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Short communication

G2P[4] the most prevalent rotavirus genotype in 2007 winter season in an European non-vaccinated population

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ABSTRACT

Background: Recently, a high prevalence of G2P[4] rotavirus (RV) infection was reported from Brazil, and linked with the universal RV vaccination programme that used the G1P[8] live oral RV vaccine.
Objective: To determine the genotypes of RV co-circulating in a non-vaccinated population, in northern Portugal in the winter season of 2007.
Study design: Prospective multicenter study of the genotypes circulating in the northwest region of Portugal during January to March 2007. Children with acute gastroenteritis, who attended the Pediatric Emergency Services of five Hospitals, were included in the study. The parents of the children completed a clinical and epidemiological data questionnaire and stool samples were collected. Stool samples positive in a RV enzyme immunoassay (EIA) were genotyped by reverse transcriptase-polymerase chain reaction.
Results: Stool samples were collected from 424 children. Two hundred and thirty-four (55.2%) stool samples were RV-positive. G2P[4] was the predominant RV type (68.6%), followed by G9P[8] (14.0%).
Conclusions: Because our population was naïve for RV vaccine, the G2P[4] predominance cannot be explained by vaccination. Rather, this high prevalence of G2P[4] may be within the normal fluctuation of RV genotypes. RV strain surveillance programmes are important for informing RV vaccination programmes.

1. Background

Rotavirus (RV) is a major cause of acute gastroenteritis (AGE) in children, being responsible for more than 500,000 deaths each year, the majority in developing countries.[1] In addition, the economic burden of RV disease is significant.[2,3]

In Europe four RV serotypes (G1P[8], G2P[4], G3P[8], and G4P[8]) have represented more than 90% of RV-associated acute gastroenteritis.[4] The previously rare genotype, G9P[8], has emerged in the past several years.[5] In Portugal, in 2006, G9P[8] was the predominant strain in a Hospital-based study.[6] Also, recently, a high prevalence of G2P[4] was reported from Brazil, and linked with the universal RV vaccination programme that used a G1P[8] live RV vaccine.[7] The authors of this study[7] speculate that the use of a G1P[8] vaccine might have eradicated vaccine-related serotypes, allowing G2P[4] to emerge.

2. Study design

We conducted a prospective multicenter study of RV genotypes in a region of Portugal, from January 1 to March 31, 2007. The region is located in the north-western part of Portugal, bounded to the north by Galiza (Spain) and to the west by the Atlantic Ocean; the climate is temperate.

Children less than 15 years of age, who presented with AGE to the Paediatric Emergency Room of one of the five Hospitals involved in

Abbreviations: RV, rotavirus; EIA, enzyme immunoassay; RT-PCR, reverse transcription-polymerase chain reaction; RNA, ribonucleic acid; AGE, acute gastroenteritis.

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the study (Braga-Central Hospital, Barcelos, Famalicão, Guimarães and Viana do Castelo), were enrolled in this study. Children hospitalized for reasons other than AGE, who had diarrhea starting after admission, were also included. Acute diarrhea was defined as three or more watery stools a day, for no longer than 3 weeks. Nosocomial infection was considered when the symptoms started 72 or more hours after admission. The parents completed a questionnaire that was designed to collect clinical and epidemiological data. They also provided signed consent authorizing the collection of a stool sample. The study protocol was approved by the local Ethical Committee.

Group A RV antigen in stool was detected with commercial enzyme immunoassay (EIA) kits—VIKIA® Rota-Adeno (bioMérieux, France) following the manufacturers' instructions. RV-positive specimens were genotyped by reverse transcription-polymerase chain reaction (RT-PCR). Genotyping was performed using methods published elsewhere.[8] Stool samples were prepared as 10% suspensions in balanced salt solution, and nucleic acid was extracted. Reverse transcription was performed as previously described.[8,9] The genotypes were determined in Braga, Portugal, and confirmed in the UK (Virus Reference Department, Health Protection Agency, London).

3. Results

From 512 children identified with AGE in all the five hospitals, stool samples were collected from 424 and tested for RV antigen. RV was detected by EIA in 231/424 (54.5%) samples. Three samples that were negative for RV by EIA, which were sent for genotyping by RT-PCR in error, had detectable RV ribonucleic acid (RNA). These were classified as false negative EIA results. Therefore, a total of 234 stool samples with detectable RV RNA (55.2%) were available for genotyping.

The AGE attributable to RV in January was 33.8%, February 30.8% and March 33.3%; missing 2.1%. Boys represented 58.7% of the cases. The median age of the patients was 13 months (maximum: 12 years; minimum: 1 month).

From the 234 children with RV-positive stool samples, 16 were classified as nosocomial infections (6.8%). Only one child had been vaccinated against RV; this child was aged 8 months and presented with a mild AGE.

RV RNA was not detected in 16/234 (6.8%) samples sent for genotyping; 3 of these were from suspected nosocomial infections. These 16 samples were classified as false-positives in the EIA through an inability of detection RV RNA in stools (RT-PCR was performed two times in these samples), and were excluded from the analysis. Eleven of the remaining 218 samples (5.0%) had insufficient quantity for genotyping. The general distribution of RV genotypes, including genotypes available from 13 nosocomially acquired cases, is shown in Table 1. G2P[4] was the RV type most prevalent (68.6%), and it was also the most prevalent in all age groups (69.6% in children under or equal 24 months; 65.2% in children under or equal 60 months old). G2P[4] was the most prevalent type in four of the five hospitals; in one hospital, Famalicão, G9P[8] was more common (55%).

