Acute Rotavirus Gastroenteritis in Portugal: A Multicentre Study

Gastroenterite Aguda por Rotavírus em Portugal: Estudo Multicêntrico

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Resumo

Introdução: Os dados sobre diarreia por rotavírus em Portugal são limitados. Este estudo teve como objetivo estimar a proporção de gastroenterite aguda por este vírus em crianças observadas em serviços de urgência de vários hospitais do país e analisar as suas características clínicas e moleculares.

Métodos: Estudo prospetivo, multicêntrico, observacional, incluindo crianças com menos de 5 anos, com gastroenterite aguda, observadas em 10 serviços de urgência pediátricos, entre outubro de 2008 e setembro de 2009. Foram recolhidos dados demográfico e clínicos. As amostras positivas para rotavírus foram genotipadas por reação em cadeia da polimerase.

Resultados: Foram incluídas 1846 crianças, 58% do sexo masculino, com idade média de 19,3 ± 14,4 meses. Foi identificado rotavírus nas fezes em 28,3% (intervalo de confiança 95%, 26,2-30,4%), com maior proporção no inverno e na primavera e em crianças com idade de 7-24 meses. Os genótipos mais frequentes foram G4P[8] (46%) e G1P[8] (37%), com variações de norte para sul. As crianças com gastroenterite por rotavírus tinham probabilidade significativamente superior ($p < 0,001$) de ter febre, vómitos, perda de peso, desidratação e necessidade de internamento, comparativamente aos casos negativos para rotavírus.

Discussão: A gastroenterite aguda por rotavírus em crianças portuguesas com idade inferior a 5 anos associou-se a maior morbilidade e hospitalização do que nos casos sem identificação de rotavírus. Houve diferenças importantes na distribuição dos genótipos entre as regiões. Na era das vacinas contra o rotavírus, este conhecimento é importante para as decisões relativas à prevenção da doença e para monitorizar tendências da epidemiologia molecular do rotavírus.

Palavras-chave: Rotavírus; Gastroenterite; Doença Aguda; Infeções por Rotavirus; Criança; Portugal

Abstract

Introduction: In view of the limited data on rotavirus disease in Portugal, this study aimed to estimate the proportion of acute rotavirus gastroenteritis among emergency services visits in various centres in the country and to characterise its clinical and molecular profile.

Methods: In this prospective, multicentre, observational study of children aged <5 years with acute gastroenteritis, attending 10 paediatric emergency services between October 2008 and September 2009, demographic and clinical data were collected. Rotavirus-positive samples were genotyped by polymerase chain reaction.

Results: A total of 1846 children were included, 58% male, mean age 19.3 ± 14.4 months. Stools tested positive for rotavirus in 28.3% (95% confidence interval 26.2-30.4%), with a higher proportion in winter and spring and in children aged 7 to 24 months. The most frequent genotypes were G4P[8] (46%) and G1P[8] (37%), with a geographical trend from north to south. Children with acute rotavirus gastroenteritis were more likely to have fever, vomiting, weight loss, dehydration and need for hospitalization ($p < 0.001$ for all comparisons) than rotavirus-negative cases.

Discussion: Acute rotavirus gastroenteritis in Portuguese children aged <5 years was associated with greater morbidity and hospitalisation as compared to rotavirus-negative cases. There were important differences in

genotype distribution among regions. In the era of rotavirus vaccines, this knowledge is important for policy decisions concerning disease prevention and to monitor trends of rotavirus molecular epidemiology.

Keywords: Rotavirus; Gastroenteritis; Rotavirus Infections; Acute Disease; Child; Portugal

Introduction

Diarrhoea is the second most common cause of death among children worldwide, mainly in Africa and South Asia,[1] and, although the number of deaths due to this condition has decreased in the last two decades,[1-3] morbidity remains high.[4] In developed countries, acute gastroenteritis (AGE) is rarely associated with mortality, but affects nearly every child before the age of five.[1,3,5]

Before the introduction of vaccines, rotavirus (RV) was the main pathogen responsible for AGE hospitalisation, in both developed and developing countries.[1-3,6] A study published in 2006 estimated that in the 23.6 million European children aged less than 5 years, 3.6 million episodes of acute rotavirus gastroenteritis (RVGE) occur and that it is responsible for 231 deaths, >87,000 hospitalisations and nearly 700,000 outpatient visits annually.[7] Children aged 4 to 24 months are most vulnerable to RV infection, and it has been estimated that all children under 5 years of age will be infected at least once.[5]

