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Detection of the first G6P[14] human rotavirus strain from a child with diarrhea in Egypt

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ABSTRACT

We report the first detection of a G6P[14] rotavirus strain in Egypt from the stool of a child participating in a hospital-based diarrhea surveillance study conducted throughout the year 2004. Rotavirus infection was initially detected using a rotavirus group A VP6 enzyme immunoassay; the P (VP4) and G (VP7) genotypes of the strain were identified by RT-PCR. We sequenced the VP7 gene and the VP8* portion of the VP4 gene and the strain displayed the strongest identity to the VP7 [>94% nucleotides (nt), >97% amino acids (aa)] and VP4 (>93% nt, >98% aa) sequences of PA169, a novel G6P[14] strain first isolated from a child in Italy during the winter of 1987. Additional sequencing and analysis of the other remaining structural (VP1–VP3, VP6) and non-structural (NSP1–NSP5) proteins support this animal-to-human reassortment theory. According to the full genome classification system, the G6P[14] strain (EGY3399) was assigned to G6-P[14]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotypes. The greatest similarity of EGY3399 NSP4 and NSP5 gene sequences were to those of ovine and simian origin, respectively. Coupled with other observations, our results suggest G6P[14] isolates rarely cause severe diarrhea in Egyptian children, and support other studies that indicate animal rotavirus contribute to the genetic diversity of rotavirus detected from humans through interspecies transmission and single or multiple segments reassortment.

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1. Introduction

Group A rotaviruses are the most important etiological agent of acute and severe dehydrating diarrhea in infants and young children, annually causing 352,000–592,000 deaths (median, 440,000 deaths) in children <5 years of age (Parashar et al., 2006). Rotavirus is a member of the *Reoviridae* virus family and all strains possess 11 segments of double-stranded RNA enclosed within a triple-layered shell. Each segment encodes one polypeptide (moncistronic), except genome segment 11 which encodes two, for a total of six nonstructural and six structural proteins (Estes and Kapikian, 2007). Outer capsid serotype-specific proteins are referred to as VP7 (a glycoprotein, designated G) and VP4 (a protease-sensitive protein, designated P) encoded by genome segment 7, or 8, or 9, and 4, respectively (http://www.reoviridae.org/dsrna_virus_proteins//Rotavirus.htm) (Estes and Kapikian, 2007). A binary classification system for rotaviruses using G and P designations has been adopted to assign strains to either established or newly identified and evolving sero-/geno-types. Recently, the Rotavirus Classification Working Group (RCWG) proposed an extensive classification system that takes into account all 11 rotavirus genome segments (Matthijnssens et al., 2008).

Currently, at least 25 G genotypes and 33 P genotypes have been identified by nucleotide sequencing (Abe et al., 2011; Esona et al., 2010; Maes et al., 2009; Matthijnssens et al., 2008), and of these, 12 G types (G1–G6, G8–G12, G20) and 15 P types (P[1]–P[11], P[14], P[19], and P[25]) have been detected in humans (Esona et al., 2009; Gentsch et al., 2005; Matthijnssens et al., 2009; Solberg et al., 2009). Globally, the major G genotypes identified in humans are G1, G2, G3, G4 with a recent emergence of G9. Genotypes G5, G6, G8, G10, G11 and G12 are globally rare but may be regionally common (Gentsch et al., 2005; Malek et al., 2010). However, rare human G genotypes are commonly associated with animals, such as cattle (G6, G10, G15), chickens (G9), horses (G13, G14) and swine (G5) (Browning et al., 1991). Although G6 and G10 are rarely described in humans, they represent the two most important genotypes isolated from cattle (Howe et al., 2008; Reidy et al., 2006). The most common human P genotypes include P[4], P[6]

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and P[8] (Fang et al., 2002; Gentsch et al., 2005; Steele and Ivanoff, 2003) while uncommon genotypes such as P[9], P[11] and P[14] are increasingly detected in humans in different areas of the world (Gentsch et al., 1992; Gentsch et al., 2005; Santos and Hoshino, 2005).

G6 rotaviruses have been found to be an infrequent cause of human disease. The first cases of G6 rotavirus infection were reported from Italy during the winter season of 1987, in two children with acute gastroenteritis. Subsequently, these viral strains were genotyped as G6P[9] (strain PA151) and G6P[14] (strain PA169) (Gerna et al., 1992). Nearly a decade later (1996–1999), 17 G6 strains, representing 1.4% of the total strains characterized, were reported from Hungary (Bányai et al., 2003a). Characterization of the VP7 sequence of six of the strains resulted in their classification into three distinct clusters by phylogenetic analysis; a single PA169-like strain, three PA151-like strains and, two novel G6 strains (Bányai et al., 2003b). Most recently, a human G6P[6] rotavirus infection was reported from a 13-month-old child hospitalized with severe diarrhea in Belgium (Rahman et al., 2003). Outside of Europe, the first G6P[14] human rotavirus strain (MG6) was isolated from a 16-month-old Australian child hospitalised with acute gastroenteritis in 1993 (Palombo and Bishop, 1995). Two more G6P[14] rotavirus strains (MG6.01 and AG6.01) were isolated in Australia during 1996–1997 (Cooney et al., 2001; Diwakarla et al., 2002). In the United States from 1996 to 1999, a single G6P[9] human rotavirus strain, most similar to the Italian bovine-like strain, PA151 was reported (Griffin et al., 2002). The sole report of human G6 rotavirus strains in Asia comes from India, where six strains were isolated from patients with diarrhea and were characterized (Kelkar and Ayachit, 2000; Kelkar et al., 2004). G6 rotaviruses have been predominantly isolated from cattle (Chang et al., 1996; Falcone et al., 1999), with some studies indicating a prevalence greater than 50% (Chang et al., 1996; Falcone et al., 1999; Okada et al., 2002). The most common P type specificities reported with bovine G6 strains include P[1], P[5], and P[11] (Chang et al., 1996; Falcone et al., 1999).

