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Characterization of unusual G8 rotavirus strains isolated from Egyptian children

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Summary. We report the first detection of P[14], G8 rotaviruses isolated in Egypt from the stool of children participating in a 3 year study of rotavirus epidemiology. Two strains, EGY1850 and EGY2295, were characterized by a serotyping enzyme immunoassay (EIA), virus neutralization, and sequence analysis of the genes encoding VP7 and the VP8* portion of the VP4 gene. These two strains shared a high level of homology of their VP7s (87.8% nucleotide [nt], 97.2% amino acid [aa]) and VP4s (89.6% nt, 97.1% aa) and had the highest VP7 identity to serotype G8 (>82% nt, >92% aa) and VP4 identity to genotype P[14] (≥81% nt, >91% aa) strains. Serological results with a VP7 G8-specific and VP4 P[14]-specific neutralizing monoclonal antibodies supported the genetic classification of EGY1850 and EGY2295 as P[14], G8. Genogroup analysis supports earlier findings that human G8 rotaviruses may be genetically related to bovine rotaviruses. These findings demonstrate that our understanding of the geographic distribution of rotavirus strains is incomplete, emphasize the need to monitor rotavirus serotypes, and extend the known distribution of serotype G8 and genotype P[14] strains in Africa.

Introduction

Rotavirus is the major etiologic agent of severe diarrhea in children worldwide. The extensive disease burden of rotavirus diarrhea, which is estimated to cause 600,000–800,000 deaths and one third of all hospitalizations for diarrhea among

children throughout the world, has made development of an effective rotavirus vaccine a global priority [21].

The proteins considered most important for rotavirus vaccine development are the outer capsid proteins VP4 and VP7 which are encoded by RNA segments 4 and 7, 8, or 9, respectively. Both proteins elicit serotype-specific neutralizing antibody responses and segregate independently during reassortment events [28, 36–38]. Consequently, Estes and Cohen (1989) proposed a dual typing system for rotaviruses consisting of G and P serotypes based on the VP7 (glycoprotein) and VP4 (protease-sensitive) proteins [13]. Presently, there are 14 G serotypes of which only 10 have been reported in humans. Routine P serotyping is less clear because a correlation between P genotypes and serotypes has not been clearly established [12]. Currently, there are 20 P genotypes of which 10 serotypes have been correlated to certain genotypes. Although there are 14 different G serotypes and 10 P serotypes recognized in humans and animals, only four G/P serotype combinations (G1–G4) have been reported as important causes of diarrhea in children worldwide [16].

Since serological characterization of P types is tedious, genetic methods have been developed to identify the most common human rotavirus VP4 genes (P genotypes) from children with diarrhea, and their genotypes correlate well with serotypes as far as investigated [15, 30]. However, recently described exceptions to the correlation between P serotypes and P genotypes confirm that nucleic acid based typing methods can only be used to identify the most common VP4 genes in circulation and cannot be used to assign serotypes [27, 32]. A recent survey of the literature demonstrated that the most common P genotypes are P[8], which is found associated with serotypes G1, G3 and G4, and P[4] which is usually found with serotype G2 [16]. However, in some regions, uncommon G serotypes and P genotypes have also been identified. For example, serotypes G5 (Brazil), G9 (India and Bangladesh) and P[6] (India, Brazil and Bangladesh) have been reported frequently in these settings and the large number of G and P combinations of rotavirus strains in these countries has demonstrated that rotavirus serotypes may be more diverse than previously believed [22, 31, 41, 49, 51, 52]. Furthermore, recent reports of serotype G9 strains from multiple cities in the United States has raised the prospect that some of these rare or unusual serotypes are underdiagnosed and may be more prevalent than previously appreciated [7, 24, 35, 42, 52].

In 1997, a review of rotavirus epidemiology in Africa indicated that G1 (42%) was the most common serotype in circulation followed by G2 (16%) and G3 (4%) [10]. Similarly, in a study of Egyptian infants <1 year old, the most common rotavirus serotypes were G1 (17.7%) and G4 (17.7%); however, 38.7% of the specimens were nontypeable [40]. The inability to type a large number of strains may be due to technical reasons such as the absence of recognizable epitopes of the monoclonal antibodies used in the typing ELISA or due to the presence of inhibitory factors in the stool. Although these data are consistent with the global pattern of circulating strains, recent investigations suggest that some countries may have other serotypes in circulation such as P[6], G8; P[8], G5; P[6], G2; P[9], G1; P[9], G3; G8 and G10 [9, 16, 31, 41, 43]. Since the first vaccines for

rotavirus are targeted to provide serotype-specific immunity against strains of the four major P/G combinations, surveillance and identification of other strains are essential to determine whether the vaccines will work against these strains or if vaccine coverage for additional serotypes will be required.

