APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2003, p. 3919–3923 Vol. 69, No. 7
0099-2240/03/$08.00+0 DOI: 10.1128/AEM.69.7.3919–3923.2003

Group A Rotavirus in Sewage Samples from Barcelona and Cairo: Emergence of Unusual Genotypes

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Received 29 October 2002/Accepted 31 March 2003

The presence of rotavirus strains in sewage samples from Cairo, Egypt (November 1998 to October 1999), and Barcelona, Spain (November 1998 to December 2002), was investigated by using a generic molecular detection method based on amplification of a VP6 gene fragment. Overall, 85.7 and 66.9% of the sewage samples from Cairo and Barcelona, respectively, were positive. Positive samples were characterized further, and VP7 and VP4 genotypes were determined. Although 30% of the positive samples from Cairo were G untypeable, the distribution of G types in the positive samples was 69.6% G1, 13% G3, 8.7% G4, and 8.7% G9. The percentage of untypeable samples was much higher for the Barcelona samples (56.5%), and the distribution in the positive samples was 56.4% G1, 31.5% G3, 6% G9, 4% G2, and 2% G5. When the P types were examined, 26.7% of the positive samples from Cairo were untypeable, and the distribution of types in the positive samples was 53.3% P[8], 30% P[6], and 16.6% P[4]. In Barcelona, 27.2% of the samples were P untypeable, and the frequencies of the types detected were 49.7% P[8], 37.2% P[4], 8.8% P[6], and 4.2% P[9]. The distribution for strains from Cairo was 38.5% P[8]G1, 27% P[6]G1, 11.5% P[4]G1, 11.5% P[8]G3, 7.7% P[6]G4, and 3.8% P[8]G9. Strikingly, equivalent frequencies of common and uncommon strains were observed for Barcelona samples, and the distribution was 38.8% P[8]G1, 30.6% P[4]G1, 11.6% P[8]G3, 6.6% P[4]G3, 5.8% P[6]G1, 1.6% P[6]G3, 1.6% P[9]G1, 0.8% P[4]G2, 0.8% P[6]G9, 0.8% P[8]G9, and 0.8% P[8]G5. Additionally, two P[−]G5 strains were isolated in Barcelona, and the porcine or human origin of these strains was unclear. Rotavirus variability exhibited not only a geographic pattern but also a temporal pattern.

Group A rotaviruses are the leading cause of infantile diarrhea worldwide (24) and are associated with more than 600,000 deaths annually, mainly in developing countries (15). Rotavirus strains may be serotyped on the basis of two outer capsid proteins that are the targets of neutralizing antibodies produced following natural infection; these are glycoprotein VP7, which determines G serotypes, and the protease-sensitive protein VP4, which determines P types (24). Fourteen rotavirus G serotypes, including 10 serotypes that infect humans, have been identified (11). A good correlation between G serotypes and genotypes has been demonstrated (16), and a new genotype of bovine origin has been recognized (30). The correlation is less clear for VP4, since while 13 P serotypes have been detected (11), at least 20 genotypes, including 8 genotypes that infect humans, have been characterized (11). Genotyping studies have indicated that four G-P combinations, P[8]G1, P[4]G2, P[8]G3, and P[8]G4, are common worldwide. Two emerging combinations, P[8]G9 and P[6]G9, are becoming common (1, 2, 12, 15, 23). The distribution of rotavirus types may vary between distinct geographic and socioeconomic regions of the world or even between years in a given community. Since the rotavirus serotypes (genotypes) circulating in a given region have a direct influence on the predicted efficiency of a potential vaccine for the region, an examination of the G and P type distribution is necessary.

The aim of the present work was to ascertain the G and P type distributions in two different urban areas, Greater Cairo in Egypt from November 1998 to October 1999 and Barcelona in Spain from October 1998 to December 2002, by using sewage samples as the virus sources.