RV mixed infection occurred in 5.3% of cases. The majority of the mixed infections were with G1 and G2 RV strains, 54.5% of the mixed infections (Table 2). Unusual G/P associations were observed: 1-G1P[4], 2-G2P[8], 1-G4P[9], 1-G9P[4], and 2-G9P[9].

Table 1
Molecular characterisation of rotavirus strains.

Genotype	Number	Percent (%)
G1P[8]	10[a]	4.8
G1P[4]	1	0.5
G2P[4]	142[b]	68.6
G2P[8]	2	1.0
G4P[9]	1	0.5
G9P[4]	1	0.5
G9P[8]	29[c]	14.0
G9P[9]	2	1.0
Mixed types	11[d]	5.3
Partially typed[e]	8[f]	3.8
Total	207	100.0

[a] 2 nosocomially acquired cases.
[b] 6 nosocomially acquired cases.
[c] 1 nosocomially acquired case.
[d] 3 nosocomially acquired cases.
[e] Partially typed—unclassifiable strains (G type or P type): 4 unclassifiable G type; 4 unclassifiable P type.
[f] 1 nosocomially acquired case.

Table 2
Rotavirus mixed infection.

G type	P type	Number	% of mixed infections
G1 + G2	P[4]	5	45.4%
G2 + G9	P[4]	2	18.2%
G1 + G2	P[4] + P[8]	1	9.1%
G1	P[4] + P[8]	2	18.2%
G9	P[4] + P[8]	1	9.1%
Total		11	100.0%

4. Discussion

G2P[4] was the most prevalent RV type detected during the 2007 RV season in northwest Portugal. The predominance of G2 genotype differs from other recent studies in Europe, in which G1 was the most prevalent.[10–15] However, G2 has been the prevalent RV type in the past in some countries in Europe.[4] Recently, a high prevalence of G2P[4] was reported from Brazil and linked with the universal RV vaccination programme using a G1P[8] live oral RV vaccine.[7] Because our population was naïve for RV vaccine, the G2P[4] predominance cannot be explained by the introduction of vaccination and this high prevalence may be within the normal fluctuation of co-circulating RV genotypes, which is in line with reports from other countries and areas, in non-vaccinated population, that show exactly the same.[4,11]

RV was detected in almost half of the children with AGE enrolled in this study, and was also identified as a common nosocomial agent.[16–19] The genotype distribution was similar to the distribution in those admitted for community-acquired AGE, which seems logic because it was the genotype circulating in the area and in the period of the study.

Although G2P[4] was the most prevalent (68.6%) in all age groups, the majority of the unusual G/P associations were observed in children equal or under 24 months.

The four major G–P combinations in Europe (G1P[8], G2P[4], G3P[8] and G4P[8]), representing more than 90% of the RV infections in the past in Europe,[4] were identified in only 73.4% of our subjects. G9 strains, which have recently entered the human population,[4] and appear to be increasing in incidence worldwide,[11] were identified in 16.9%, which may explain why the proportion of the four most prevalent combinations in Europe is lower in this study. The detection of G9 strains has increased dramatically in the past decade, and its efficient adaptation to the human population has led to the spread of such strains throughout the world.[11] G9P[8] was more frequently identified in one hospital in this multicenter study. Among all the samples, G9P[8] was detected in only 14.0%, contrary to what was found in a study from a 2006 Portuguese Hospital-based study, in the center of Portugal, in which G9P[8] was found to be the most prevalent genotype, accounting for 90% of cases.[6] Although interesting, this regional differences have

already been documented by other authors.[11] G3P[8] and G4P[8], two of the most prevalent types reported worldwide,[4] were not detected in this study. Uncommon RV types in Europe, such as G1P[4] and G2P[8], which have been detected at relatively high frequencies in different parts of the world,[4] were also found in this study, albeit rarely. The frequencies of these reassortant types range from 0% to 11.3%,[4] which is in line with our results (G1P[4], 0.5%; G2P[8], 1.0%). These unusual G/P type combinations may have arisen from zoonotic transmission or by reassortment between common human strains during dual infection of a single cell.[20]

Non-typeable RV strains have been reported in almost every epidemiological survey around the world, regardless of the methodology employed.[10] Recently, the number of non-typeable RV strains has steadily declined[3] as better and more comprehensive test systems have become available. In this study, 3.8% of the strains were non-typeable.

Mixed infections with two RVs occurred in 5.3% of the children, and is similar to that reported previously, ranging from 1% to 26.4%.[3] These mixed infections occur more frequently in areas of high incidence of RV infection. The majority of the mixed infections were with G1 and G2, different from that found in other European studies, in which G9 and G3 or G9 and G1 were more frequently associated with multiple infections.[3] This is likely to reflect mixed infections occurring with the most prevalent genotypes co-circulating at the time of the study that provided opportunities for reassortment.

Because diverse RV strains co-circulate in the human population, it is important that RV strain surveillance programmes continue to monitor this diversity in order to understand their possible implications for RV vaccination programmes. The present results confirm that, in the same country, in nearby areas, circulating RV genotypes may be highly variable, which supports the need for a vaccine effective against all RV types, not only against those described as the most prevalent in one country, one area or one season. These results, obtained from a non-vaccinated population, also points out the hypothesis, assumed by some authors,[7] that the use of a G1P[8] vaccine might have eradicated vaccine-related serotypes, allowing G2P[4] to emerge.

Conflict of interest

None.

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