Distribution of Rotavirus VP7 Serotypes and VP4 Genotypes Circulating in Sousse, Tunisia, from 1995 to 1999: Emergence of Natural Human Reassortants

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Rotavirus strains circulating in Sousse, Tunisia, between 1995 and 1999 were characterized antigenically by monoclonal antibodies to the VP6 subgroup and the VP7 serotype. The VP4 genotype was determined by reverse transcription-PCR, as were the strains with untyped VP7. Only 17% of 375 children were shedding rotavirus as determined by latex agglutination assay. Most rotavirus strains were G1P[8] (50%), followed by G4P[8] and G4P[6]. Reassortant G1P[4] strains emerged in Sousse during the 1998–1999 season.

In this study, we conducted the characterization of the human rotavirus strains found in Sousse, Tunisia, between 1995 and 1999.

MATERIALS AND METHODS

Patient sample. Fecal specimens were collected between February 1995 and May 1999 from 375 infants and young children in Sousse, Tunisia. The specimens were collected from infants and young children between 1 month and 60 months of age presenting with acute diarrheal illness to the Hopital Farhat Hached. Rotavirus detection was performed on 10 to 20% suspensions of the fecal specimens in phosphate-buffered saline, using a latex agglutination assay (Rotavirus Slide; BioMerieux, Marcy l’Etoile, France). The test was performed as specified by the manufacturers.

Polyacrylamide gel electrophoresis. The rotavirus-positive fecal specimens were analyzed by polyacrylamide gel electrophoresis (PAGE) to identify the rotavirus strains in circulation. In brief, the 10 to 20% fecal suspensions were mixed with an equal volume of phenol-chloroform to disrupt the viral particles and release the viral double-stranded RNA (dsRNA) genome (19). After centrifugation at 1,200 × g for 5 min, the aqueous phase containing the dsRNA was precipitated in absolute ethanol overnight at −20°C. Following centrifugation, the dsRNA pellet was reconstituted in 0.01 M Tris–EDTA buffer and the enzymatic reaction was read at 450 nm.

Rotavirus strains in circulation. In limited North African studies, rotavirus has been reported as a significant etiological agent of severe dehydrating diarrhea in young children. In Egypt, the epidemiology of rotavirus infection was reported to occur in the cooler months of the year and in very young infants and was more significantly associated with watery diarrhea and vomiting as presenting features (3). An early study to characterize Egyptian rotaviruses showed that subgroup II (SG II) strains were more common than SG I strains (14). The most recent study, which analyzed the rotavirus VP7 serotype with monoclonal antibodies, showed that serotypes G1 and G4 predominated and that mixed G1-G4 strains were common (17). Furthermore, G8P[14] strains have also been recently identified in Egyptian children (10). Rotavirus infection has been also reported in North Africa from Algeria (16, 24) and Morocco (23), although no characterization of the rotavirus strains was performed.

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were blocked with 2.5% skim milk (Carnation) in phosphate-buffered saline before the stool specimens were added for incubation overnight at 4°C. After washing the plates, a 1:10,000 dilution of a rabbit antirotavirus hyperimmune serum (kindly donated by Taka Hoshino, National Institute of Allergy and Infectious Diseases) was incubated at 37°C for 1 h to bind to the captured rotavirus particles. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Dako) was added as the detector antibody, and the presence of the enzyme was measured spectrophotometrically at 450 nm using the TMB enzymatic kit (Roche) as the substrate.

The untyped specimens were analyzed by the nested PCR-typing method of Gouvea et al. (7), which has been widely utilized for genotyping the VP7 gene of human rotaviruses (4, 25).

**VP4 genotyping.** The VP4 genotype of the rotavirus strains was determined in selected numbers by the typing method devised by Gentsch et al. (5). Initially, the viral RNA was extracted by a phenol-chloroform method, followed by ethanol precipitation and then purification by RNAid (Bio 101 and Southern Cross Biotechnology). The purified RNA was reverse transcribed with avian myeloblastosis virus reverse transcriptase (Promega) at 43°C for 25 min in the presence of primers to the VP8* region of the gene (con2 or con3). The cDNA was amplified with the same primers in a Perkin-Elmer thermal cycler (4800) at 95°C for 1 min to denature the cDNA, 48°C for 2 min to anneal the primers, and then 72°C for extension of the strands. A nested PCR was then performed using separate primers specific for regions of the VP8* gene which are divergent in the distinct VP4 gene alleles of rotavirus, but conserved within members of the same genotype (5). The commonly identified human rotavirus VP4 genotypes were included (i.e., P[8], P[4], P[6], P[9], and P[10]).

