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# Genetic characterization of a rare bovine-like human VP4 mono-reassortant G6P[8] rotavirus strain detected from an infant in Bangladesh

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ABSTRACT

During an ongoing diarrhea etiology surveillance in Mirzapur, Bangladesh, a rare human G6P[8] RVA strain (RVA/Human-wt/BGD/KH2288/2011/G6P[8]) was detected in a stool sample of a 7-month-old infant with acute diarrhea. Complete genotype analyses revealed that KH2288 possessed the G6-P[8]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotype constellation. Sequence analysis of the VP7 gene revealed a close phylogenetic relationship with bovine G6 strains from India, whereas, the VP4 gene segment was nearly identical to typical human P[8] strain circulating in Bangladesh and the rest of the world. Phylogenetic analysis of the remaining nine gene segments revealed a close relatedness to either animal or animal derived human RVA strain. We speculated that, strain KH2288 was a monoreassortant between a human RVA strain and a RVA strain typically infecting member of the Artiodactyla, such as cattle, goat or sheep. To our knowledge, this is the first complete genotyping report of a naturally occurring G6P[8] RVA strain, worldwide.

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## 1. Introduction

Group A rotaviruses (RVAs) are an important cause of diarrheal diseases, and cause significant morbidity and mortality in young children and animals worldwide, especially in developing countries (Estes and K.A., 2007) . It was estimated that 12–14% of childhood hospitalizations in Bangladesh were due to RVA (Tanaka et al., 2007). Among country estimates of RVA related deaths among children, Bangladesh ranked 9th with 9857 deaths per year among those less than 5 years, and accounting for 2% of the total global mortality (Unicomb et al., 1997).

The virus belongs to the family *Reoviridae*, and possess a genome of 11 segments of double stranded RNA, encoding six structural (VP) and six non-structural proteins (NSP). Traditionally, RVAs are characterized based on their outer capsid proteins, VP7 (G genotypes) and VP4 (P genotypes) (Estes and K.A., 2007) . Based on the full genome sequences of all 11 genome segments, a RVA genotyping classification system has been proposed and so far at least 27 G (VP7), 37 P (VP4), 17 I (VP6), 9 R (VP1), 9 C (VP2), 8 M (VP3), 16 A (NSP1), 10 N (NSP2), 12 T (NSP3), 15 E (NSP4), and 11 H (NSP5/6) genotypes have been described (Matthijnssens et al., 2008b; Matthijnssens et al., 2011; Trojnar et al., 2013).

To date, at least 12 G- (G1–G6, G8–G12, G20) and 14 P- (P[1], P[3]–P[11], P[14], P[19], P[25], and P[28]) genotypes have been detected from humans, but only G1–G4 and G9 are currently of epidemiological importance worldwide (Estes and K.A., 2007; Matthijnssens et al., 2008a). Notably, the frequent detection of G12 RVA strains in recent years suggests the emergence of this genotype globally (Matthijnssens et al., 2010a; Rahman et al., 2007a).

Based on the complete RVA genome sequence comparisons, two major genotype constellations among human, Wa-like (I1-R1-C1-M1-A1-N1-T1-E1-H1) and DS-1-like (I2-R2-C2-M2-A2-N2-T2-E2-H2) have been described (Matthijnssens and Van Ranst, 2012). The majority of the human Wa-like gene segments are believed to have a common ancestor with porcine RVA strains, whereas several of the human DS-1-like gene segments are believed to have a common ancestor with bovine RVA strains (Matthijnssens et al., 2008b). A third minor human genotype constellation; AU-1-like (I3-R3-C3-M3-A3-N3-T3-E3-H3) is believed to have originated from cats or dogs (Nakagomi et al., 1990). Additionally, many unusual RVA genotype constellations

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have been described in humans, such as (i) reassortants possessing typical human Wa and DS-1 gene segments,(ii) typical animal genotype constellations or (iii) mixed animal × human gene constellation, but these are usually unable to spread efficiently among humans (Ghosh and Kobayashi, 2011; Martella et al., 2010; Matthijnssens and Van Ranst, 2012).

G6 is considered a typical bovine RVA genotype, but is occasionally detected in humans all over the world in combination with a wide range of human or animal P-genotypes, most notably P[14] (Banyai et al., 2003; Ciarlet et al., 2008; Gerna et al., 1992; Griffin et al., 2002; Kelkar and Ayachit, 2000; Matthijnssens et al., 2008a; Palombo and Bishop, 1995; Rahman et al., 2003; Van Damme et al., 2007). To date, the complete genomes have been determined for 12 human G6 RVA strains: 7 G6P[14], 2 G6P[9], 1 G6P[11], 1 G6P[1] and 1 human-animal reassortant G6P[6] RVA strain (Banyai et al., 2009; Doan et al., 2013; El Sherif et al., 2011; Matthijnssens et al., 2009; Matthijnssens et al., 2008c; Steyer et al., 2013; Yamamoto et al., 2011). Genetic analysis revealed that many of these human G6 RVA strains possess a genotype constellation (I2-R2/5-C2-M2-A3/11/13-N2-T6-E2/E12-H3) which is shared with RVA strains detected from cattle or other members of the artiodactyla such as goat, antelope, buffalo, sheep or the guanaco (Ciarlet et al.,2008; Matthijnssens and Van Ranst, 2012).