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MATERIALS AND METHODS

Sewage samples. Raw sewage from three sewage treatment plants (Balaks, Zenin, and El Berka) in Cairo, Egypt, was sampled monthly from November 1998 to October 1999 ($n = 35$). Raw sewage from the Sant Adrià del Besòs sewage treatment plant in Barcelona, Spain, was sampled during a 4-year period. From November 1998 to October 1999 a mean of 10 samples per month were obtained ($n = 125$), from November 1999 to May 2000 a mean of 25 samples per month were analyzed ($n = 174$), and from November 2000 to October 2001 and from November 2001 to December 2002 a mean of two samples per month were obtained ($n = 30$ and $n = 28$, respectively).

Different concentration procedures were used in Cairo and Barcelona. In the first case, viruses from 3 liters of sewage were concentrated in 75 ml of 50 mM glycine buffer with 3% beef extract (Oxoid) by adsorption-elution to negatively charged nitrocellulose membranes (Schleicher and Schuell) (31) and were reconcentrated by organic flocculation in 1 ml of 0.14 N Na_2HPO_4 (pH 7) (25). Lyophilization was employed to concentrate raw sewage in Barcelona. Samples were concentrated by freeze-drying 50 ml and resuspending the dried material in 500 μl of distilled water (13, 28).

Rotavirus detection. RNA was purified from 50-μl portions of concentrated sewage samples by guanidine thiocyanate extraction, as previously described (5).

For generic detection of rotaviruses, a reverse transcription (RT)-PCR–hybridization method based on amplification of a VP6 fragment and confirmation by Southern blot hybridization with a digoxigenin-labeled internal probe was used. Primers VP6-3 (5′-GCTTTAAAACGAAGTCTTCAAC-3′; positions 2 to 23 of human strain Wa [accession number K02086]) and VP6-4 (5′-GGTAAA TTACCAATTCCTCCAG-3′; positions 187 to 166 of human strain Wa [accession number K02086]), each at a concentration of 1 μM, were used in an RT reaction in a 10-μl (final volume) mixture containing 4 U of Moloney murine leukemia virus enzyme (Promega), each deoxynucleoside triphosphate at a concentration of 0.2 mM, and 5 μl of a denatured (5 min at 99°C) double-stranded RNA sample. The reaction mixture was incubated for 60 min at 50°C. Five microliters of the RT product was processed by PCR by using 3.5 U of the Expand High Fidelity PCR system (Roche) in a 50-μl (final volume) mixture

TABLE 1. Distribution of rotavirus G types in sewage samples from Cairo and Barcelona

City	Sampling dates	No. of positive samples (%)									
		G1	G2[a]	G2[b]	G2 total	G3	G4	G5	G8	G9	Total
Cairo	Nov. 1998–Oct. 1999	16 (69.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (13.0)	2 (8.7)	0 (0.0)	0 (0.0)	2 (8.7)	23 (100.0)
Barcelona	Nov. 1998–Oct. 1999	7 (38.9)	0 (0.0)	2 (11.1)	2 (11.1)	8 (44.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.5)	18 (99.9)
	Nov. 1999–May 2000	59 (62.1)	0 (0.0)	0 (0.0)	0 (0.0)	30 (31.6)	0 (0.0)	2 (2.1)	0 (0.0)	4 (4.2)	95 (100.0)
	Nov. 2000–Oct. 2001	15 (55.5)	0 (0.0)	4 (14.8)	4 (14.8)	7 (25.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)	27 (99.9)
	Nov. 2001–Dec. 2002	3 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (22.2)	0 (0.0)	1 (11.1)	0 (0.0)	3 (33.3)	9 (99.9)
	Total	84 (56.4)	0 (0.0)	6 (4.0)	6 (4.0)	47 (31.5)	0 (0.0)	3 (2.0)	0 (0.0)	9 (6.0)	149 (99.9)

[a] All samples were tested with primers RVG9 and aCT2.
[b] All samples that were P[4] positive and G2 negative with primers RVG9 and aCT2 were also tested with primers 9 Con 1 and 9T1-2.

supplemented with each primer at a concentration of 1 μM and each deoxynucleoside triphosphate at a concentration of 2 mM. The PCR program included a 9-min denaturation step at 95°C and 40 cycles of amplification for 1 min at 94°C, for 1 min at 50°C, and for 1 min at 72°C, followed by a final elongation step of 7 min at 72°C. In order to confirm the rotaviral nature of the 186-bp amplimer, Southern blot hybridization with a digoxigenin-labeled probe (5′-CAAATGAT AGTTACTATGAATGG-3′; positions 129 to 151 of human strain Wa [accession number K02086]) was performed.