**RESULTS**

Overall, rotavirus antigen was detected in 65 of 375 stool specimens (17.3%) collected from infants and young children who presented with diarrhea to the Hopital Universitaire in Farhat Hached, Sousse, Tunisia.

Second, rotavirus infection was found to occur predominantly in the cooler season in Tunisia, with most cases occurring between November and February (Fig. 1). Third, all but three of the rotavirus-positive children were less than 2 years of age, with the majority (92%) under 12 months of age (Fig. 2).

**PAGE.** PAGE was performed on all the rotavirus-positive specimens to examine the genomic diversity of the dsRNA of the strains. In total, 52 of the 65 rotavirus-positive specimens (78%) yielded an RNA electropherotype and showed a limited number of rotavirus strains circulating in the city. There was a major, long RNA electropherotype that circulated every year during the study, with five minor long variations present. In 1999, one of these strains appeared in numbers equal to those of the predominant strain of 1995 to 1998. Only two specimens with a short RNA electropherotype were detected, one each in 1998 and 1999.

Three specimens were seen which carried more than the 11 dsRNA segments, and they were classified as dual infections with more than one strain of rotavirus. Two of these were dual infections between a long and a short RNA electropherotype. VP6 SG specificity. VP6 SG II strains were identified in 50 cases (77%) and predominated overall, as was expected from the overwhelming majority of long RNA electropherotypes seen. In fact, only three SG I strains were identified during the study. Furthermore, two of the SG I strains were identified as G2P[4] strains. In two more cases both SB I and SB II monoclonal antibodies reacted in obvious mixed infections, where strains with both long and short RNA electropherotypes were observed (Table 1).

**VP7 serotype specificity.** In total, 51 of the strains were serotyped by the monoclonal antibodies used in this study or genotyped by a nested PCR assay for the VP7 serotype. In
1995 to 1997, VP7 serotype G1 viral strains were found to be predominant and were detected in 25 of 34 (73%) of the specimens (Table 1). Serotype G4 strains were detected in four cases, one of which was a dual infection with a G1 strain, which was confirmed by the presence of more than 11 RNA segments in the gel. However, G4 strains became more common during 1999 with the emergence of a new electrophoretic strain. Only two G2 serotypes were seen, one in each of the last 2 years of the study. In 1996 and 1998, G1-G2 mixed infections were detected. No serotype G3, G8, or G9 strains were seen, although 14 strains (42%) could not be typed by the monoclonal antibodies.

**VP4 genotype.** The VP4 genotype assay was performed on 25 selected strains from different RNA profiles. P[8] was the predominant gene allele circulating in Sousse during the study and comprised 52% of the strains identified. The second most commonly seen VP4 genotype was P[6], which was associated with G4 strains in 1995 and 1999. VP4 P[4] was detected in the G2 specimens in 1998 and 1999 (Table 1). VP4 P[4] genotypes were detected more commonly during the last year of the study, but were associated with a G1 VP7 serotype.

**DISCUSSION**

In this study, we report the first antigenic and genotypic characterization of group A rotaviruses in Tunisia. First, group A rotaviruses were detected in the stools of 17% of the infants and young children who presented with acute diarrheal disease to the Hopital Farhat Hached. This figure is lower than the 40% reported in Egypt (3) or 33% in Sicily (1). However, the prevalence of rotavirus infection in this study is similar to that reported in other countries in North Africa, such as Algeria (16, 24), Morocco (23), and, in an earlier study, Egypt (17). In the present study, rotavirus infection was detected by a latex agglutination assay, compared to the ELISA techniques used elsewhere, and this may account for the generally slightly lower levels of rotavirus detection observed. This has been described previously (21).

Second, rotavirus infection was found to occur predominantly in the cool, dry season in Tunisia. This is also similar to the pattern seen in other North African countries where rotavirus infection has been recorded, such as Morocco (23), Algeria (16, 24), and Egypt (3), where epidemiological studies showed the same seasonal trend. Third, this study confirms that rotavirus infection in this region occurs in children less than 2 years of age, and predominantly in infants less than 12 months of age, as described for Egypt (17). Interestingly, in other Mediterranean areas and countries, such as Sicily (1) and France (5), rotavirus infection has shown similar trends, peaking in the cooler months of the year and predominantly infecting younger children.

PAGE of the rotavirus RNA showed the presence of limited strains circulating in the city. A single, long RNA electrophoretic pattern predominated and was seen in 63% of all cases. This strain also persisted throughout the 5 years of the study, although in 1999 another long RNA electropherotype was detected commonly. In addition, only two short RNA patterns were seen in stool specimens, indicating an unusual absence of these strains over a relatively long period of time. Two mixed infections with long and short electropherotypes were observed. This contrasts with the situation described in Casablanca, Morocco (23), where in just 1 year, nine different
the potential for reassortment between these two viruses to generate the G1P[4] strain is clear from the number of these reassortants found in 1998–1999. Other studies have also demonstrated the potential for human rotavirus reassortants, which in some areas appear to occur at high levels (25).