Several studies have reported acute RVGE prevalence in children seeking medical attention in primary care, emergency departments and hospitals worldwide. In the USA, in an active population-based surveillance programme, RV was responsible for 44% of AGE cases in children under 3 years of age.[8] In Europe, several studies have reported similar proportions, including 40.6% in the REVEAL study involving selected areas of Belgium, France, Germany, Italy, Spain, Sweden and the UK[9] and 43.4% in SHRIK, a multicentre study conducted in 12 hospitals in France, Germany, Italy, Spain and the UK.[6] In Portugal, published studies showed rates of 55.2% in the northwest region[10] and 45% in a single-centre study performed in central Portugal.[11]

These figures emphasise the importance of acute RVGE as a public health problem and of vaccination to prevent RV infection and to reduce the burden of disease.[9,12]

The two available RV vaccines, Rotarix® (GlaxoSmithKline Biologicals) and RotaTeq® (Sanofi Pasteur MSD), included in the national immunisation programmes of various countries worldwide or recommended for routine immunisation,[13-15] have been shown to have a significant impact on disease burden.[16-19] Both vaccines have been licensed in Portugal in 2006, are available, under prescription, on the private market, and are recommended for routine immunisation by the Portuguese Society of Paediatrics,[14] but to date are not included in the national immunisation programme.

A rational planning of immunisation strategies and subsequent evaluation of its impact is only feasible if there are national data on the epidemiology of a specific disease. Some previous studies in the country, although reporting proportions of acute RVGE, were performed in a single institution or region,[10,11,20-22] which does not provide a broad and representative picture of the disease in Portugal.

This study aimed to estimate the proportion of acute RVGE among ES visits of children <5 years due to AGE, in various centres in the country. The secondary objectives were to describe the clinical features and vaccination status of RV-positive AGE cases and to compare them with RV-negative cases, and to describe RV genotypes.

Methods

Study design

This was a prospective, multicentre, observational study of children aged <5 years admitted with AGE to paediatric emergency services (ESs) of selected hospitals all over the country, between October 2008 and September 2009 (12 months).

Selection of study areas

The study areas were selected according to the geographical distribution of the population, comprising four tertiary paediatric services and six services in medium-sized cities (100,000-250,000 population) from all regions - North, Central, South and Islands (Madeira and the Azores).

Inclusion and exclusion criteria

Children aged <5 years, living within the hospital catchment area and attending the ES with symptoms of AGE, defined as three or more watery or looser than normal stools within a 24-hour period, with or without fever or vomiting, lasting less than seven days and preceded by a symptom-free period of 14 days, with available stool sample, were included. Written informed consent signed by their parents/guardian was required. Participation in the study was voluntary and proposed for all eligible children.

If a child visited the ES more than once during the AGE episode, the visit relating to the highest level of care was considered. Different AGE episodes in the same children were considered as new cases.

Children were excluded if they had chronic gastrointestinal tract disease or an immunodeficiency, hospitalisation in the week previous to the onset of symptoms, or no stool sample available, or if they had travelled to another country in the week before the ES visit.

Data collection

A questionnaire with demographic and clinical data based on the hospital's medical records was completed for each child. RV vaccination status was also recorded.

RV detection and genotyping

Stools were obtained during the stay in the ES or within 48 hours if the child was admitted to the hospital.

Detection of RV was based on a rapid immunochromatographic test (VIKIA® Rota-Adeno, Biomérieux, France), performed at each participating centre. Samples identified as positive for RV were kept at +4ºC and later sent to a European reference laboratory (Virus Reference Department, Health Protection Agency, London), and genotyping was done by polymerase chain reaction, following published protocols (http://www.eurorota.net/docs.php).

Statistical analysis

The statistical analysis was performed using SPSS 16 software. Quantitative data were summarised with mean, standard deviation, median, minimum and maximum, and qualitative data in frequency tables.

The proportion of RV cases was calculated along with 95% confidence intervals (CI). Comparisons between RV-positive and -negative cases were performed using the Mann-Whitney test for quantitative data and the chi-square and Fisher tests for qualitative data, assuming a 0.05 significance level.

Ethical approval

The protocol was reviewed and approved by the local Ethics Committee of each participating centre.