Rotavirus typing. VP6-positive samples were analyzed further, and the G type and P type were determined by using the methods described by Gouvea et al. (16) and Gentsch et al. (14), respectively. The cocktail of primers used in the multiplex reaction allowed determination of the G1, G2, G3, G4, G5, G8, and G9 VP7 types (16, 19) and the P[4], P[6], P[8], and P[9] VP4 types (14). Additionally, a monoplex reaction for G2 typing was performed with the samples that were P[4] positive and G2 negative as determined by the multiplex nested PCR. Two separate monoplex nested PCR G2 typing reactions, either with the RVG9 and aCT2 primers (16) or with the 9 Con 1 and 9T1-2 primers (10), were used. On the other hand, a monoplex nested PCR with the P[8]-specific primer 1T-1D was performed with all the P[8]-negative samples (22). Several samples were specifically analyzed for the presence of the porcine P[6] (P2B), P[7], and P[13] genotypes. The P[6] (P2B) and P[7] genotypes were detected by using the procedure described by Gouvea et al. (18), while the P[13] genotype was detected by using the specific primer 501-P[13] (5′-CCTCCRTATTTCATTGCAGCTT G-3′; positions 524 to 501 of the Po/A46 strain [accession number AY050274]) and the RVG9 common primer described by Gouvea et al. (16). As positive controls the following human reference strains were used: Wa (G1P[8]), Ito (G3P[8]), VA70 (G4P[8]), and DS-1 (G2P[4]). The OSU strain (G5P[7]) was used as a control for porcine viruses.

Sequencing. The sequences of the amplimers generated in the nested G typing reaction were determined for 100 and 60% of the G5 and G2 samples, respectively. The sequences of the nested products of the P typing reaction for 15% of the P[4] samples and 7% of the P[8] samples were also determined. Portions (40 μl) of the nested PCR products were electrophoresed on a 1% agarose gel, and the DNA was purified with a High Pure PCR product purification kit (Roche) used according to the manufacturer's instructions. The nucleotide sequences were determined by using 2 to 7 μl of the purified DNA with an ABI PRISM Big Dye terminator cycle sequencing Ready Reaction kit (version 2.0; Perkin-Elmer) and an ABI Prism 7700 automated DNA sequencer (Perkin-Elmer). Sequences of both chains were used for genetic analysis of the fragments mentioned above.

Phylogenetic analysis of the G5 sequences. Each nucleotide sequence was compared to the sequences of reference strains by using the BLAST program (National Center for Biotechnology Information). Multiple alignments of nucleotide sequences were constructed by using the CLUSTALW program (European Bioinformatics Institute). Nucleotide distance matrices were calculated by the pairwise distance method by using the Molecular Evolutionary Genetics Analysis 2.1 software (MEGA, version 2.1) (26). Phylogenetic relationships were analyzed with and without bootstrap analysis of 100 replicates.

RESULTS

Rotavirus detection. Overall, 30 (85.7%) of 35 sewage samples from Greater Cairo were positive for rotavirus, as determined by a generic VP6 detection method. In Barcelona, 239 (66.9%) of 357 samples were positive. Negative samples were randomly distributed throughout the year in both studies.

Rotavirus G typing. A total of 21 of 30 Egyptian rotavirus-positive samples could be G typed, so 30% of the samples were untypeable. Overall, the most frequent type was G1 (69.6%), followed by G3 (13.0%), G4 (8.7%), and G9 (8.7%) (Table 1). The percentage of samples containing more than one type was 9.5%.

