Arch Virol (2007) 152: 833–837
DOI 10.1007/s00705-006-0874-0
Printed in The Netherlands

Archives of Virology

Brief Report

Diversity of human rotaviruses detected in Sicily, Italy, over a 5-year period (2001–2005)

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Received August 11, 2006; accepted October 9, 2006; published online December 11, 2006

Summary

Rotavirus infection was detected in 39.9% of 1030 children hospitalized with gastroenteritis in Palermo, Italy, in the period 2001–2005. Rotavirus strains belonging to G1, G4 and G9 types were continually detected, with G1 being the most common type in 2001, 2002 and 2004. A G4 epidemic occurred in 2003, while G9 was predominant in 2005. G2 strains displayed a low prevalence, except in 2003. G3 rotaviruses accounted for 2.7–17% of the gastroenteritis episodes in 2002–2005. The P-type of a subset of 166 strains confirmed the circulation of the usual G/P combinations, but single G1P[6], G9P[9] and G6P[9] strains were also found.

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Group A rotaviruses (HRV) are common agents of enteritis in infants and young children worldwide [16]. Classification of HRV strains is based on the antigenic differences of the two outer capsid proteins VP4 and VP7, both of which induce neutralizing antibodies, and are encoded by the genomic segments 4 and 9, respectively [12, 16]. The segmented nature of the rotavirus genome enables the outer capsid genes to segregate independently. Thus far, at least 15 G (VP7-specific) and 26 P (VP4-specific) rotavirus types have been recognised, of which 11 and 13, respectively, have been found in various combinations in humans [12, 17, 19, 20, 22]. HRV strains with G1P[8] specificity are universally acknowledged as the most prevalent and ubiquitous. Strains with G2P[4], G3P[8] and G4P[8] specificity are widely dispersed but their incidence varies regionally and temporally [12]. Since 1996, HRV strains of the G9P[6] or G9P[8] type have been identified as the fifth most common strain globally, while other G types and G/P combinations seem to be sporadic or only of regional relevance [11].

Surveillance of the HRV types involved in gastroenteritis episodes throughout the world has been used to define the appropriate vaccine formulations to prevent HRV infections in paediatric populations. Taking into account the antigenic heterogeneity of

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rotaviruses, vaccines matching the most common rotavirus antigenic profiles have been developed, and recently, two of these (RotaTeq and Rotarix) have been also licensed [24, 27].

In Italy, limited data are available on the patterns of distribution of HRV G and P types. However, in Palermo, Sicily, epidemiological surveillance for HRV enteritis in children has been carried out uninterruptedly since 1985 [1–3, 8]. Over a 20-year period, the rate of HRV infections has been constantly high in Sicily, with HRV being responsible for approximately 25–50% of paediatric hospitalizations due to enteritis. Strains with G1–G4 specificities accounted for more than 90% of HRV infections until 1999, when HRVs of the G9P[8] type emerged and became the third most frequent HRV G type [3].

Here, we report the data on the surveillance activity carried out in Palermo in the years 2001–2005. A total of 1030 stool samples were obtained from patients, aged less than five years, admitted with acute gastroenteritis at the "G. Di Cristina" Children's Hospital of Palermo in the years 2001–2005. HRV-positive specimens, detected by enzyme immunoassay (IDEIA Rotavirus; DakoCytomation Ltd, Ely, UK), were characterized for VP7 specificity by serotyping; G1–G4 monoclonal antibodies (RV4:2, RV5:3, RV3:1 and ST3-3:1) were used as described previously [9]. Subsets of G-serotyped HRVs and all of the G-unserotypeable strains were submitted to genotyping with type-specific primers by a semi-nested PCR strategy. In the first-round PCR, the full-length VP7 gene was amplified using conserved oligonucleotide primer pair Beg9/End9, and the G types were subsequently predicted in a second-round PCR using a pool of internal primers specific for the G1–G4 and G9 genotypes in combination with the reverse consensus primer [14, 15, 19].

Characterization of the VP4 specificity was carried out similarly using the generic primers Con3 and Con2 and the internal primers specific for the P[4], P[6], P[8], P[9] and P[11] genotypes [13, 15].

HRV infection was detected in 411 (39.9%) out of 1030 children examined. The yearly prevalence ranged from 24.7% in 2003 to 53.4% in 2005. Most of the infections (73%) occurred between November and April. The distribution of the various G types is described in Table 1. G1 was the most common serotype detected throughout the study. G1 HRVs were predominant in 2001 (66.0%), in 2002 (88.0%) and in 2004 (78.0%). G2 prevalence was low (0–4.9%), except in 2003, when it was the second most common G type (22.6%). Strains of the G3 type were not detected in 2001, while they accounted for 2.7, 17.0, 7.3 and 16.9% of the infections in 2002, 2003, 2004 and 2005, respectively. Similar to G1 strains, G4 and G9 strains were detected over the entire study period. G4 represented the most common G type (41.5%) in 2003, while G9 was the most common G type in 2005 (60.8%). Mixed G types were rarely detected (1.7%). Six HRV strains were untypeable by both serotyping and genotyping.