Further studies to determine the VP7 serotype of circulating strains of rotaviruses are needed in developing countries for a number of reasons. First, the recent development of an effective group A rotavirus vaccine, although withdrawn from use by the company currently, has stimulated further research for a rotavirus vaccine. However, the successful implementation of a vaccine requires an understanding of the VP7 serotype epidemiology of rotaviruses in Africa. This study is the first to report the VP7 serotype and VP4 genotype of circulating rotavirus strains in Tunisia; however, further studies are planned to investigate the epidemiology of human rotavirus VP7 serotypes in North Africa.

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REFERENCES


RNA electropherotypes were observed, with a clear shift noted during the year.

The apparent lack of genomic diversity, which would be expected in an urban setting, may be due to the limited numbers included in this study. Nevertheless, minor RNA genome patterns were seen each year and one appeared to be competing successfully by the last year of the study.

SG II rotaviruses were identified commonly, as was to be expected. Other studies have reported a predominance of VP6 SG II strains in this region, in Egypt (14), in Sicily (1), and elsewhere (6, 20, 27).

Reports of the distribution and epidemiology of the human rotavirus VP7 serotypes and VP4 genotypes in North Africa are limited in number. In this study, the vast majority of strains were typed as G1P[8] strains, with G4P[6] and G1P[6] strains the next most common strains. Only two G2 strains were identified, one in 1999 and one in 1998. The predominance of G1 strains reflects both the earlier study performed in Egypt (17) and the trend seen globally (6). In the only similar study undertaken in North Africa, where the VP7 serotype of rotaviruses in Egypt was examined, G1 and G4 strains also predominated and G1-G4 mixed strains were detected quite commonly (17). In recent studies in France (5) and Sicily (1), G4 strains were most common.

In this study, a few apparently “reassortant” viruses were identified which carried a G1P[4] background. Most human rotavirus strains show a strong association of the G1 serotype with a P[8] VP4 genotype, and the P[4] genotype is normally determined in viruses with a G2 VP7 serotype (6, 13, 26). These G1P[4] reassortant rotaviruses have been described to occur at a low level in other settings, such as in Sicily (1), the United States (5), and Japan (27). It was surprising that although G2P[4] strains occurred in such low numbers in Sousse during this study, there were two dual infections with a G2P[4] and G1P[8] strain. It is unclear why this should be so. Nevertheless,

<table>
<thead>
<tr>
<th>Year/No. of Specimens</th>
<th>RNA Profileb</th>
<th>VP6 Subgroup</th>
<th>VP7 Serotype</th>
<th>VP4 Genotypec</th>
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<tbody>
<tr>
<td>1995/17</td>
<td>TL1 (6) II</td>
<td>G1 (6)</td>
<td>P[8] (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL2 (4) II</td>
<td>G4 (3)</td>
<td>P[6] (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL3 (4) II</td>
<td>G1 (4)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed (1) II</td>
<td>G4/1 (4)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1996/14</td>
<td>TL1 (10) II</td>
<td>G1 (10)</td>
<td>P[8] (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL3 (1) II</td>
<td>G1 (1)</td>
<td>P[8] (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TL4 (1) Not typed</td>
<td>G1 (1)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed (1) II</td>
<td>G1/2 (1)</td>
<td>P[4]/P[6] (1)</td>
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<tr>
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<td>TL1 (2) II</td>
<td>G1 (2)</td>
<td>ND</td>
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<tr>
<td></td>
<td>TL3 (1) II</td>
<td>G1 (1)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TS1 (1) I</td>
<td>G2 (1)</td>
<td>P[4] (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed (1) I/II</td>
<td>G1/G2 (1)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>1999/21</td>
<td>TL1 (6) II</td>
<td>G1 (6)</td>
<td>P[8] (3); P[4] (3)</td>
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<tr>
<td></td>
<td>TL6 (1) I</td>
<td>G4 (1)</td>
<td>P[6] (1)</td>
<td></td>
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<tr>
<td></td>
<td>TS2 (1) I</td>
<td>G2 (1)</td>
<td>P[4] (1)</td>
<td></td>
</tr>
</tbody>
</table>

* Values enclosed in parentheses are the numbers of specimens typed.
* The RNA electropherotypes are described as TL1 to TL5 for Tunisia long profiles, differentiated as numbers 1 to 5, and TS1 and TS2 for the two Tunisian short profiles.
* NT, not typed; ND, not done.