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Short communication

Genomic characterization of a rotavirus G8P[1] detected in a child with diarrhea reveal direct animal-to-human transmission

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ABSTRACT

Group A rotavirus is a major cause of severe gastroenteritis in children and young animals. During a retrospective analysis of samples collected from Paraguayan children under 5 years old with diarrhea, and previously negative for rotavirus and norovirus, we detected the presence of bovine rotavirus sequences by viral metagenomics. Nucleic acid was extracted direct from stool sample and determined to be G8P[1]. The genomic analyzes revealed that the strain presents an Artiodactyl-like genome (G8-P[1]-I2-R2-C2-M1-Ax-N2-T6-E12-H3) suggesting a direct animal-to-human transmission.

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, 2013). Recently, an extended genotyping system based on genetic differences within all 11 segments was established. This new classification system has provided valuable information on the RVA genetic diversity and allowed the precise identification of the 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 patients in a large private hospital from Asuncion, 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

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		NSP2	NSP3	NSP4	NSP5	VP1	VP2	VP3	VP4	VP6	VP7
Human (G1P[8], G2P[4], G4P[6])	RVA/Human-tc/USA/Wa/1974/G1P1A[8]	83.0	78.0	79.7	94.8	76.1	81.5	95.7	63.0	80.2	69.6
	RVA/Human-wt/JPN/KU/1999/G1P[8]	ND	77.5	78.8	94.2	78.5	82.9	85.1	64.0	83.5	69.9
	RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	88.4	78.6	83.3	89.7	84.3	85.0	76.6	64.2	88.4	69.3
	RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]	87.5	82.4	83.1	90.3	83.4	84.3	74.5	64.1	88.4	69.9
	RVA/Human-tc/GBR/ST3/1975/G4P2A[6]	83.6	80.2	79.1	92.9	77.3	82.2	85.1	62.2	78.5	70.0
Human (G8P[4], G8P[6], G8P[8])	RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]	88.4	81.9	83.5	90.3	82.5	82.6	73.4	64.8	83.5	82.1
	RVA/Human-wt/CMR/6782/2000/G8P[6]	ND	80.2	80.6	94.8	76.4	81.9	98.9	63.5	77.7	81.9
	RVA/Human-wt/CMR/6809/2000/G8P[6]	84.2	80.2	81.3	94.8	76.4	81.5	98.9	63.0	85.1	81.7
	RVA/Human-wt/COD/DRC86/2003/G8P[6]	88.8	80.8	82.4	89.7	82.2	84.0	75.5	63.8	85.1	81.5
	RVA/Human-wt/COD/DRC88/2003/G8P[8]	88.4	81.3	82.0	90.3	82.8	84.0	74.5	61.9	86.0	81.4
	RVA/Human-wt/CMR/6780/2000/G8P[8]	83.6	80.2	80.4	94.8	75.2	81.9	98.9	64.2	77.7	82.4
	RVA/Human-wt/CMR/6787/2000/G8P[8]	84.2	80.2	79.7	94.8	75.8	81.5	92.6	64.2	78.5	82.2
	RVA/Human-wt/TUN/6862/2000/G8P[8]	85.1	80.2	79.3	94.8	76.1	81.2	98.9	64.4	78.5	82.2
	RVA/Human-wt/TUN/6854/2002/G8P[8]	84.2	80.2	79.3	94.8	76.1	81.5	98.9	64.4	76.9	82.2
	RVA/Human-wt/CIV/6736/2004/G8P[8]	85.1	79.7	79.3	94.2	75.8	81.9	98.9	64.4	76.9	82.3
	RVA/Human-wt/BRA/R291/2002/G8P[4]	ND	ND	81.9	ND	ND	ND	ND	63.3	ND	82.0
	RVA/Human-wt/ETH/6810/2004/G8P[8]	83.3	80.2	79.3	94.8	76.1	81.2	92.6	64.4	85.1	82.3
Human (G12P[8])	RVA/Human-wt/ARG/Arg7500/2009/G12P[8]	83.9	81.3	80.0	93.5	77.0	81.9	88.3	64.5	80.2	72.2
	RVA/Human-wt/ARG/Arg6653/2008/G12P[8]	83.9	81.3	79.1	93.5	76.1	81.9	87.2	64.5	80.2	72.2
	RVA/Human-wt/ARG/Arg6627/2008/G12P[8]	83.3	80.2	79.3	92.9	77.0	81.9	87.2	64.5	80.2	72.1
Artiodactyla (G6P[1], G6P[5], G6P[14], G8P[1], G8P[14], G15P[11])	RVA/Cow-tc/NGA/NGRBg8/1998/G8P[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	81.5
