCR

RNA was extracted from the stool suspension using the QIAamp Viral RNA mini kit (Qiagen/Westburg, Leusden, The Netherlands) according to the manufacturer's instructions. RT-PCR was carried out using the Qiagen OneStep RT-PCR Kit (Qiagen/Westburg). The primers specific for the group B rotavirus NSP2 gene (Bl, 5′-CTATTCAGTGTGTCGTGAGAGG-3′; and B4, 5′-CGTGGCTTTGGAAAATTCTTG-3′) were used as described by Gouvea et al. (1991). The reaction was carried out with an initial reverse transcription step at 45 °C for 30 min, followed by 40 cycles of amplification (30 s at 94 °C, 30 s at 50 °C, 1 min at 72 °C), and a final extension of 7 min at 72 °C in a thermal cycler. PCR products were run on a 1.5% ethidium bromide stained agarose gel and visualized under UV-light. Specific segment size (489 bp) for group B rotavirus DNA product was observed on stained gel.

2.4. Nucleotide sequencing

The PCR products were purified with the QIA quick PCR purification kit (Qiagen/Westburg), and sequenced in both directions using the dideoxy-nucleotide chain termination method with the ABI PRISM® BigDye Terminator Cycle Sequencing Reaction kit (Perkin-Elmer Applied Biosystems, Foster City, California) on an automated sequencer (ABI PRISM™ 310). The consensus primers GrB_VP7_25F (5′-CTTCTCGTCCTTGCTGCTG-3′; strain Bang373, accession number AY238385, nt 25–43), GrB_VP7_814R (5′-GGGTTTTTACAGCTTCGGC-3′; strain Ban373, nt 796–814), GrB_VP4_13F (5′-GCTATGTTGACGTATTTACG-3′; strain Bang373, accession number AY238388, nt 13–32), and GrB_VP4_1178R (5′-GTATAACCAGAAGCGTC CAC-3′; strain Bang373, nt 1159–1178) were used for amplifying and sequencing of the VP7 and VP4 gene segments. The NSP2 primers used in the RT-PCR were employed for NSP2 gene sequencing.

2.5. DNA and protein sequence analysis

The chromatogram sequencing files were inspected using Chromas 2.23 (Technelysium, Queensland, Australia), and consensus sequences were prepared using SeqMan II (DNASTAR, Madison, WI). Nucleotide and amino acid sequence similarity searches were performed using the National Center for Biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) BLAST (Basic Local Alignment Search Tool) server on GenBank database, release 153.0 (Altschul et al., 1990). Multiple sequence alignments were calculated using CLUSTALX 1.81 (Thompson et al., 1997). Sequences were manually edited in the GeneDoc Version 2.6.002 alignment editor (Nicholas et al., 1997).

2.6. Phylogenetic analysis

Phylogenetic analyses were conducted using the MEGA Version 2.1 software package (Kumar et al., 2004). The dendrograms were constructed using the neighbor-joining method.