Naficy et al. (manuscript in prep.) recently reported a longitudinal study of rotavirus diarrhea in children from Abu Homos, Egypt in which the most prevalent strains were P[4], G2 and P[8], G1. In this investigation, 22% of rotavirus isolates could not be G or P typed, and six of these strains were culture adapted and further analyzed by polyacrylamide gel electrophoresis (PAGE) as well as subgrouping and serotyping enzyme immunoassays (EIA). These rotaviruses all possessed an unusual combination of subgroup I specificity and long RNA electropherotype which is a combination often found in animal strains [53]. Therefore, two strains, EGY1850 and EGY2295, have been further characterized by serotyping EIA, neutralization tests, and sequence analyses of the VP7 gene and the VP8* portion of the VP4 gene.

Materials and methods

Viruses

Specimens for this laboratory investigation came from a longitudinal study of diarrhea in a cohort of Egyptian children 0–2 years of age from Abu Homos who were followed for 1 year (Naficy et al., manuscript in prep.). Of the 64 rotavirus strains, 46 (72%) were fully typed (G serotype and P genotype), 4 (6%) were partially typed (G serotype), 6 (9%) were untypeable and the remaining 8 (13%) had inadequate RNA to analyze further. Stool specimens from 2 children that were positive for rotavirus RNA by gel electrophoresis and silver staining but negative for G types 1–4, 9 and P genotypes [4], [6], [8] by reverse transcription polymerase chain reaction (RT-PCR) and untypeable for G serotypes using monoclonal antibodies (MAbs) were adapted to grow in MA104 cells for further examination [44]. Strain EGY1850 was isolated from a fecal specimen of a 30-month-old female child with diarrhea, and strain EGY2295 was isolated from the stool of a 17-month-old male child who did not have diarrhea. Tissue culture-adapted standard human rotavirus strains used in this study include Wa (P1A[8], G1), DS-1 (P1B[4], G2), P (P1A[8], G3), ST-3 (P2[6], G4), B37 (P[not determined], G8), 69M (P4[10], G8), NCDV (P6[1], G8), HAL1166 (P[14], G8), and PA169 (P[14], G6) [5, 12, 18, 19, 56].

Serotyping EIA

Viral isolates were serotyped with a MAb-based EIA initially using G serotype-specific MAbs including KU-4 (G1), S2-2G10 (G2), YO-1E2 (G3), ST-2G7 (G4) and subsequently with B37:1, a G8 serotype-specific MAb [47, 50]. Strains were subgrouped using the same EIA procedure with two subgroup-specific MAbs 255-60 (SG I) and 631-9 (SG II) [25]. The immunoassay was conducted as previously described with minor modifications [47, 55]. Plates were blocked with 5% skim milk in 0.01 M phosphate-buffered saline (PBS), pH 7.2 for 2 h at 37 °C, and dilutions of pooled rabbit serum against the four serotypes and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G were prepared in 1% skim milk-PBS. Serotype-specific reactions were defined by a 2-fold higher O.D. value against the homologous MAb compared to the heterologous MAb; i.e. positive/negative (P/N)>2 [47].

Electropherotypes

The double-stranded (ds) RNA profiles of the fecal and tissue culture-adapted viruses were analyzed by PAGE and bands were visualized by silver staining (Bio-Rad Silver Staining Kit, Hercules, CA) [33].

Fluorescent focus neutralization reduction assay (FFN)

Antigenic characterization of the tissue culture-adapted strains was performed by the FFN as previously described by Coulson et al. (1985) using the VP7 MAb, B37:1, and the VP4 MAb 2H4 [8, 19, 50]. The neutralizing antibody titer was expressed as the reciprocal of the dilution giving a 50% reduction in the number of infected cells compared with that in the virus control wells.

RT-PCR

Rotavirus dsRNA was extracted from rotavirus-infected MA104 cells with phenol-chloroform-1% sodium dodecyl sulfate, precipitated with ethanol, and concentrated by a glass powder procedure as previously described [15]. To obtain initial templates for sequencing, full-length VP7 genes were amplified by RT-PCR with the primer pair degenerate beg9 and degenerate end9 [23]. An 877 base pair (bp) fragment of VP4 (nucleotides 11-887), which includes the entire VP8* subunit plus 40 amino acids of the VP5* subunit, was amplified by RT-PCR using the consensus primer pair Con3 and Con2 [15].