VP4 characterization was performed on a subset of 166 HRV strains. As expected, almost all of the G1, G3, G4 and G9 strains tested exhibited the P[8]-type and all the G2 strains were P[4]-type. Two unusual G/P combination (G1P[6] and G9P[9]) were found. A strain exhibiting the P[9]-type could be

Table 1. Distribution of group A rotavirus G types circulating in infants and young children (<5 years) with acute gastroenteritis in Palermo, Italy, during a 5-years follow-up (2001–2005)

Year	No. of samples collected	No. of HRV positive	G1 (%)	G2 (%)	G3 (%)	G4 (%)	G9 (%)	Non typeable (%)
2001	159	53 (33.3)	35 (66)	0	0	9 (17)	7 (13.2)	2 (3.8)
2002	181	75 (41.4)	66 (88)	1 (1.3)	2 (2.7)	2 (2.7)	3 (4)	1 (1.3)
2003	214	53 (24.7)	7 (13.2)	12 (22.6)	9 (17)	22 (41.5)	2 (3.8)	1 (1.9)
2004	199	82 (41.2)	64 (78)	4 (4.9)	6 (7.3)	5 (6.1)	3 (3.7)	0
2005	277	148 (53.4)	24 (16.2)	1 (0.7)	25 (16.9)	6 (4)	90 (60.8)	2 (1.3)
Total	1030	411 (39.9)	196 (47.7)	18 (4.4)	42 (10.2)	44 (10.7)	105 (25.5)	6 (1.5)

defined as G6 only after sequence analysis of the first-step VP7 amplicon (data not shown).

This study confirms the important role of HRVs as agents of enteritis in the Italian paediatric population and highlights the high prevalence of strains of the G1–G4 and G9 types. These findings mirror the epidemiological data described in the European countries, in North America, in Australia and in New Zealand and differ from those observed in developing countries [10, 15, 25]. In the years 2001–2005, G1 and G9 types represented the most diffuse HRV strains in the paediatric population of Palermo. G1 strains were predominant in 2001 (66%), 2002 (88%) and 2004 (78%), and their prevalence decreased in 2003 when a G4 epidemic occurred. Since 1985, a similar trend had been observed in Italy in 1991, 1993 and 1999 [4, 5, 16].

G9 HRV strains were detected for the first time in Italy in 1999 [3]. In 2005, G9 represented the most common isolates in Sicily, accounting for 60.8% of HRV infections. Likewise, high rates of detection of G9 strains were reported in Bari, Italy, in 2001–2002 [18], in Hungary in 2002–2003 [7], and in Belgium in 2000–2001 and 2002–2003 [21]. G9 HRVs were also found to be predominant (up to 89.2%) in Salvador, Brazil, in 1999, 2000, and again in 2002, thus demonstrating that they can maintain predominance during multiple consecutive rotavirus seasons [26]. Accordingly, the pattern of fluctuation of G9 HRVs appears to be similar to the patterns exhibited by G1 and G4 HRV strains rather than to that of G2 strains. Indeed, since their emergence, G9 HRV strains have been detected every year in Italian children, while G2 strains have completely disappeared in some years, as observed in 1987, 1990–1991, 1994, 2000 and 2001 [1–3, 5]. G2 was the most common type only in 1996, while in 2003 it was the second most diffuse type (22.6%). Likewise, low circulation rates have been reported for the G2 serotype HRVs in Europe [10].

Sequence analysis of the VP7 and VP4 genes of the HRV strains detected in Sicily over 20 years has revealed different evolutionary patterns for rotavirus G1, G2 and G4 strains. Intra-serotypic variation appears to be much higher within G1 than G2 and G4. The continuous predominance of G1 strains over consecutive years was apparently related to alternate circulation of an heterogeneous population of lineages and sub-lineages, with repeated introductions of novel G1 strains [6]. Conversely, homogeneous populations of G4 and of G2 strains have been circulating for one or more consecutive years, and peaks of prevalence by G4P[8] and G2P[4] HRVs were due to the introduction in the Sicilian infantile population of new rotavirus strains rather than to the re-emergence of old epidemic strains [4, 5].

In the past, in Italy, G3 HRVs have been detected sporadically or at very low prevalence [1–3, 8]. However, in the years 2003 and 2005 they accounted for 17% of gastroenteritis episodes, thus acquiring an important epidemiological role. Increased prevalence of infections by G3 HRVs in children has been also described in recent years in Ireland and Japan [23, 28].

In summary, the findings of this study extend the data on rotavirus diversity in Italy. Based on these results, polyvalent vaccine formulations including the G1–G4 types would be appropriate, but future vaccine candidates may also need to include a G9 component, as G9 HRVs are continuously circulating in Italy. There is concern that the introduction of human rotavirus vaccines could alter the forces and balances that drive rotavirus evolution. Continuous epidemiological surveillance is paramount for understanding the short- and long-term effects of the vaccines on rotavirus ecology and implementing future vaccine strategies.

Acknowledgments

This work was supported by grants from the Ministero della Sanità (Italian Ministry of Health) (Progetto Finalizzato 2003: "Diversità genetica ed antigenica dei rotavirus, studio dei meccanismi evolutivi ed implicazioni ai fini diagnostici e vaccinali") and from the Ministero dell'Istruzione, dell'Università e della Ricerca (Italian Ministry of Education, University and Research) (Fondi di Ateneo ex 60%).

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