Regardless of the G genotypes, the P[8] RVA genotype with a Wa-like genotype constellation has been observed as the most common RVA genotype worldwide during at least three decades. Several studies investigating the complete genome of human G1P[8], G3P[8], G4P[8], G9P[8] and G12P[8] RVA strains have shown the consistent presence of Wa-like genotype constellations (Heiman et al., 2008; Matthijnssens et al., 2008b; Matthijnssens et al., 2008d; McDonald et al., 2009, 2011; Rahman et al., 2010). It has been speculated that this genotype constellation is the reason of their successful spread among humans. Furthermore, small numbers of human G5, G8, G10 and G11 RVA strains have also been described with P[8] possessing the Wa-like genotype constellation (Esona et al., 2009; Heiman et al., 2008; Matthijnssens et al.,2010c).

In 2011, during an ongoing diarrhea etiology surveillance in Mirzapur, Bangladesh, we identified a rare human G6P[8] strain, RVA/Human-wt/BGD/KH2288/2011/G6P[8] in an 7-month-old child with acute diarrhea. It has been shown before for animal derived G9 and G12, that the acquisition of the P[8] genotype in combination with a Wa-like genotype constellation can result in a rapid spread of such novel reassortant RVA strains around the world (Matthijnssens et al., 2010a). To investigate the genotype constellation of our unusual G6P[8] RVA strain, and its potential for successful dissemination among the human population, we conducted phylogenetic analysis of all the 11 genome segments of this strain. This is the first report of the full genetic analysis of a human G6P[8] RVA strain.

## 2. Methods

### 2.1. Rotavirus strain

Fecal specimen KH2288 was collected in 2011 in the framework of a systematic diarrhea etiology surveillance study conducted by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The surveillance was approved by the Research Review Committee and Ethical Review Committee of icddr,b.

The sample was identified as positive for RVA by a solid-phase sandwich-type enzyme immunoassay against VP6 described elsewhere (Rahman et al., 2007b).

### 2.2. RNA Extraction and RT-PCR

Viral RNA was extracted using the QIAamp Viral RNA mini kit (Qiagen, Leusden, The Netherlands) according to the manufacturer's instructions. In order to identify the VP7 and VP4 genotype, traditional multiplex reverse transcription (RT)-PCR was performed with G1, G2, G9, G12, P[4], P[6] and P[8] genotyping primers described elsewhere (Gentsch et al., 1992; Rahman et al., 2007b). The extracted RNA was denatured at 97 °C for 3 min and reverse transcription followed by polymerase chain reaction (RT-PCR) was carried out using the Qiagen OneStep RT-PCR kit (Qiagen). Primers used in this study are listed in Supplementary file 1. The RT-PCR reaction was carried out with an initial reverse transcription step at 45 °C for 30 min, followed by PCR activation at 95 °C for 15 min, 35 cycles of amplification and a final extension of 10 min at 72 °C. For VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 the amplification cycle conditions were as follows: 45 s at 94 °C, 60 s at 48 °C and 2 min at 72 °C. For VP1, VP2, VP3 and VP4, the cycle conditions were 45 s at 94 °C, 45 s at 48 °C and 2 min at 72 °C.

### 2.3. Sequence analysis

Nucleotide sequencing was carried out in an automated ABI 3500 xL Genetic Analyzer (Applied Biosystem, Foster City, CA) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem), as per kit protocol. The electropherogram files were inspected using Chromas 2.23 (Technelysium). Sequence similarity searches were performed using the National Center for Biotechnology Information (National Institutes of Health, Bethesda, MD, USA) Basic Local Alignment Search Tool (BLAST) server on GenBank database release 2.2.13. Sequences were aligned by using the ClustalW program located in the BioEdit 7.0.5 suite of programs (Hall, 1999).

### 2.4. Phylogenetic analysis

Phylogenetic trees were constructed using the neighbor-joining method. The bootstrap probability at a branching point was calculated with 1000 pseudoreplicate data sets. Genetic distances at the nucleotide level were calculated using the Kimura two-parameter method. The nucleotide sequences of the 11 gene segments of strain KH2288 have been deposited in GenBank under the accession numbers KC986962-KC986972 for the genes encoding VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5, respectively.

## 3. Results

A seven-month-old Bangladeshi male infant was submitted to the out-patient department of Kumudini Hospital, Mirzapur, Bangladesh on June 14th, 2011 with complaints of watery diarrhea for 7 days, vomiting, fever, cough and anal excoriation. The mother did not mention a history of abdominal pain, or convulsion. On physical examination, the child had fever (38.7 C), radial pulse 100/min, respiratory rate 36/min, but no sign of dehydration. The child was immunized against tuberculosis, polio, diphtheria, pertussis, tetanus, H. influenzae B (Hib), and hepatitis B. The child was well nourished and was treated with ORS, zinc, and paracetamol at the facility.

### 3.1. G and P-genotype analyses

The VP7 gene of KH2288 strain could not be genotyped by the multiplex RT-PCR method and was therefore subjected to sequence

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