Nucleotide sequencing

For nucleotide sequencing, ethidium bromide-stained RT-PCR products were extracted and purified from a 1.2% Seakem GTG agarose gel (FMC Bioproducts, Rockland, ME) using the QIAquick Gel Extraction Kit procedure (Qiagen, Chatsworth, CA). The DNA was sequenced by the dideoxynucleotide chain termination method, using the PRISM TM Ready Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA) on an automated sequencer (Applied Biosystems model 377). The oligonucleotide primers used for cycle sequencing were synthesized in the CDC Biotechnology Core Facility, Centers for Disease Control and Prevention. After initial sequence data were obtained with degenerate primers beg9 and end9 (VP7) and Con2 and Con3 (VP4), additional primers were designed for the strains to complete sequencing in both directions of both VP7 and the VP8* portion of the VP4 genes.

The sequencing data for the Egyptian strains were compared to sequences of other rotavirus strains using the pileup and distances programs of the University of Wisconsin Genetics Computer Group software suite [11]. The VP7 and partial VP4 gene sequences of strains EGY1850 and EGY2295 have been deposited in the GenBank sequence database and assigned accession numbers: AF104102 (strain EGY1850, VP7), AF104101 (strain EGY1850, VP4), AF104104 (strain EGY2295, VP7), and AF104103 (strain EGY2295, VP4).

Genogroup analysis

Northern hybridization analysis was carried out with a whole genome probe derived from the virus strain EGY2295 (P[14], G8). The probe was generated by labeling cDNA, derived by reverse transcription of purified dsRNA with random hexanucleotide primers and digoxigenin (DIG)-11-dUTP. Fifty ng/ml of probe was used for hybridization as described previously [39]. Viral dsRNA was electrophoresed in a 10% (w/v) polyacrylamide gel, denatured, and transferred to a nylon membrane. Prehybridization and hybridization at 50 °C were followed by posthybridization stringency washes at room temperature and 50 °C. Bound

probe was detected using anti-DIG antibody conjugated to alkaline phosphatase (Boehringer Mannheim, Indianapolis, IN) and the chemiluminescent substrate CSPD (Boehringer Mannheim).

Results

Sequence analysis of the VP7 genes of strains EGY1850 and EGY2295

For the six previously nontypeable strains, we selected one strain each from a patient with diarrhea (EGY1850) and a child without diarrhea (EGY2295) to sequence the gene encoding VP7 for comparison with representative strains of other serotypes (Table 1). Strains EGY1850 and EGY2295 exhibited 87.8% nt and 97.2% aa sequence identity to each other and had the greatest homology with the VP7 sequences from both human and bovine G8 rotaviruses (>82% nt, >92% aa). The VP7 sequences showed less identity to strains of other G serotypes (72.7–76.4% nt, 75.1–84.7% aa).

The VP7 sequence comparisons of strain EGY2295 indicated that it was more closely related to the bovine G8 strain A5 (94.4% nt, 96.6% aa similarity) than to other human G8 strains (≤87.4% nt). In contrast, while the VP7 nucleotide sequence of strain EGY1850 was most similar (88.8%) to the Nigerian human rotavirus strain HMG89, the deduced amino acids of this strain were closest (96.6%) to strain A5. This level of amino acid homology was also noted with other G8 strains including HAL1166, 69M and 678.

The predicted amino acid sequences in the three antigenic regions A, B and C of the VP7 proteins of strains EGY1850 and EGY2295 were aligned with seven G8 strains (A5, HMG89, HAL1166, DG8, 678, 69M, B37), three G3 strains (AU-1, MO, YO), one G9 strain (WI61) and one G11 strain (YM) (data not shown). Serotype G8 rotavirus strains HAL1166, DG8, 678, and B37 contained identical A, B and C regions. Strain EGY2295 and A5 were identical in antigenic region A as were strains EGY1850 and HMG89 but differed from all of the other G8 strains by a single substitution at position 87 except strain 69M which had an additional substitution at position 94. The antigenic C region was totally conserved for all G8 strains, while all but two strains (A5 and HMG89) contained an identical antigenic B region. Strains A5 and HMG89 had single substitutions at positions 148 and 147, respectively.

