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Distribution of Rotavirus VP4 Genotypes and VP7 Serotypes among Nonhospitalized and Hospitalized Patients with Gastroenteritis and Patients with Nosocomially Acquired Gastroenteritis in Austria

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To assess the potential benefits of a reassortant tetravalent rotavirus vaccine, we investigated stool specimens from children in three different groups by reverse transcription-PCR (RT-PCR) for rotavirus G and P types: (i) children not hospitalized with community-acquired rotavirus-acute gastroenteritis (RV-AGE), (ii) children hospitalized for RV-AGE, and (iii) children with nosocomially acquired RV-AGE. From a total of 553 samples investigated, 335 were positive by enzyme-linked immunosorbent assay, of which 294 (88%) were positive by RT-PCR. Among the RT-PCR-positive samples, the predominant types were G1P[8] (84%), followed by G4P[8] (9%) and G3P[8] (2%). No differences between the three groups were observed, suggesting that community vaccination will diminish the most cost-relevant cases of hospitalizations and nosocomial infections.

Rotavirus (RV) is responsible for 6% of deaths among children under 5 years of age and for 25% of deaths due to diarrheal disease in developing countries (6, 15). Immunization against RV with a potent vaccine (the licensed RV vaccine had to be withdrawn because of its possible association with intussusception [5]) will have a dramatically beneficial effect. In developed countries, where RV-acute gastroenteritis (RV-AGE) is usually mild (4, 13, 16, 26) but causes enormous socioeconomic costs (2, 13, 23), assessment of the need for a RV vaccine will require national estimates of the burden of disease. Therefore, we performed a prospective study on RV disease which provides information about the incidence of the disease, hospitalizations, and nosocomial infections in Austria. A child's risk of contracting a community-acquired case of RV-AGE was 1.3 per 100 children-years and that of contracting a nosocomial infection was 2.59 per 1,000 hospital-days. These data will be central for considerations in introducing a vaccine in Austria. In addition to epidemiologic information, data on the diversity of RV in Austria need to be collected, since the reassortant vaccine is based on serotype-specific protection against the four most common serotypes of RV prevalent worldwide. RV is composed of two double-capsid layers that surround the virus core, which contains 11 segments of double-stranded (ds) RNA. The two outer capsid proteins of the virus, VP7 (encoded by gene segment 9) and VP4 (encoded by gene segment 4), are capable of inducing production of neutralizing antibodies (7). The major neutralizing antigen, VP7, is a glycoprotein and carries the G-serotype specificity, while the minor neutralizing antigen, VP4, carries the P-serotype specificity. Since serotypic specificity is defined and characterized by serological methods, the terms G type and P type (genotype) are used for typing of RV by molecular biological methods. To date, at least 14 G types and 18 P types have been identified in humans and animals. G serotypes 1 to 4 are the most prevalent in humans, with between 71 and 97% of the strains characterized (8). Eight P genotypes have been found in humans, although the majority of strains belong to only two P genogroups (genogroups P[4] and P[8]) (8). European data on RV P types are available only for Italy, but data from Germany and Switzerland will soon be available. In view of the possibility of genetic shift and drift, it is essential to determine the P and G types of RV, both before and after mass immunization, for detection of vaccine failure. As yet, there are no national data about serotype distribution in Austria. In order to fill this gap in information, we investigated in a prospective study 335 stool samples by reverse transcription-PCR (RT-PCR) to assess the potential benefit of a reassortant tetravalent RV vaccine for Austria and to evaluate differences between community-acquired nonhospitalized, hospitalized, and nosocomial cases.

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MATERIALS AND METHODS

Accurate diagnosis of an RV-AGE was made by investigating stool specimens by enzyme-linked immunosorbent assay (ELISA) (TestPack; Abbott, Delkenheim, Germany) as part of a prospective study to evaluate the RV disease burden in Austria. The study was performed in Innsbruck and Leoben between December 1997 and May 1998 and included children between 0 and 48 months of age with diarrhea who consulted a pediatrician participating in this study or who were hospitalized either at the University Hospital of Innsbruck or at the Children's Hospital of Leoben. Furthermore, all nosocomial cases which occurred in one of these hospitals were documented and stool specimens were collected. Written informed consent was obtained from the parents of the subjects before investigation. All ELISA-positive as well as some ELISA-negative stool specimens were further investigated by RT-PCR at the Institute for Microbiology and Virology, University of Bochum, Bochum, Germany. Briefly, double-stranded RNA extracted from stool specimens was isolated by phenol-chloroform extraction and was subsequently purified with an RNAid PLUS KIT (Dianova, Hamburg, Germany) according to the instructions of the manufacturers. The purified RNA was used as a template for G and P typing by RT-PCR. G and P typing was done by the RT-nested PCR method as described by Gentsch et al. (9) and Gouvea et al. (14). The primers and conditions were the same as described by them.

RESULTS

General. A total of 553 specimens were collected during the study period from children with AGE; 335 samples were positive for RV group A antigen by ELISA (Table 1). Further analyses of the ELISA-positive samples by RT-PCR detected the G and P types in 294 of 335 samples (88%), whereas 41 (12%) were negative for both the G and P types. Overall, strains of G type 1 in combination with P type 8 (G1P[8]) were the most prevalent (84%), followed by strains of G4P[8]) (9%)

TABLE 1. RV gastroenteritis detected by ELISA and RT-PCR

Condition	No. of children			
	Outpatients	Hospitalized cases	Nosocomial cases	Total
Total with AGE	142	355	56	553
RV positive				
ELISA	52	255	28	335
RT-PCR	38	232	24	294
RV negative	94	96	28	218

and G3P[8]) (2%) In two samples the G type could be determined but the P genotypes could not, and in two further samples, the P genotypes could be determined but their G types could not.