	RVA/Cow-tc/USA/NCDV/1967/G6P6[1]	93.3	89.6	82.2	97.4	95.2	87.5	74.5	92.2	87.6	73.0
	RVA/Cow-tc/FRA/RF/1982/G6P[1]	92.7	91.2	82.0	97.4	94.6	87.5	74.5	91.6	86.8	73.2
	RVA/Cow-tc/VEN/BRV033/1990/G6P6[1]	90.6	89.0	79.1	96.1	89.7	83.6	75.5	87.5	81.8	70.7
	RVA/Cow-tc/GBR/UK/1973/G6P7[5]	90.0	81.3	81.5	96.8	78.5	87.1	73.4	64.5	86.8	73.0
	RVA/Cow-tc/USA/WC3/1981/G6P[5]	95.1	91.8	80.6	97.4	94.6	87.1	72.3	63.4	88.4	73.1
	RVA/Cow-wt/ZAF/1603/2007/G6P[5]	90.0	92.9	82.4	98.1	90.6	91.6	75.5	66.0	85.1	84.7
	RVA/Cow-wt/ZAF/1605/2007/G6P[5]	90.0	92.9	82.2	98.1	90.6	91.6	75.5	66.2	86.0	71.7
	RVA/Cow-wt/ZAF/1604/2007/G8P[1]	94.5	84.6	82.0	98.1	92.7	88.2	74.5	90.1	85.1	71.7
	RVA/Cow-tc/THA/A5-13/1988/G8P[1]	ND	ND	ND	ND	ND	ND	ND	76.9	ND	82.4
	RVA/Cow-wt/ARG/B383/1998/G15P[11]	95.4	87.9	95.3	100.0	78.9	82.9	71.3	49.4	84.3	70.7
	RVA/Goat-tc/BGD/GO34/1999/G6P[1]	88.4	90.1	83.5	98.1	82.2	86.4	71.3	78.4	81.8	74.9
	RVA/Goat-tc/BGD/GO100/1999/G6P[1]	ND	ND	83.5	98.1	ND	ND	ND	78.4	81.8	74.9
	RVA/Goat-tc/BGD/GO102/1999/G6P[1]	ND	ND	83.5	98.1	ND	ND	ND	78.4	81.8	74.7
	RVA/Antelope-wt/ZAF/RC-18-08/G6P[14]	91.2	87.4	82.6	97.4	90.0	92.0	73.4	61.1	87.6	73.0
	RVA/Guanaco-wt/ARG/Rio_Negro/1998/G8P[1]	87.2	87.4	92.9	97.4	79.2	90.2	72.3	79.5	86.0	87.0
	RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14]	90.6	92.3	98.2	98.7	78.9	90.9	70.2	63.7	87.6	86.7
	RVA/Vicuna-wt/ARG/C75/2010/G8P[14]	90.3	92.9	71.9	ND	95.2	91.3	70.2	63.5	88.4	86.5
	RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]	89.7	90.1	83.5	97.4	85.8	89.9	71.3	62.0	87.6	86.2
	RVA/Rhesus-tc/USA/PTRV/1990/G8P[1]	94.5	90.7	81.7	98.1	95.5	87.5	70.2	91.6	84.3	87.0
Human infected Artiodactyla-like rotaviruses	RVA/Human-tc/ITA/PA169/1988/G6P[14]	89.4	92.9	83.3	98.1	88.8	88.2	78.7	62.6	86.8	72.8
	RVA/Human-tc/AUS/MG6/1993/G6P[14]	90.6	89.6	82.9	98.1	94.6	86.8	75.5	62.7	87.6	73.5
	RVA/Human-wt/HUN/Hun5/1997/G6P[14]	89.1	90.1	82.2	97.4	83.1	90.2	72.3	62.6	85.1	73.8
	RVA/Human-wt/BEL/B10925/1997/G6P[14]	89.1	90.1	83.5	98.1	84.6	89.5	70.2	63.0	86.8	73.2
	RVA/Human-wt/ITA/111-05-27/2005/G6P[14]	91.8	90.1	83.5	98.1	85.2	89.5	74.5	63.1	86.8	73.1
	RVA/Human-tc/KEN/B12/1987/G8P[1]	91.2	93.4	81.3	98.7	93.4	96.5	75.5	79.2	88.4	86.7
	RVA/Human-tc/IND/69M/1980/G8P4[10]	91.2	80.2	82.2	94.8	83.7	90.6	77.7	68.6	83.5	85.1
	RVA/Human-wt/HUN/BP1062/2004/G8P[14]	88.4	92.3	83.5	98.1	82.8	85.7	75.5	62.7	90.1	81.4
	RVA/Human-wt/BRA/R239/200X/G10P[9]	ND	ND	97.6	ND	ND	ND	ND	62.0	ND	74.6
	RVA/Human-wt/ARG/Arg4605/2006/G4P[6]	86.0	79.1	79.5	94.8	81.0	80.8	86.2	62.7	78.5	69.9
	RVA/Human-wt/ARG/Arg4671/2006/G4P[6]	86.0	78.0	80.0	95.5	79.2	81.5	86.2	62.9	78.5	68.6
	RVA/Human-tc/NGA/HMG035/2000/G8P[1]	ND	ND	ND	ND	ND	ND	ND	81.7	ND	81.4
	RVA/Pig-tc/USA/OSU/1977/G5P9[7]	84.8	79.7	80.2	94.8	79.5	81.5	87.2	71.8	83.5	74.7
Perissodactyla (G3P[12], G13P[18], G14P[12])	RVA/Horse-wt/ARG/E30/1993/G3P[12]	87.8	84.1	92.4	87.7	84.9	88.9	80.9	68.9	78.5	73.4
	RVA/Horse-wt/IRL/03V04954/2003/G3P[12]	87.2	80.2	82.0	87.7	83.7	89.5	80.9	68.9	80.2	73.7
	RVA/Horse-tc/GBR/L338/1991/G13P[18]	83.3	74.2	77.7	91.0	78.2	78.4	79.8	73.9	74.4	71.0
	RVA/Horse-wt/IRL/04V2024/2004/G14P[12]	88.1	80.2	81.7	87.1	83.4	87.5	79.8	68.9	77.7	73.3
	RVA/Horse-wt/ARG/E403/2006/G14P[12]	88.4	81.9	92.2	87.7	84.3	88.9	80.9	69.3	81.8	74.8
	RVA/Horse-wt/ZAF/EqRV-SA1/2006/G14P[12]	87.8	79.7	81.7	87.1	83.7	88.2	79.8	69.5	78.5	72.8
	RVA/Horse-wt/ARG/E4040/2008/G14P[12]	88.8	82.4	92.0	87.1	84.3	87.5	81.9	69.1	82.6	74.7