Sequence comparisons of the VP7 genes of strains EGY1850 and EGY2295 showed a moderate degree of similarity at the amino acid level (>82%) to representative G3, G9, and G11 strains. To determine if this similarity extended to the antigenic regions, alignments of regions A, B and C were compared (data not shown). Serotype G3 strains AU-1, MO and YO possessed almost identical antigenic C regions when compared to the serotype G8 strains including EGY1850 and EGY2295. A single variation at position 213 was identified. Similarly, comparisons of YM (G11) and the G8 strains revealed a high degree of similarity in region C with only two substitutions at positions 212 and 220. No significant identity in regions A or B was noted for the G3 or G11 representative strains when compared to the G8 strains. Alignments with the G9 strain WI61 (P1A[8], G9)

Table 1. Comparisons of the VP7 gene sequences of strains EGY1850 and EGY2295 to different rotavirus serotypes

Rotavirus strain[a]	G Serotype	Origin	Sequence similarity (%) of virus strain			
			EGY1850		EGY2295	
			nt[b]	aa[c]	nt	aa
EGY1850	8	human	–	–	87.8	97.2
EGY2295	8	human	87.8	97.2	–	–
HAL1166	8	human	85.7	96.6	85.0	96.3
B37	8	human	85.3	94.2	83.3	92.9
69M	8	human	86.5	96.6	84.2	95.4
HMG89	8	human	88.8	95.4	87.4	95.7
DG8	8	human	85.2	96.0	85.6	95.1
A5	8	bovine	86.8	96.6	94.4	96.6
678	8	bovine	85.0	96.6	85.1	95.4
NCDV Cody I-801	8	bovine	83.5	95.4	84.7	95.4
C8008	8	bovine	83.3	94.5	82.6	94.2
Wa	1	human	72.9	77.9	72.9	78.2
S2	2	human	73.5	76.1	72.9	75.8
YO	3	human	76.3	84.7	75.7	83.8
ST3	4	human	73.2	75.1	72.7	75.1
OSU	5	porcine	75.7	82.3	75.5	81.0
PA169	6	human	75.2	84.4	75.9	83.8
WI61	9	human	76.2	82.6	75.1	82.3
Mc35	10	human	76.4	81.6	74.6	81.3
YM	11	porcine	75.9	84.4	76.2	82.9
L26	12	human	74.4	79.5	73.0	78.6
L338	13	equine	74.0	76.4	73.3	77.4
FI23	14	equine	74.6	80.4	74.0	79.8

[a]The following VP7 sequences were obtained from published reports and the GenBank database: HAL1166 (accession no. L20882); B37 (J04334); 69M ([24]; not listed); HMG89 (X98918); DG8 (AF034852); A5 (D01054); 678 (G605644); NCDV Cody I-801 (U14999); C8008 (U14998); Wa (K02033); S2 (M11164); YO (D86284); ST3 (X13603); OSU (X04613); PA169 (L20880); WI61 ([24]; not listed); Mc35 (D14033); YM (M23194); L26 (M58290); L338 (D00843); and FI23 (M61876)

[b]Nucleotides

[c]Amino acids

showed no significant similarity to the G8 strains in any of the three antigenic regions A, B or C.

Sequence analysis of the VP4 genes of strains EGY1850 and EGY2295

The nucleotide and deduced amino acid sequences of the partial length VP4 gene (nt 24–860) for strains EGY1850 and EGY2295 were compared to representative rotavirus strains of the 20 different reported P genotypes (data not shown). Strains

EGY1850 and EGY2295 exhibited 89.6% nt and 97.1% aa sequence identity to each other and a high level of homology (>83% nt, >93% aa) to the published P[14] human rotavirus strains HAL1166, PA169, Mc35 and MG6. In addition, strains EGY1850 and EGY2295 shared 81–86.9% nt and 91.8–95.8% aa similarity to the four reported lapine P[14] rotavirus strains [6]. The partial VP4 sequences of strain EGY1850 and EGY2295 were less similar to representative strains from the other 19 P genotypes (47.5–76.9% nt, 42.2–84.9% aa). Although both strains were the most similar to P[14] strains, they shared a relatively higher degree of identity (>76% nt, >83% aa) with strain AU-1 (P3[9], G3) than with any of the other representative strains of the remaining 18 P genotypes.

The amino acid sequences (12–242) were further analyzed using the Kitsch module of the PHYLIP package to estimate the phylogenetic relationships among various strains (Fig. 1). Interestingly, the P[14] strains clustered into two groups according to species origin. In addition, the relative similarity of the P[14] strains to AU-1 (P3[9], G3) was illustrated by the phylogenetic analysis.