Results

Study population

A total of 1846 children aged <5 years with AGE were included in the study: 18.6% from the Northern region of Portugal, 32.2% from the Central region, 21.2% from the South and 28.0% from the Islands.

Demographic and clinical data

Mean age was 19.3 ± 14.4 months (13-59 months) and 58% were male.

Fifty-four percent had fever, 68.0% vomiting, 27.4% weight loss and 27.1% were dehydrated (mild dehydration in 77.8%). Fifteen percent were hospitalised; their mean age was 18.8 months and mean duration of stay was 2.5 ± 2.1 days.

Twelve percent had received at least one dose of RV vaccine (53.6% Rotarix® and 46.4% RotaTeq®). Of those who were vaccinated with Rotarix®, 85.3% had two doses and 73.9% of those vaccinated with Rotateq® had three doses.

Rotavirus detection

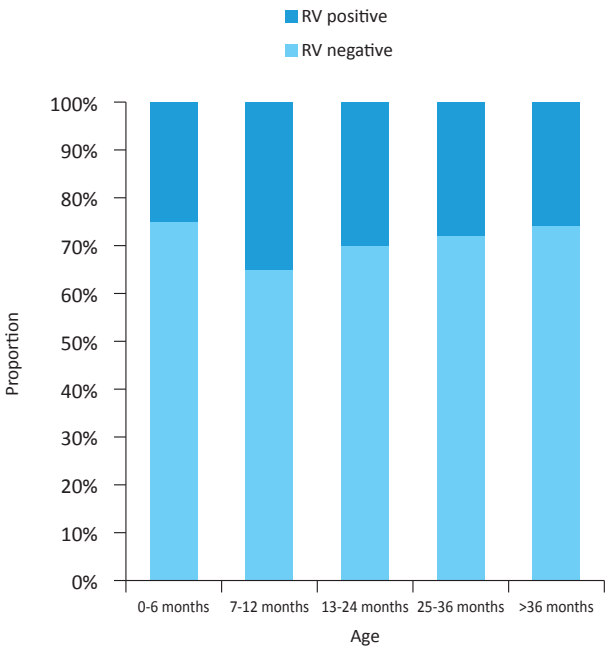
Stool samples tested positive for RV in 522 cases (28.3%; 95% CI 26.2-30.4%). The Southern and Northern regions had higher rates of RV detection, with 32.9% and 31.2% respectively, followed by the Central region (26.6%) and Islands (24.8%) (p = 0.023).

The age distribution of RV-positive and -negative cases is presented in Fig. 1.

The monthly distribution of acute RVGE cases showed seasonal differences (p < 0.001), with a higher proportion in the winter and spring (Fig. 2). The highest proportion of acute RVGE cases was observed in April, when nearly half of AGE cases were caused by RV (48.5%).

Characteristics of rotavirus-positive acute gastroenteritis cases and comparison with rotavirus-negative cases

Of the 522 acute RVGE cases, 81 (15.5%) occurred at age <6 months; 143 (27.4%) between 7 and 12 months;



RV - rotavirus.

Figure 1. Age distribution of acute rotavirus-positive and -negative gastroenteritis cases.

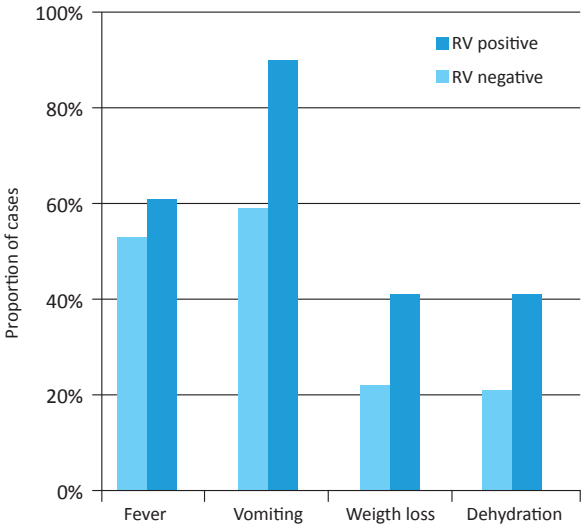
163 (31.2%) between 13 and 24 months; 67 (12.8%) between 25 and 36 months; and 68 (13.0%) in children older than 36 months. Of the 1324 RV-negative cases, 250 (18.8%) occurred at age ≤6 months; 288 (21.7%) between 7 and 12 months; 393 (29.6%) between 13 and 24 months; 179 (13.5%) between 25 and 36 months; and 214 (16.1%) in children older than 36 months. The mean age of children with acute RVGE was 18.7 months, compared to 19.6 months in RV-negative cases ($p = 0.616$).