Mixed infections. Mixed infections were detected in nine (2.7%) samples. All of them had strains of more than one G type, and only one had more than one P type. Six of nine possessed strains of genotypes G1P[8] and G4P[8], and two possessed strains of genotypes G1P[8] and G3P[8]. Strains with dual P genotypes, P[8] and P[9], were detected in only one sample in combination with strains of G types G1 and G2. All mixed infections identified were confirmed by using the genotype-specific primers in the second amplification and typing reactions both as single primers and as a primer mixture.

Serotypes in patient subpopulations. The distribution of the serotypes in the different groups of patients (those with community-acquired not hospitalized, hospitalized, and nosocomial cases of RV-AGE) is shown in detail in Table 2. There was almost no difference between the three groups in the G- and P-type distributions of their strains.

ELISA-positive and RT-PCR-negative samples. Forty-one (12%) of the RV antigen-positive samples were absolutely negative by RT-PCR. With the exception of two samples in which rotavirus RNA could be detected, all other RT-PCR-negative samples were also negative by gel electrophoresis. For these two samples, typing was not possible because we were not able to obtain a first-round PCR product which would allow typing by sequencing.

Retesting of ELISA-negative samples by RT-PCR. Twenty-three of the total of 218 (10.5%) RV antigen-negative specimens were analyzed for G and P types by RT-PCR. Of these, the negative result of the RV antigen test was confirmed for 18 samples. G- and P-type-specific amplification products were detected in five (21.7%) samples. A type G1P[8] strain was observed in three samples, a type G4P[8] strain was observed in one sample, and one sample possessed type G1 and G4 strains. The P type could not be determined for the strain in this sample.

DISCUSSION

Introduction of a reassortant tetravalent RV is under consideration in Austria. In this context, information about the prevalence of RV disease and on the serotype diversity of RV is of great relevance. Therefore, we investigated the G- and P-serotype distributions of RV strains that cause disease in this country. Molecular epidemiological methods for P genotyping were first developed in the beginning of the 1990s (9, 14), and the majority of epidemiological studies describe only the distributions of G types. To our knowledge, P-typing data are currently available only for Italy (1). The results of G typing in our study are comparable to those of studies performed in other European countries and the United States (3, 10, 18). The most prevalent strains showed G1 and G4 specificity. Gerna et al. (11) reported that 84% of all strains investigated between 1981 to 1988 were of type G1 and G4. Gentsch et al. (8) reviewed the prevalence of G serotypes and found that 71 to 97% of the strains characterized belonged to these serotypes. The high percentage (93%) of G1 and G4 serotypes among isolates found in Austria is closely similar to that in Australia (24, 25, 27), Israel (22), and The Netherlands. Since the data of the present study are the first such data for Austria, it is not possible to provide information on the year-to-year variability in the distribution of RV serotypes in this country. Further investigations will have to be done after introduction of a vaccine for early detection of vaccine failures due to such causes as genetic changes. The value of P genotyping is not yet clearly established. The combined genotyping may have advantage in identifying unusual viruses. Furthermore, it may help in clarifying the importance of VP4 in inducing protective immunity. Unusual G-type and/or P-type combinations, such as those found in Italy and Australia with G6P[13] specificities (12, 19) or like those in other countries (e.g., G1P[6] and G9P[6]) (21, 22), were not detected. Recently Gentsch et al. (8) and Parashar et al. (20) published data from a "global collection" which includes specimens from the United States, Costa Rica, Korea, Israel, China, Mexico, Bolivia, India, and Bangladesh. These data have shown that the common strain G1P[8] was predominant (53%), followed by G4P[8] (14.3%), G2P[4] (10.7%), G3P[8] (5.4%), strains of mixed genotypes (2.6%), and other genotypes (18.4%). The relatively low frequency of other genotypes or combinations in our study population indicates that the genetic diversity of the Austrian population of strains is smaller than that reported for the global collection.

The subtypes of RV strains that are responsible for community-acquired AGE not requiring hospitalization of the patient or RV strains that cause severe AGE necessitating hospitalization as well as RV strains that cause nosocomial cases of AGE had nearly identical distribution patterns. Therefore, we hypothesize that vaccination of the community will decrease the frequency of the most cost-relevant cases of gastroenteritis, namely, the hospitalized and nosocomial ones. In the face of exploding costs of health care provision, information about the cost-effectiveness of a medical procedure such as vaccination is very relevant in national health decision making. Furthermore, our study enabled a comparison between the results of ELISA and PCR. We found that the ELISA was false positive for 12% of the samples compared to the results of RT-PCR. Similar rates of false-positive results for samples tested by the TestPack ELISA were described by Lipson et al. (17).

We believe that the description of RV subtype diversity in

TABLE 2. Distribution of G and P types in Austria

Characteristic	No. (%) of children			
	Outpatients	Hospitalized cases	Nosocomial cases	Total
ELISA positive	52	255	28	335
G1P[8] positive	36 (69)	192 (75.0)	20 (71.0)	248 (74.0)
G3 P[8] positive	0	5 (2.0)	1	6 (1.8)
G4 P[8] positive	4 (8)	20 (7.8)	3 (11.0)	27 (8.0)
Double infections	2 (4)	7 (2.7)	0	9 (2.7)
Other[a]	0	4 (1.5)	0	4 (1.0)
RT-PCR-negative	10 (19)	27 (11.0)	4 (14.0)	41 (12.0)

[a] Includes strains for which the G or P type could not be determined.

different groups of children with RV-AGE in Austria is predictive for use in policy decision making and that vaccination with a reassortant tetravalent vaccine would protect the Austrian population from infection with RV.

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