Antigenic characterization

Serotyping and electropherotypes

To confirm the classification of strains EGY1850 and EGY2295 as serotype G8, cell lysates were tested in a serotyping EIA. Both strains EGY1850 and EGY2295 as well as the other four previously nontypeable Egyptian strains reacted (P/N>2) with B37:1, the G8-specific MAb and did not react with the other monoclonals which recognize serotype-specific neutralization epitopes for G types 1–4. Interestingly, the G3 control strain, P, also reacted (P/N>2) with B37:1 as has been previously reported [50]. Both EGY1850 and EGY2295 as well as the other 4 Egyptian strains also reacted strongly with the subgroup I MAb. However, by PAGE, the RNA profiles of the Egyptian strains had long patterns which is unusual for human strains considering their subgroup I specificities.

Neutralization with MAbs

The G8 strains EGY1850 and EGY2295 were characterized by FFN using VP7 and VP4 neutralizing monoclonal antibodies (N-MAbs) (Table 2). The VP7 N-MAb B37:1 neutralized both EGY1850 and EGY2295 to titers of 17,000 and 26,000, respectively, which is similar to the reported neutralization titer of the parent strain B37 [50]. In addition, both strains were neutralized by 2H4, the VP4-specific P[14] N-MAb at titers of 16,000 (EGY1850) and 13,000 (EGY2295) [19].

Genogroup analysis of strain EGY2295

To investigate the origins of all gene segments, Northern hybridization analysis was performed using a whole genome probe from strain EGY2295 with RNA from the human strains Wa (P1A[8], G1), DS-1 (P1B[4], G2), PA169 (P[14], G6) and HAL1166 (P[14], G8) and the bovine strain NCDV (P6[1], G6) (Fig. 2). Probe

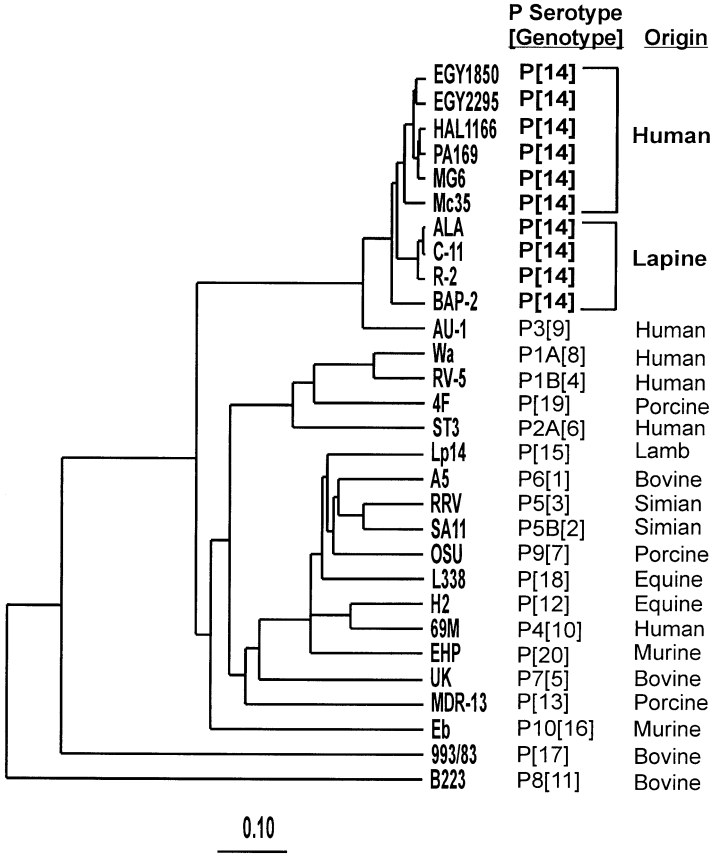

Fig. 1. Phylogenetic tree of the VP4 amino acid sequences[a] (12-242) of strains EGY1850 and EGY2295 with different rotavirus genotypes. The phylogenetic tree was created with the Kitsch module of the PHYLIP package to illustrate the relationships between the VP4s of EGY1850 and EGY2295 with different rotavirus genotypes [14]. The length of the abscissa to the connecting node is proportional to the genetic distance between sequences. [a]The following VP4 sequences were obtained from published reports and the GenBank database: HAL1166 (accession no. L20875); PA169 (L20874); Mc35 (D14032); MG6 (U22012); ALA (U62149); C-11 (U62150); R-2 (U62151); BAP-2 (U62152); A5 (D13395); SA11 (X14204) [27]; RRV (M18736); RV-5 (M32559); UK (M22306); ST3 (L33895); OSU (X13190); Wa (M96825); AU-1 (D10971); 69M (M60600) [32]; B223 (M92986); H2 (L04638); MDR-13 (L07886); Lp14 (L11599); Eb (L18992); 993/83 (D16352); L338 (L26888); 4F (L10359); and EHP (U08424)