In the case of the Spanish samples, only 104 of 239 samples could be typed, and thus the percentage of untypeable samples was much higher (56.4%). The most frequent type again was G1 (56.4%), followed by G3 (31.5%), G9 (6.0%), G2 (4.0%), and G5 (2.0%) (Table 1). Since the G2 type was detected only by using primers 9 Con 1 and 9T2-1, the sequence of 60% of the amplimers, randomly chosen, was used to confirm the actual G2 nature. In this analysis 33.9% of the positive samples contained mixed types.

Rotavirus P typing. Overall, 22 of 30 rotavirus-containing samples from Cairo could be P typed, and thus the percentage of untypeable samples was 26.7%. The frequencies of P types were as follows: P[8], 53.2%; P[6], 30.0%; and P[4], 16.6% (Table 2). Mixed types were detected in 31.8% of the sewage samples.

A total of 174 of 239 Spanish samples could be P typed, and

TABLE 2. Distribution of rotavirus P types in sewage samples from Cairo and Barcelona

City	Sampling dates	No. of positive samples (%)						
		P[4]	P[6]	P[8][a]	P[8][b]	P[8] total	P[9]	Total
Cairo	Nov. 1998–Oct. 1999	5 (16.6)	9 (30.0)	11 (36.6)	5 (16.6)	16 (53.3)	0 (0.0)	30 (99.9)
Barcelona	Nov. 1998–Oct. 1999	18 (36.7)	6 (12.2)	6 (12.2)	17 (34.7)	23 (46.9)	2 (4.0)	49 (99.8)
	Nov. 1999–May 2000	74 (39.1)	10 (5.3)	26 (13.7)	75 (39.7)	101 (53.4)	4 (2.1)	189 (99.9)
	Nov. 2000–Oct. 2001	18 (37.5)	8 (16.7)	9 (18.7)	9 (18.7)	18 (37.4)	4 (8.3)	48 (99.9)
	Nov. 2001–Dec. 2002	4 (20.0)	3 (15.0)	3 (15.0)	7 (35.0)	10 (50.0)	3 (15.0)	20 (100.0)
	Total	114 (37.2)	27 (8.8)	44 (14.4)	108 (35.3)	152 (49.7)	13 (4.2)	306 (99.9)

[a] All samples were tested with primers 1T-1 and Con 3.
[b] All samples that were negative with primers 1T-1 and Con 3 were also tested with primers 1T-1D and Con 3.

TABLE 3. Distribution of common and uncommon rotavirus strains in different countries and in Cairo and Barcelona

Strains	% of strains							
	United States (1996–1999)[a]	India (1996–1998)[b]	People's Republic of China (1998–2000)[c]	Argentina (1996–1998)[d]	Sousse (Tunisia) (1995–1999)[e]	Valencia (Spain) (1996–1999)[f]	Cairo (Egypt) (1998–1999)	Barcelona (Spain) (1998–2002)
Common strains								
P[8]G1	76	15	29	12/23	50	43	38.5	38.8
P[8]G3	2	0	13	0/0.2	0	2	11.5	11.6
P[8]G4	1	6	2	4/11	0	32	0	0
P[4]G2	11	22	31	43/30	8	6	0	0.8
Total	90	43	75	59/64	58	83	50	51.2
Uncommon strains								
P[6]G1	0.5	0	2.4	—/1.4[g]	0	0	27	5.8
P[4]G1	0.1	0	4.1	14/2.7	21	0	11.5	30.6
P[6]G4	—	0.7	2.8	—/0.2	21	0	7.7	0
P[8]G9	1	5	0.3	—	0	—	3.8	0.8
P[4]G3	—	0	4.1	0/—	0	0	0	6.6
P[9]G1	—	—	2.8	—	0	0	0	1.6
P[6]G3	—	0.7	1.7	—	0	0	0	1.6
P[9]G3	0.2	—	0	—	0	0	0	0
P[6]G9	3.2	9	3.8	—/0.4	0	—	0	0.8
P[8]G5	—	—	—	—/0.4	—	—	0	0.8
Total	5	15.4	24	14/5.1	42	0	50	48.6

