Brief Original Article

First report of a norovirus outbreak associated with the variant Sydney 2012 in Portugal

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Abstract

Introduction: This study describes the investigation of a gastroenteritis outbreak in a group of students, associated with a dinner reunion in February 2013 in Porto, Portugal.
Methodology: An anonymous structured questionnaire was developed and sent to 34 students who attended the dinner reunion. Eighteen students completed the questionnaire and thirteen met the case definition (attack rate of 72%). Stools from two students were screened for norovirus by RT-PCR using primer pairs that target the highly conserved polymerase gene and the capsid gene.
Results: Norovirus genotyping confirmed the variant Sydney 2012 as the probable cause of the outbreak.
Conclusion: This is the first report of an outbreak associated with the new variant Sydney 2012 in Portugal.

Key words: norovirus GII.4 Sydney; norovirus; foodborne; outbreak; Portugal

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Introduction

Noroviruses are today recognized as the leading cause of foodborne illnesses, and are the cause of half of all gastroenteritis outbreaks worldwide [1-3]. The emergence of the new norovirus GII.4 Sydney variant has been associated with a global increase of norovirus activity [4-7]. In Portugal, no information is available on norovirus circulation since these viruses are not routinely included in the diagnostic algorithm of acute gastroenteritis and are not searched for in food when an outbreak alert is made. To date, there is only one report of a gastroenteritis foodborne outbreak caused by norovirus in Portugal [8], supporting the notion that norovirus surveillance studies and outbreak reports are manifestly lacking in this country.

On 23 February 2013, a group of students from a faculty of the University of Porto, in Porto, Portugal, gathered for a dinner reunion at a local restaurant. On 1 March, two students reported that they were recovering from symptoms of intense vomiting and diarrhea that had started on 24 February, to the Laboratory of Microbiology of the Faculty of Pharmacy of The University of Porto. Based on their symptoms, the possibility of a foodborne outbreak was considered. Stool samples were immediately collected from both students, and a retrospective investigation of the rest of the group was initiated to evaluate the extent of the outbreak and to identify the causative pathogen, the source of infection, and the mode of transmission.

Methodology

Epidemiological investigation

A list of all the students who attended the reunion dinner was retrieved, leading to a total of 34 students who sat at two different tables. A structured questionnaire was developed and sent to all students to collect information about demographics, clinical

Table 1. Oligonucleotide primers used for the detection of norovirus in stool samples from the gastroenteritis outbreak in a group of students in Porto, Portugal

Target virus	Target genes	Primers	Primer sequence, 5'-3'	Sense	Size (bp)	Reference
Norovirus GI and GII	RdRp (region A)	JV12y	ATACCACTATGATGCAGAYTA	+	327	[9]
		JV13i	TCATCATCACCATAGAAIGAG	-		
Norovirus GII	Capsid (region C)	G2SKF	CNTGGGAGGGCGATCGCAA	+	343	[10]
		G2SKR	CCRCCNGCATRHCCRTTRTACAT	-		

G: genogroup; RdRP: RNA-dependent RNA-polymerase; bp: base pairs

symptoms, disease onset, and food items consumed at the dinner. Participation in this study was voluntary and anonymous. To obtain details on a possible second wave of infection caused by person-to-person transmission, students were also asked to report if similar gastroenteritis cases occurred in their households during the same or the following week. A case was defined as a person who ate dinner at the restaurant on 23 February 2013 and who presented at least one measurable symptom of the following: diarrhea alone, a vomiting episode plus fever, abdominal pain, or nausea between 23 February and 1 March 2013. As this outbreak was only reported on 1 May, no food samples were retrieved for study since, according to regulations, food samples are to be kept only for 48 hours.

Virological investigation

The two stools were screened for norovirus by RT-PCR, using broadly reactive primer pairs, which targets the highly conserved polymerase gene (region A [9]) and the capsid gene (region C [10]) (Table 1). Amplified products of the expected size were sequenced and the sequences were genotyped using an automated genotyping tool implemented at the National Institute for Public Health and the Environment (RIVM) [11].