produced to the human P[14], G8 strain EGY2295 did not hybridize strongly to either representative of the human genogroups Wa or DS-1, but the same probe did hybridize with 8 segments of HAL1166 thereby confirming a close relationship between the Egyptian P[14], G8 strain EGY2295 and the human P[14], G8 strains from Finland. In contrast, hybridization of the EGY2295 probe with the human P[14], G6 strains PA169 and the bovine P6[1], G6 strain NCDV was limited to 3–5 segments. The RNA hybridization pattern found with NCDV, a representative of the bovine genogroup, supports earlier findings of RNA-RNA hybridization between G8 strains and bovine rotaviruses [45].

Table 2. Reciprocal FFN titers of neutralizing monoclonal antibodies against strains EGY1850 and EGY2295

Virus	P & G Types	Monoclonal antibody	
		B37:1 (G8 MAb)	2H4 (P[14] MAb)
EGY1850	P[14], G8	17,000	16,000
EGY2295	P[14], G8	26,000	13,000
Wa	P1A[8], G1	<100	–
DS-1	P1B[4], G2	<100	<100
P	P1A[8], G3	<100	<100
ST-3	P2A[6], G4	<100	<100
PA169	P[14], G6	–	30,000
69M	P4[10], G8	21,000	–

Discussion

To our knowledge, this study represents the first detection and characterization of P[14], G8 rotavirus strains in Egypt. Our findings are based in part on sequence analysis of the genes encoding VP7 and partial VP4 of strains EGY1850 and EGY2295, which showed a strong relationship to P[14], G8 rotavirus strains, and were confirmed serologically with a serotyping EIA and FFN assays. Strains with G8 serotype specificity have been identified worldwide with human G8 rotavirus strains previously found in several countries including Indonesia, Finland, Italy, the United Kingdom, and Brazil (Table 3) [3, 18, 20, 43]. Therefore, our findings of serotype G8 in Egypt support previous reports of G8 rotaviruses in other African countries (Nigeria and Malawi) and suggest that this serotype may be more common in the developing countries of Africa than previously thought [1, 9, 10].

Rotaviruses with the uncommon VP7 serotype, G8, have been isolated from both human and bovine species [1, 18, 45, 48]. Interestingly, among all of the human serotype G8 strains characterized to date, the VP7 of EGY2295 showed the greatest nucleotide homology to a bovine strain A5 (P6[1], G8) which was isolated from a young calf with diarrhea in Thailand [48]. In addition, the VP7s of EGY1850 and EGY2295 also share a high degree of similarity with the human G8 strain HAL1166 (P[14], G8) (>85% nt, >96% aa) which was isolated from an infant with diarrhea in Finland [18].

To date, the unusual VP4 genotype P[14] has only been rarely isolated from humans and rabbits [6, 17, 19, 39]. Strains EGY1850 and EGY2295 were shown to possess the P[14] genotype through sequence analysis of part of the gene encoding VP4 and exhibited the greatest similarity to previously reported human P[14] strains HAL1166 (P[14], G8), PA169 (P[14], G6), MG6 (P[14], G6) and Mc35 (P[14], G10). The VP4s of these Egyptian strains shared the highest identity (>97% aa) to strain HAL1166. Our findings of the P[14] genotype are supported by earlier studies of Mphahlele and Steele (1995) who detected rotaviruses of the P[14] genotype among six infants from South Africa [34]. Thus, our findings