[a] Data from reference 20.
[b] Data from reference 23.
[c] Data from reference 12.
[d] Data from reference 3/data from reference 4.
[e] Data from reference 32.
[f] Data from reference 7.
[g] —, no data available.

thus the percentage of untypeable samples was 27.2%. The distribution of the P types found was as follows: P[8], 49.7%; P[4], 37.2%; P[6], 8.8%; and P[9], 4.2% (Table 2). In a first screening performed with primers Con 3 and 1T-1, the P[8] type was detected at a low frequency (14.4%). However, the frequency of this type significantly increased when primers Con 3 and 1T-1D were used. Several (7%) of the additional P[8] strains were confirmed by sequencing. Furthermore, since most of the P[4]-positive samples unexpectedly did not contain G2 types (see below), the sequence of 15% of the P[4] amplimers, randomly chosen, was used to confirm the actual P[4] nature. More than one P type was detected in 64.9% of the samples.

Combinations of P and G types. Given the complexity of the sewage samples, which could contain several strains from different individuals, only the samples containing either a single G or a single P type were used for evaluation of potential strains (83 single G or P samples of 116 G- and P-typed samples [71.5%]). The most frequent combination found in Cairo was P[8]G1 (38.5%), followed by P[6]G1 (27.0%), P[4]G1 (11.5%), P[8]G3 (11.5%), P[6]G4 (7.7%), and P[8]G9 (3.8%) (Table 3).

The distribution of strains in the Barcelona area was 38.8% P[8]G1, 30.6% P[4]G1, 11.6% P[8]G3, 6.6% P[4]G3, 5.8% P[6]G1, 1.6% P[9]G1, 1.6% P[6]G3, 0.8% P[4]G2, 0.8% P[8]G9, 0.8% P[6]G9, and 0.8% P[8]G5 (Table 3). Interestingly, three G5-containing samples were detected. One of these samples was associated with a P[8] type, while the other two could not be associated with any P type. To rule out the possibility that the latter two strains could be of porcine origin, specific typing reactions with primers for the P[6] (P2B), P[7], and P[13] genotypes were performed, and all of the assayed samples were again negative. To further analyze these strains, a phylogenetic analysis was performed to compare the sequences of these G5 Barcelona isolates with the G5 sequences available in the GenBank database. The closest strains were OSU and Po/A34, both of porcine origin.

It is worth mentioning that several of the combinations were detected on only few occasions, such as P[6]G3 in August 1999, P[8]G3 in January and March 2000, P[9]G1 in November and December 1999 and January 2000, P[9]G3 in November and December 1999, P[8]G5 in August 2002, P[8]G9 and P[6]G9 in November 2002, and P[−]G5 in December 1999 and March 2000.

DISCUSSION

In this work, the presence of rotavirus strains in sewage samples from Cairo and Barcelona was investigated. Although a higher percentage of positive samples was obtained for the samples from Cairo than for the samples from Barcelona, negative samples were randomly distributed throughout the year in both areas, which corroborated previous data for Barcelona raw sewage, in which seasonal distribution of rotavirus could not be detected (6), and clinical data for Cairo, in which reduced levels of detection were obtained both in winter months (January and February) and in a summer month (July) (29).

Although serotyping is a classification method based on neutralization of virus infectivity, the available information for gene sequences of rotavirus strains allows prediction of the serotype of a given strain by PCR with type-specific primers (14, 16).