Children with acute RVGE were more likely to have fever, vomiting, weight loss and dehydration ($p < 0.001$ for all comparisons) than children who were RV-negative (Fig. 3). Hospitalisations occurred in 23.3% of RV-positive children versus 11.4% of RV-negative cases ($p < 0.001$).

The proportion of acute RVGE in children who did not receive RV vaccine was more than four times higher than that observed in the vaccinated group (31.0% versus 7.3%, respectively, $p < 0.001$). Of the 16 acute RVGE cases who had received RV vaccine, a 4-month-old child was hospitalised, with a concomitant respiratory infection and no signs of dehydration.

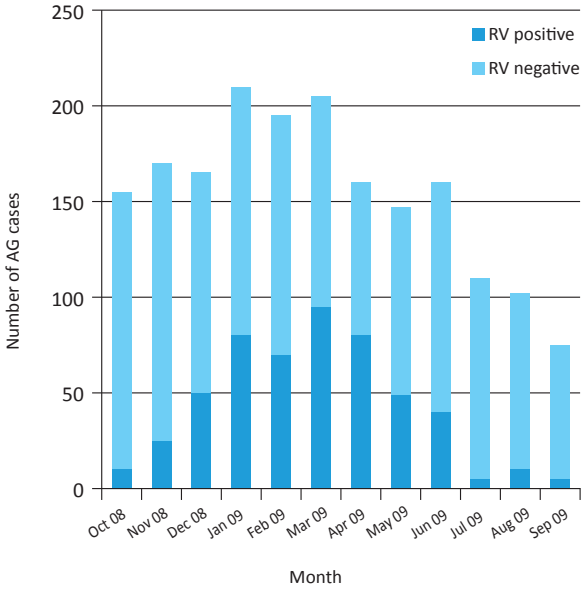
RV genotypes

Of the 522 acute RVGE cases, 509 were available for genotyping. The most frequent genotypes were G4P[8], in 46% of cases (95% CI 42.5-51.1%) and G1P[8], in 37% (95% CI 32.7-41.1%) (Fig. 4). Co-infection was detected in 2.6%, with G1+G4P[8] as the most frequent combination. Fig. 5 presents genotype distribution by hospital. From North to South, the proportion of G4P[8] increased and the proportion of acute RVGE cases due to G1P[8] decreased. G2P[4] was detected in only two hospitals and G9P[8] in three. Genotype G12P[8] was found in 14 children from seven centres. Genotypes G3P[8], G8P[4] and G9P[9] were found only in the Islands. Vaccinated children had genotypes G4P[8] and G1P[8].



RV, rotavirus

Figure 3. Clinical characteristics of acute rotavirus-positive and -negative gastroenteritis cases.



AG - acute gastroenteritis; RV - rotavirus

Figure 2. Monthly distribution of acute rotavirus gastroenteritis cases.

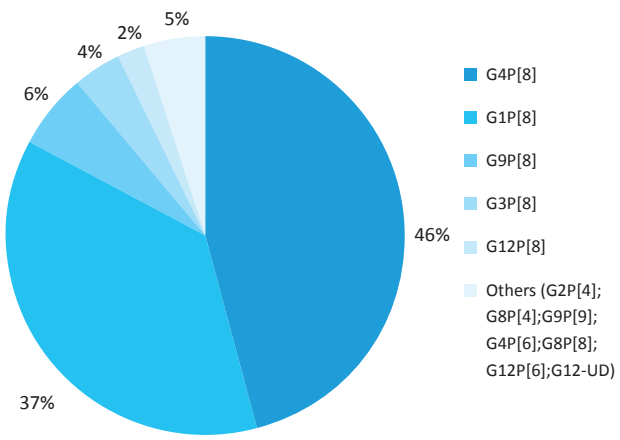


Figure 4. Overall genotype distribution in Portugal.