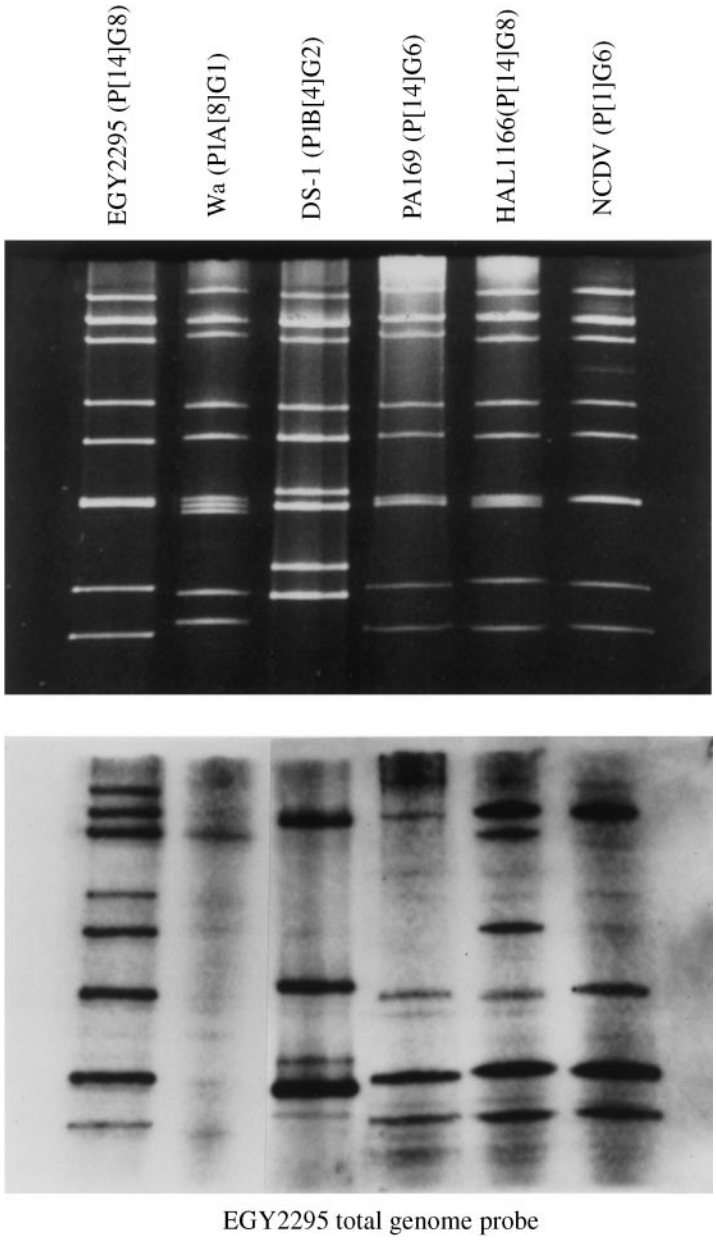

Fig. 2. Northern hybridization analysis of rotavirus strains using DIG-labeled total genome probe of EGY2295. RNA was electrophoresed in a 10% (w/v) polyacrylamide gel, stained with ethidium bromide (top), denatured, transferred to a nylon membrane and hybridized using the whole genome probe of strain EGY2295 (bottom)

suggest that this genotype is circulating in Africa and may not be uncommon. In addition, sporadic detections of P[14] rotaviruses in Finland, Italy, Thailand, Australia, United States and South Africa (Table 4) demonstrate that genotype P[14] is distributed globally and may have greater epidemiological significance [6, 20, 34, 39].

Table 3. Summary of serotype G8 rotavirus strains isolated from humans

Country	Collection year	Characterization		Number of children	Prototype strain(s)	Ref.
		Subgroup	PAGE			
Indonesia	1978–79	I	super short	2	B37, B38	[2]
Indonesia	1979–81	I	super short	1	69M	[26]
Finland	1983–86	I	long	6	HAL1166	[18]
Italy	1987	II	long	1	PA171	[18]
United Kingdom	1983–94	ND	ND	2	not listed	[3]
Nigeria	1993–95	ND	short	1	HMG89	[1]
Brazil	1996–97	ND	short	2	not listed	[43]
Malawi	1997–98	ND	short	51	not listed	[9]
Egypt	1995–96	I	long	6	EGY1850, EGY2295	

ND Not determined

Table 4. Summary of genotype P[14] rotavirus strains isolated in humans and animals

Country	Collection year(s)	Origin	Characterization			Number of cases	Prototype strain	Ref.
			PAGE	Subgroup	G Serotype			
Finland	1983–86	Human	long	I	G8	6	HAL1166	[19]
Italy	1987–88	Human	long	I	G6	1	PA169	[17]
Thailand	1987–89	Human	long	I	G10	1	Mc35	[52]
South Africa	1987, 1989, 1991	Human	ND	ND	ND	4, 1, 1	not listed	[34]
Australia	1993	Human	long	I	G6	1	MG6	[39]
United States	1989	Lapine	ND	ND	G3	3	BAP-2	[6]
Japan	not listed	Lapine	ND	ND	G3	1	R-2	[6]
Egypt	1995–96	Human	long	I	G8	6	EGY1850, EGY2295	