When G typing was used, a significant proportion of the rotavirus-positive samples could not be characterized. In the case of samples from Cairo, the percentage of untypeable samples is consistent with previously published data (29), which showed that 38.7% of stool samples collected from August

1992 to October 1993 were untypeable with an enzyme immunoassay covering serotypes G1 to G4. The overall distribution of rotavirus G types in Cairo is similar to the distributions reported in other parts of the world. However, the results presented here reveal the occurrence of G9 strains in Egypt for the first time, although this does not necessarily imply emergence of new strains, since this serotype was not investigated in previous studies (21, 29). The absence of G2 strains in sewage from Cairo could be the result of either a lack of G2 strains circulating in the population or, more likely, low-level circulation of such strains, as has been reported previously (29). Another interesting observation was the shift from G4 strains to G1 strains; in a study performed in 1992 and 1993 (29) these two serotypes were found at equal frequencies, but in the present survey, performed in 1998 and 1999, G1 was detected at a significantly higher frequency.

The number of untypeable strains in sewage from Barcelona was significantly higher than the number of untypeable strains in samples from Cairo. Whether this was the result of technical problems associated with the concentration of RT-PCR inhibitors in the freeze-dried samples or reflected the actual occurrence of untypeable strains is not known. However, in a previous study (13) conducted to evaluate different methods for removing inhibitors, lyophilization appeared to be a satisfactory procedure. Additionally, it should be kept in mind that all the samples assayed were previously selected as VP6-positive samples by RT-PCR and Southern blotting. However, the procedure involved a different RT-PCR, performed with different primers and amplifying a much shorter fragment. A more plausible explanation may be the level of rotavirus in sewage. The proportions of samples that were determined to be positive only after Southern blot hybridization (no gel band corresponding to the amplimer was visualized) were 3% in Cairo and 39% in Barcelona. Rotavirus typing was difficult in these samples having very low virus contents.

The lack of G8 and G4 strains in sewage from Barcelona was surprising, since the G4 type appeared to be one of the most common types in previous studies conducted in Barcelona (October 1996 to October 1997) (33) and Valencia (September 1996 to May 1999) (7). However, in the latter study, the G4 frequency decreased during the period studied, and the prevalence of this type at the conclusion date, just when our sampling started, was significantly lower. The occurrence of the G3 type as the second most common type is also remarkable, since similar findings have been described previously only for the People's Republic of China (12).

From a technical point of view, it should be noted that the G2 type was not detected, either in a multiplex or a monoplex nested reaction, by using the RVG9-aCT2 pair of primers. In contrast, when primers 9 Con 1 and 9T1-2 were used in a monoplex nested reaction, several samples were G2 positive.

The present study provided the first available information on the distribution of P types in Egypt, and the data are similar to those found in other countries, such as Tunisia (32) and India (23). There was a strikingly high frequency of P[6], which was not detected either in Spain (7) or in Ireland (27), and only reduced frequencies of P[6] were detected in the United States (20) and the People's Republic of China (12).

The P-type data for Barcelona sewage isolates suggest that there was a shift from P[8] to mainly P[4] and also to P[6] and P[9] in Spain, since Buesa and coworkers (7) described the occurrence of 82% P[8] strains, 5.5% P[4] strains, and 12.4% untypeable strains in Valencia, which is located 300 km south of Barcelona, and Wilhemi and colleagues (33) described the occurrence of 100% P[8] strains in a rural area located 100 km north of Barcelona. High levels of the P[4] genotype have also been described for the People's Republic of China (12) and India (23); in the latter country there was also a high level of the P[6] genotype.

Interestingly, completely different epidemiological patterns for the P[8] type were obtained depending on the primers used. When the degenerate 1T-1D primer (22) was used instead of the 1T-1 primer in the nested PCR, a significant increase in the level of the P[8] type was observed, mainly in Barcelona, indicating that there was a higher incidence of strains whose sequences could not be amplified with 1T-1 in Barcelona than in Cairo.