Statistical analysis

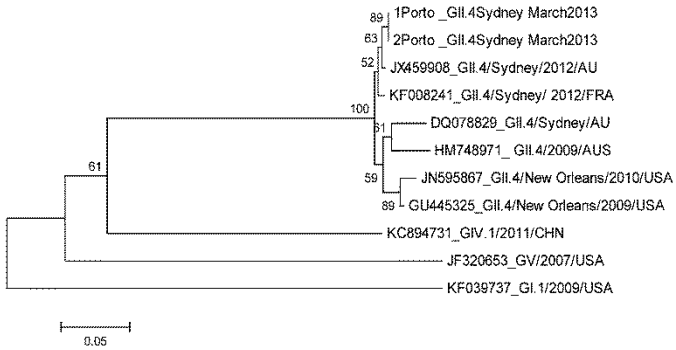
Bivariate analysis was performed by means of odds ratios (OR) with 95% confidence intervals (CI) to evaluate the statistical strength of the associations between disease and food item consumption. P values less than 0.05 were considered statistically significant. Analyses were performed using Epicalc package in the R software (R 2.15.1)

Results

Of the 34 students, 18 completed the questionnaire, for a response rate of 52.9%. Among these 18 students, 13 met the case definition, yielding an overall attack rate of 72%. The reported clinical symptoms were vomiting (n = 11, 84.6%), diarrhea (n = 10, 76.9%), nausea (n = 10, 76.9%), asthenia (n = 9, 69.2%), abdominal pain (n = 9, 69.2%), and fever (n = 8, 61.5%). The first cases and the epidemic peak occurred on 24 February (n = 7, 53.8%), and the last cases on 25 February (n = 6, 46.2%), which corresponds with an incubation period of 24–48 hours. Clinical symptoms lasted one day (n = 2, 15.4%), two days (n = 4, 30.8%), three days (n = 6, 46.2%), or four days (n = 1, 7.7%). There was no information reporting the existence of secondary cases.

The stool samples from the two students tested positive for norovirus, and both isolates were identical after alignment of both RdRp and capsid regions consensus sequences. Norovirus genotyping results were GII.Pe in the polymerase region and GII.4 Sydney 2012 in the capsid region (Figure 1), indicating the variant Sydney 2012 as the probable cause of this outbreak.

Figure 1. Neighbor-joining tree of norovirus sequences (region C) from samples identified in a gastroenteritis outbreak in a group of students in Porto, Portugal. The phylogenetic analysis was performed using the Kimura two-parameter model with gamma distribution of rate variation among sites and 1,000 bootstrap replicates with Mega 6.05 software. The scale bars represent the number of substitutions per site. GenBank accession numbers were as follows: GII.4 Sydney (KF008241), GII.4 Sydney (JX459908), GII.4 Sydney (DQ078829), GII.4 (HM748971), GII.4 New Orleans (JN595867), GII.4 New Orleans (GU445325), GI.1 (KF039737), GIV.1 (KC894731), GV (JF320653).



Concerning the food risk assessment, the data obtained through the questionnaires on the food items consumed at the dinner showed pizza as the most likely source of the outbreak (OR: 13.1; 95% CI: 1.1-169.6; p = 0.046).

Discussion

In the present study, we describe the investigation of a foodborne gastroenteritis outbreak potentially caused by norovirus GII.4 Sydney in a group of university students. One of the drawbacks of this study was that other enteric pathogens were not tested for in the stools. However, the clinical and epidemiological characteristics of this outbreak, including the abrupt onset of symptoms – diarrhea and vomiting in the majority of cases – an attack rate of 72%, incubation period of 24 to 48 hours, and duration of illness ranging from one to four days are in accordance with norovirus symptoms. The prolonged viral fecal excretion typical of norovirus infections allowed us to identify the agent of this outbreak in the stools of the two symptomatic students, collected six days after the onset of illness. Unexpectedly, statistical analysis suggested pizza as the most likely source of this outbreak. Despite being a warm product, contamination by a food handler or a contact surface cannot be excluded. Unfortunately, it was not possible to confirm the presence of norovirus in food since the outbreak was reported six days after the dinner, and food samples are required by law to be kept for only 48 hours. To our knowledge, this is the first report of an outbreak of gastroenteritis associated with norovirus 2012 Sydney variant in Portugal. This report represents an important contribution to the scarce information about norovirus activity in Portugal.

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