ND Not determined

Our results provide new insights on the role of interspecies transmission of rotavirus strains between humans and animals. The VP7s of strains EGY1850 and EGY2295 might have a common origin with other P[14] strains. Both strains EGY1850 and EGY2295 have a VP7 G8 serotype that has been commonly isolated from cattle [48]. Likewise, the reported human P[14] strains [MG6 (P[14], G6), PA169 (P[14], G6), HAL1166 (P[14], G8) and Mc35 (P[14], G10)] all possess G serotypes that are common in cattle [15, 46]. Additional studies on these P[14] strains have reported that their G6, G8 or G10 VP7-serotype specificities

were acquired from bovine sources [4, 20, 39, 53]. Similar to strains EGY1850 and EGY2295, the four reported human P[14] strains possess the unusual combination of subgroup I specificity with a long electropherotype, which is characteristic of bovine rotaviruses [53]. In addition, our EGY2295 total genome probe hybridized with 3–5 segments of the bovine strain NCDV and 8 segments of HAL1166 thereby supporting the idea that strain EGY2295 may also be a reassortant between a human and bovine rotavirus strain. Browning et al. (1992) previously reported that the Finnish strains including HAL1166 hybridized to 7 segments of a NCDV probe [4]. Thus, the close relationship between EGY2295 and HAL1166 as revealed through RNA-RNA hybridization studies extends earlier findings that human G8 rotaviruses may be genetically related to bovine rotaviruses. Therefore, our genetic analysis of the VP7 gene and the genogrouping results support the concept that all human P[14] strains reported to date, which all share bovine-associated VP7 serotypes, may possibly be natural reassortants formed as a consequence of interspecies transmission of rotaviruses between humans and cattle.

The origin of the P[14] VP4 gene is unclear. Since this genotype is not common in human rotaviruses and has not been reported in bovine rotaviruses, one hypothesis is that the VP4 P[14] gene may be from a third species such as rabbits [6]. Ciarlet and coworkers demonstrated that all rabbit P[14] rotavirus strains analyzed to date have VP4 genes closely related to human P[14] VP4s but also contain serotype G3 VP7 genes [6]. These investigators proposed that a reassortment event as a consequence of interspecies transmission in humans, lapine or bovine species may have generated the P[14] strains found in humans [6]. The high degree of similarity of the partial fragment of the VP4 genes (>93% aa) of all of the human P[14] rotaviruses, in combination with their shared bovine G serotypes, adds further support to the idea that gene reassortment may occur among human, lapine and bovine rotaviruses. Unlike the VP7 similarities, identities of the VP4s between the human Egyptian strains and the bovine strain A5 was low. This finding suggests that A5-like strains could be only one possible parent of the human strains thereby requiring a reassortment event with a P[14] strain perhaps of lapine origin. This shared similarity between the VP4 P[14] gene may possibly be a means for rotavirus antigenic evolution which may help explain the unusual P[14], G8 combination found only in humans. Since VP4 plays an important role in rotavirus infectivity, perhaps the P[14] VP4 has unusual properties that promote interspecies transmission events. A second possibility that explains the unusual combination of the P[14] VP4 gene with distinctly different VP7 genes between the P[14] human and lapine rotavirus strains is a common origin that has since diverged.

Hum et al. (1989) previously reported that the antigenic region C of serotype G8 strain B37 showed a close similarity to serotype G3 strains. Our deduced amino acid sequence data for strains EGY1850 and EGY2295 reconfirm the similar antigenic C region of VP7. This similarity may possibly be explained on the basis of a common ancestor for G3 and G8 rotaviruses and agrees with earlier suggestions that there may be a genetic and antigenic connection between

the VP7s of G3, G8 and G11 rotavirus strains [29]. In addition, this link may eventually be helpful in understanding the evolutionary relationships between lapine and human P[14] rotaviruses.

This study indicates that unusual rotavirus strains such as P[14], G8 exist in Egypt and may be epidemiologically important representing 11% (unpubl. data) of the total strains typed [10, 40]. In addition, a recent review of rotavirus diarrhea in Africa indicated that 26% of strains were nontypeable for VP7 serotype raising the possibility that rotavirus strains other than the four major types G1–G4 may be prevalent in Africa [10]. Thus, our findings together with the limited number of serotyping studies with many nontypeable strains underscore the importance of monitoring the circulating serotypes of rotavirus in Africa. It will be important to characterize the various strains in circulation throughout Africa and subsequently establish the epidemiological importance of uncommon strains to assess whether the tetravalent rotavirus vaccine provides heterotypic protection against unusual types such as the P[14], G8 type described in this report or whether additional reassortant vaccine strains will need to be included in future vaccines [54].

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