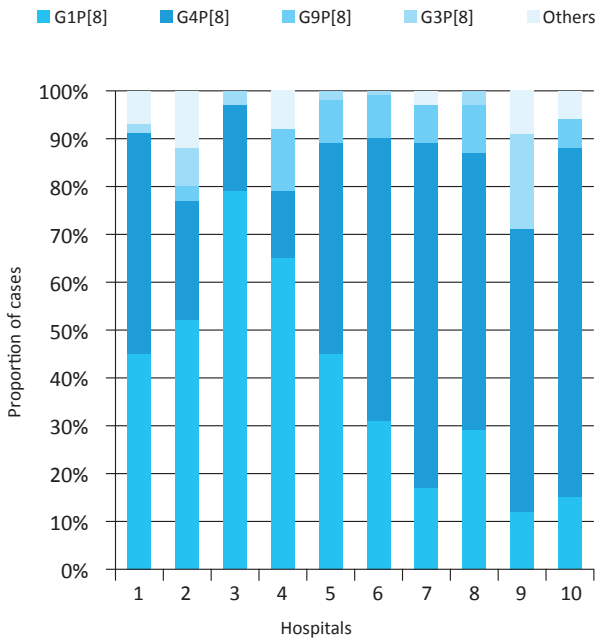


Figure 5. Genotype distribution by hospital.

Discussion

This is the first multicentre Portuguese study that estimates the proportion of acute RVGE in children, describes clinical features of RV infection and reports the distribution of genotypes in all regions in the country.

The overall proportion of acute RVGE in children aged <5 years observed in paediatric ESs was 28.3%, slightly higher in the Northern and Southern regions and higher in winter and spring. These figures are similar to those found in other southern European countries like Spain and Italy.[12,23-25]

Of the acute RVGE cases, 15.5% occurred in children aged <6 months, which highlights the importance of early vaccination, and 74% occurred in children aged <2 years, similar to what was found in the SHRIK study (15.9% and 81%, respectively).[6]

Children with RV presented in more severe clinical condition and had a higher rate of hospitalisation.

It has been reported that several RV strains may co-circulate.[12,26] Worldwide, types G1 to G4 and G9 are associated with the majority of RV infections; in Europe, G1 is responsible for 70% of acute RVGE cases.[3,9,26] Previous studies in Portugal showed G9P[8] and G2P[4] to be the predominant types in winter.[10,11] Co-circulation of several genotypes and important variations from season to season in the same area were also demonstrated in the Central region of the country.[21,22] In the present study, the most frequent genotypes were G4P[8] and G1P[8], in 46% and 37% of cases, respectively. Emergence of genotype G12P[8] has been reported in several countries, confirming that it has the potential to become a sixth common genotype across the world.[27-29] Interestingly, rare genotypes such as G8P[8] and G9P[9] were found only on the islands of Madeira and the Azores. Also of interest is the geographical trend in genotype distribution observed from the north to the south of the country.

There were some children with acute RVGE who had received the vaccine but only one case, with a concomitant respiratory infection, was hospitalised. This is in agreement with efficacy studies that showed both vaccines were highly efficacious against severe RVGE but provided less protection against RVGE of any severity.[30,31]

Some study limitations should be taken into account. More children were included from the Central region and the Islands than the North and South regions. Although a variety of ESs of national hospitals participated in this study, only children seeking health care at public hospitals were included, which could lead to underestimation of the real prevalence of acute RVGE. Admission may also have been influenced by variation in clinical management of AGE in each hospital. Although these results refer to 2008-2009, they constitute a baseline for the country, from a period when there was moderate vaccine use, similar to the situation in subsequent years. A single-centre study performed early after vaccine introduction showed some fluctuations in the proportion of acute RVGE but no clear trends.[22]

In conclusion, acute RVGE was associated with greater morbidity and hospitalisation as compared to RV-negative cases. Our data highlight the need for early protection, since 15.5% of acute RVGE cases occurred in children younger than 6 months. Considering the genotype profile identified in previous national studies, the predominance of G1 and G4 types in this study confirms the recognised variability in strains over time within the same geographic area.

In the era of RV vaccines, this knowledge is important for policy decisions concerning disease prevention and to monitor trends in RV molecular epidemiology.

Conflicts of Interest

The Associação de Saúde Infantil de Coimbra, Portugal, but not Fernanda Rodrigues, has received funding for research conducted by Fernanda Rodrigues from Sanofi Pasteur MSD, for consultancy and postgraduate speaking and travel bursaries to attend educational meetings from Sanofi Pasteur MSD and GSK, the manufacturers of licensed rotavirus vaccines in Europe.

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Protection of human and animal subjects
The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data
The authors declare that they have
followed the protocols of their work center on the publication of patient data.

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