Global rotavirus strain surveillance, using standardized detection and typing techniques (www.who.int/vaccines-documents/) paired with automated sequencing, has allowed researchers to monitor the epidemiology of rotavirus and track the emergence of novel P and G genotypes as well as record atypical P–G combinations. Surveillance data provides strong evidence that group A rotavirus exhibit extensive diversity and dynamic potential for reassortment. As vaccines are introduced it will be important to monitor for potential increases in rotavirus diversity, or possible emergence of novel strains that escape vaccine immunity. Although a national rotavirus vaccination program is yet to be introduced in Egypt, a predictable 60% of the well to do in the community seek immunization through the private sector. Through surveillance, the evolution of new rotavirus strains can be evaluated as a result of selection pressure exerted by the vaccine or via the natural genotype fluctuation of the virus.

2. Materials and methods

2.1. Rotavirus antigen detection

Stool samples were collected in 2004 at Abu Homos District Hospital as part of an ongoing hospital-based diarrhea surveillance study among Egyptian children aged under 5 years in Abu Homos district of the Nile river delta (Wierzba et al., 2006). A "diarrheal episode" was defined to begin on the first day of loose or liquid stools after at least 3 consecutive non-diarrheal days and completed when there were 3 consecutive non-diarrheal days after a diarrheal day. Suspensions of stool (10–20%, w/v) were made from 391 stool samples in phosphate-buffered saline and screened for rotavirus with the Premier Rotaclone EIA kit (Meridian Diagnostics, Cincinnati, OH, USA) that detects the VP6 protein of the virus. The assay was performed according to the manufacturer's instructions. Results were read spectrophotometrically at a wavelength of 450 nm. According to the manufacturer's protocol, specimens with an absorbance greater than 0.150 were considered positive.

2.2. RNA extraction

Viral RNA was extracted from 10–20% stool suspension (made from 0.1 g or 100 μl stool in 2 ml of a 1:1 vertrel/water solution) using a NucliSens automated extractor (Biomerieux, Durham, NC, USA) according to the protocol specified by the manufacturer and method described previously (Boom et al., 1990).

2.3. RT-PCR

Extracted dsRNA was denatured at 95 °C for 5 min, and then RT-PCR for the VP7 (complete) and VP4 (fragment) genes of sample EGY3399 was performed using a One-Step RT-PCR kit (Qiagen, Inc., Valencia, CA, USA). The VP7 and VP4 forward and reverse primers and cycle conditions described previously were used (Das et al., 1994; Gentsch et al., 1992; Iturriza-Gómara et al., 2004). An additional pair of P[14] oligonucleotides (jrg234 (11–32, +), 5′-TGG CTT CTT TGA TCT ACA GAC A-3′ and jrg236 (887–868, −), 5′-ATC TCT GAC CAC TTG TAT CC-3′) were also used (Gentsch, J.R., personal communication). As shown in Table 1, the different rotavirus gene segments (complete or partial) were amplified using previously published rotavirus specific consensus primers for VP1, VP2, VP3, VP6, NSP1, NSP2, NSP3, NSP4 and NSP5 (Bányai et al., 2009; Matthijnssens et al., 2006).

Table 1
Genotype and grouping of the structural and non-structural protein encoding genes of human strain EGY3399.

Gene	Nucleotide position sequenced based on the strain Hun5 (base pair obtained)	Genotype	Group	Animal origin	Accession no.
VP7	129–925	G6	Hun5 like	Bovine	FJ262986
VP4	11–892	P[14]	Hun5 like	Ovine	FJ262987
VP6	43–1214	I2	DS-1 like	Bovine/simian	HM113528
VP1	19–2988	R2	DS-1 like	Bovine/simian	HM113525
VP2	114–2654	C2	DS-1 like	Bovine	HM113526
VP3	50–2554	M2	DS-1 like	Bovine/ovine	HM113527
NSP1	32–1504	A11	Hun5 like	Ovine	HM113520
NSP2	47–997	N2	DS-1 like	Bovine	HM113521
NSP3	28–963	T6	Hun5 like	Bovine/ovine/lapine	HM113522
NSP4	42–566	E2	DS-1 like	Bovine/simian	HM113523
NSP5	22–616	H3	AU-1 like	Bovine/ovine/lapine	HM113524