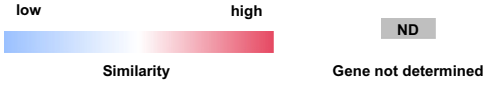

Fig. 1. Heat map showing the genetic differences between the Paraguayan G8P[1] strain and other rotavirus strains detected in various animals and humans. The nomenclature suggested by the rotavirus classification-working group (RCWG) was used for each sample. Information on host-specie, genotype, place and date of isolation 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from five fecal samples were pooled together. Viral metagenome library was prepared by ScriptSeq™ v2 RNA-Seq Library Preparation Kit (Epicentre) and run on the Miseq Illumina platform. Illumina reads and assembled contigs >100-bp were compared to the GenBank protein databases using BLASTx. A stringent *E* value of 10^{-10} was used as the cut-off for highly significant sequence similarity to known viruses. In one of the pools nine sequences showed significant BLASTx matches with bovine rotaviruses. The pool also presented multiple reads from human norovirus and 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 noroviruses a set of generic primers targeting the RNApol gene (primers Mon431/Mon432 and Mon433/Mon434) were used (Amarilla et al., 2007; Galeano et al., 2013). To further investigate the presence of bovine rotavirus and human noroviruses in those samples, RNA was extracted from a 10% fecal suspension from each of the five samples using Boom's method (Boom et al., 1990), and analyzed by