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Highlights

- Analyses reinforce the notion of signature NSP4 genotype for ungulates from South America.
- Surveillance in humans and animals will help in understanding rotavirus epidemiology.
Group A rotaviruses (RVAs) are one of the major pathogens causing diarrhea in children and young animals worldwide. The genome of rotaviruses consists of 11 double-stranded RNA (dsRNA) segments that encode 6 structural (VP1–4, VP6, VP7) and 6 non-structural proteins (NSP1–6). RVA present a triple-layered protein capsid, and the most outermost surface proteins, VP7 and VP4, independently induce neutralizing antibodies. Genetic and antigenic differences within these two proteins have been used to classify rotaviruses in G and P types, respectively (Estes and Greenberg, 1991). The genomic diversity and allowed the precise identification of the RVA genetic relationships among human and animal rotaviruses (Matthijnssens et al., 2008a).

Surveillance of rotavirus has revealed that five strains (G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]) largely predominate in humans worldwide (Santos and Hoshino, 2005). The detection of uncommon strains in humans has been linked to animal strains, and their increasing frequency has raised concerns for massive vaccination programs (Degiuseppe et al., 2013; Martella et al., 2010; Weinberg et al., 2012). On this regard, G8 strains have been associated with diarrhea in cattle and other species from the Artiodactyl order (Fukai et al., 1999; Okada and Matsumoto, 2002; Parreno et al., 2004), but also have been found circulating in humans at high frequencies in Africa (Cunliffe et al., 1999), and sporadically in Europe, the Americas, and Asia (Ahmed et al., 2013; Banyai et al., 2009b; Delogu et al., 2013; Park et al., 2011; Pietsch et al., 2009). This study describes the detection of a G8P[1] rotavirus strain in stool sample from a Paraguayan child presenting acute gastroenteritis (AGE).

A total of 118 fecal samples collected from children ≤5 years old with non-bacterial AGE were analyzed by Next Generation Sequencing (NGS) to identify potential new viruses associated with this disease. The patients were admitted as ambulatory or hospitalized in a large private hospital from Asunción, Paraguay between January 2004 and December 2005. The samples were randomly selected from a set of 205 previously tested negative for rotavirus and norovirus. (Amarilla et al., 2007; Galeano et al., 2013; Parra et al., 2005). A total of 140 µl of the stool supernatant was filtered through a 0.45-µm filter (Millipore), and the filtrate was treated with a cocktail of DNases (Turbo DNase from Ambion, Baseline-ZERO from Epicentre, and Benzonase from Novagen) and RNase A (Fermentas) to digest unprotected nucleic acids. Viral nucleic acids were extracted with the QiAamp spin-columns following manufacturer’s instructions (Qiagen). The nucleic acids from five fecal samples were pooled together. Viral metagenome

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Enteric adenoviruses. Of note is that initial screening of rotaviruses was done by detection of characteristic viral dsRNA in polyacrylamide gel electrophoresis (PAGE) while for monovalent and wild-type strains, a set of RT-PCR using primers for the NGS gene from rotaviruses was performed. In order to confirm the transmission of rotavirus G8P[1] from human to human, we assessed the origins of the rotavirus G8P[1] detected in a child with diarrhea. The monovalent strains were detected in various animals and humans. The mammalian species infected by rotavirus G8P[1] are described in their names. Maximum and minimum values of similarities were selected for each gene for the heat map. Color legend is indicated at the bottom of the figure. Additionally, the figure shows the similarity obtained in the BLAST analysis. The cut-off for highly significant sequence similarity was 10%.

![Heat map of rotavirus G8P[1] similarity](image)