The distribution of P-G combinations in Cairo isolates is more or less in agreement with the distribution in Tunisia (32), the only preexisting data for North Africa, where P[8]G1 was the most prevalent strain and where P[6]G4 and P[4]G1 strains emerged. However, while P[6]G4 strains were detected at a low frequency in Cairo, in Tunisia such strains appeared to be quite common. The P[8]G9 combination, detected at a rate of 4.8% in Cairo sewage, is being increasingly detected worldwide (1, 20) since its first description in the United States (8), and very recently it has been implicated in a large waterborne outbreak (C. Villena, R. Gabrieli, R. M. Pintó, S. Guix, D. Donia, E. Buonomo, L. Palombi, F. Cenko, S. Bino, A. Bosch, and M. Divizia, submitted for publication).

The most surprising conclusion of the Barcelona study was the similar frequencies observed for the combinations considered common (51.2% in Barcelona) and uncommon (48.6% in Barcelona) worldwide, mainly because P[4]G1 and P[4]G3 strains are still considered novel or reassortant strains. The reason for the replacement of P[4]G2 strains by these two novel [P4] combinations is not known. However, these novel strains have been detected worldwide since the early 1990s (3, 15), and in some countries they are the most common combinations, among G1 and G3 genotypes, after P[8]G1 and P[8]G3, respectively (12). In particular, P[4]G1 strains are being detected increasingly; they accounted for 4 and 14% of the strains in the People's Republic of China (12) and Argentina (3), respectively, and they also are considered emerging in Tunisia (32). With this background, the fact that the immigrant population in Barcelona has dramatically increased in the last few years must be taken into account, and the immigrants have originated mainly from North Africa, South America, and East Asia. Studies performed in other parts of Spain (i.e., Valencia) did not result in the same conclusions (7), probably because most of the immigrants in Barcelona started to arrive in 1999 or 2000.

The other uncommon strains detected in sewage from Barcelona have also been found in other countries; these include P[6]G1 strains in the People's Republic of China (12) and the United States (20), P[9]G1 strains in the People's Republic of China (12), P[6]G3 strains in the United Kingdom (9) and the People's Republic of China (12), P[6]G9 strains in India (22), the People's Republic of China (12), and the United States (20), P[8]G5 strains in Brazil (2, 15) and Argentina (4), and P[8]G9 strains worldwide, as previously mentioned.

Interestingly, three G5 strains were isolated from Barcelona sewage. The G5 strains were originally derived from pigs and are not very common in humans, since they have been found only in Brazilian (17) and Argentinean (4) children. One of the Barcelona G5 isolates was associated with a P[8] type, as previously described in Argentinean (4) and Brazilian children (15). The other two G5 strains were P[−]G5. The VP7 genes of these two Barcelona G5 strains were closely related to the VP7 genes of porcine strains OSU and Po/A34. The actual origin of the these two Barcelona G5 strains is still unknown, since although they are closely related to porcine strains, the sewage sample was from an urban area and consequently most likely was of human origin.

Antigens for the prevalent rotavirus genogroups all over the world should be included in vaccine preparations to ensure protection against all circulating strains. The emergence of new rotavirus strains, which occur in developing countries and also occur in developed countries, and the strain variability, which may exhibit not only a geographic pattern but also a temporal pattern, pose additional difficulties in the design of efficient new rotavirus vaccines.

Important environmental virology issues, such as the differential stability of a given rotavirus genotype, could result in a certain level of distortion in these studies. Additionally, due to the kind of sample analyzed, only the viruses that are more prevalent in the population and thus excreted in higher numbers, are likely to be detected. However, it seems reasonable to assume that molecular characterization of rotavirus isolates in raw sewage may provide an overview of the epidemiology of rotaviruses circulating in the community and at the same time reveal the occurrence of asymptomatic infections.

ACKNOWLEDGMENTS

C. Villena is a recipient of a BRD fellowship from the University of Barcelona. This study was supported in part by grants QLRT-1999-0634 and QLRT-1999-0594 from the European Union, by grant 1999SGR 00022 from the Generalitat de Catalunya, and by the Centre de Referència de Biotecnologia de Catalunya (CeRBa), Generalitat de Catalunya.

We acknowledge the technical expertise of the Serveis Científic-Tècnics of the University of Barcelona.

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