Ten gene segments were successfully amplified using primers (Degiuseppe et al., 2013; Fujii et al., 2012; Matthijnssens et al., 2006a) and newly designed (available upon request), we have no success amplifying the NSP1 gene. Amplicons were purified from agarose gels using QIAquick Gel Extraction Kit (Qiagen), and sequencing was performed by Macrogen Inc. (Seoul, Korea). The phylogenetic trees were constructed using the Neighbor-Joining method and Kimura 2-parameter as a model of nucleotide substitution using the software MEGA v5.2. The statistical significance was estimated by bootstrap method (1000 pseudo-replicates). The Paraguayan G8P[1] strain is indicated by a black square. Branches of the trees were color-coded according to the host: human (light blue); horses (green); different species from the Artiodactyl order (pink). Artiodactyl-like strains detected in humans (dark blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Phylogenetic trees of genes of G8P[1] rotavirus. The phylogenetic trees from the genes segment of structural proteins (A) and non-structural proteins (B) were constructed using the Neighbor-Joining method and Kimura 2-parameter as a model of nucleotide substitution using the software MEGA v5.2. The statistical significance was estimated by bootstrap method (1000 pseudo-replicates). The Paraguayan G8P[1] strain is indicated by a black square. Branches of the trees were color-coded according to the host: human (light blue); horses (green); different species from the Artiodactyl order (pink). Artiodactyl-like strains detected in humans (dark blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Phylogenetic analyses classified the VP1 genes from the Para-
Guayan G8P[1] Paraguayan strain presented a G8-P[1]-J2-R2-C2-M1-
Ax-N2-T6-E12-H3 genomic constellation (Fig. 2), which was simi-
lar to the ones described for Artiodactyls species (Matthijnssens
et al., 2008a), and mainly composed by DS-1 gene segments.
Phylogenetic analyses classified the VP1 genes from the Para-
guan G8P[1] closely related to other rotavirus strains detected
in a South American camelid and clustered only with other Artio-
dactyl or Artiodactyl-like strains (Fig. 2A). VP2 and VP6 genes
grouped with bovine-like strains detected in humans but relatively
distant from other South American Artiodactyl strains (Fig. 2B). In
contrast, the VP3 gene clustered with typical human strains and
presented the genotype 1, suggesting an adaptation to the new
host. The NSP2 gene also showed genotype 2 (N2), closely related
to a bovine strain detected in Argentina. The NSP5 gene was the
only segment that belonged to genotype 3 (H3), and the G8P[1]
Paraguayan strain clustered with South American bovine strain
bearing the genotypes G15P[11]. The combination of genes belong-
ing to genotype 2 and 3, is a common feature of the genomes from
rotavirus strains detected in Artiodactyl species (Matthijnssens
et al., 2008a). The NSP3 gene was found to belong to the T6 geno-
type, which is phylogenetically distant from T2 to T3, but is in a
cluster typically associated with rotavirus strains detected in artio-
dactyl animals (Fig. 2B). The NSP4 gene clustered within the geno-
type E12 (Fig. 2B), closely related to the G10P[9] Brazilian strain
(R239) detected in humans, and the strains B383 (G15P[11]) and
Chubut (G8P[14]) detected in cows and guanacos from Argentina.
The E12 genotype was first described in Guanaco and Bovine rota-
virus in Argentina (Matthijnssens et al., 2009), and recently has
been characterized as part of a large conserved genome constell-
ation in Argentinean horses (Fig. 2B) (Garaicoechea et al., 2011;
Matthijnssens et al., 2012). Thus, these findings expand the num-
ber of countries where strains bearing the E12 genotype were

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detected, and also reinforce the notion that this is a signature genotype for ungulates from South America.

The genotype P[1] is commonly found in cattle and caprine (Carl.et al., 1997; Fukai et al., 1999; Ghosh et al., 2010; Okada and Matsumoto, 2002), and in South America was described in camelids (Parreno et al., 2004) and porcine (Parra et al., 2008).

The VP4 from the Paraguayan G8P[1] clustered with high bootstrap values (100%) with bovine rotavirus strains presenting G8 and G6 genotypes and detected in three different continents. (Fig. 2A). The gene from the VP7 of G8 strains is divided in three lineages (Fukai et al., 2004). Interestingly, the Paraguayan G8P[1] was clustered in the root of lineage I, which also group the G8 strains detected in Argentinean camelids (Fig. 2A), reinforcing the geographic clustering of the strains analyzed.

G8 strains causing AGE have been detected at high frequencies in the African continent, specially associated with DS1- and WA-like genes (Enosa et al., 2009; Matthijnssens et al., 2006b). G8P[1] rotaviruses were mainly found in cattle (Carl.et al., 1997; Fukai et al., 1999; Okada and Matsumoto, 2002), and rarely reported in other species (Ghosh et al., 2010; Lourie Uartre et al., 2014). Only five cases of G8P[1] strains, all with constellation of genes more similar to animals, were reported in humans, being only three of them detected in diarrhea (Banyai et al., 2009a; Ghosh et al., 2011). The sequence and phylogenetic analyses of the genes analyzed from the 492Srotavirus-positive sample provides evidence for a direct interspecies transmission animal-to-human, with a potential reassortment with human strains as evidenced by the human-like VP3. Hoshino et al. proposed the role of VP3 in host range restriction (Hoshino et al., 1995), and reassortment between bovine and human strain yielding viable virus has been previously reported (Matthijnssens et al., 2008b, 2010). Thus, the introduction of human VP3 in a bovine backbone could enhance the infectivity of this strain in a human host. However, because we also detected norovirus in this sample it is difficult to determine the real cause of diarrhea in this child. Thus, continue monitoring of circulating rotavirus strains in humans and animals will be required to shed light into the mechanism by which rotavirus transmit from different species.

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