An outbreak of acute norovirus gastroenteritis was detected and epidemiologically linked to a Christmas dinner reunion of 22 recent graduate students in a restaurant in Porto, Portugal, in December 2008. A retrospective cohort study was carried out using online standardised questionnaires. Sixteen primary and three secondary cases were identified and the risk ratios with 95% confidence intervals for each food item were calculated. The response rate to the online questionnaires was 96%. The outbreak met all four Kaplan’s criteria and the attack rate was 73%. Norovirus GII.4 2006b was detected in stools and emesis samples of two primary cases. The ingestion of soup and lettuce salad was considered a risk factor for this norovirus outbreak, as determined by statistical analysis. Our investigation demonstrated two routes of transmission of norovirus starting with foodborne exposure followed by secondary person-to-person spread. To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.

Background
Noroviruses are the leading cause of foodborne outbreaks of acute gastroenteritis and the most common cause of sporadic infectious gastroenteritis among persons of all ages [1-6]. In the present study we describe the investigation by statistical and virological methods of what we think to be the first report of a foodborne norovirus outbreak in Portugal. On 27 December 2008, a group of 22 former students of the University of Porto, now living in different regions of Portugal and abroad, gathered at a Christmas dinner party. This meeting was the only person-to-person contact that this group had had in months. They sat at two different tables (with 4 and 18 individuals, respectively) and were served separately without any contact between the two tables during the meal. Symptoms of loose stools and vomiting appeared 24 hours after the dinner in a 28-year-old couple from the group. This couple had not shared any other meal since they had spent Christmas holidays away from each other. The dehydration was so severe that they required hospitalisation. They received intravenous fluid therapy and oral loperamide in order to recover fluid balance, oral metoclopramide for nausea and emesis and oral omeprazol for gastric and duodenal protection. Both developed fever (39.0ºC - 39.5ºC) and received intravenous paracetamol and antibiotic therapy with oral ciprofloxacin, which was maintained for seven days. At that time no laboratory diagnosis was made for gastroenteritis pathogens. The two patients spent the night in the hospital for observation and received further intravenous fluids now with acetylsalicylic acid for the fever. At that time and based on the symptoms the possibility of a foodborne outbreak was considered. Preliminary investigations of the couple led to the Christmas dinner served to another 20 persons as the most probable origin of infection. A retrospective study was initiated in order to find the full extent of the outbreak and its probable source.

Methods
Epidemiological investigation
A list of people who attended the Christmas dinner was retrieved from the index cases, the 28-year-old couple who presented with vomiting, diarrhea, abdominal pain, nausea and fever. A structured questionnaire was developed and emailed to the 22 participants of the dinner to obtain information about sex, age, food intake, etc.

Table 1
Comparison of Kaplan’s criteria with the primary cases of an outbreak of gastroenteritis in Porto, Portugal, December 2008 (n=16)

<table>
<thead>
<tr>
<th>Kaplan’s criteria</th>
<th>Outbreak in Porto</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Vomiting in &gt; 50% cases</td>
<td>Vomiting in 94% of the cases</td>
</tr>
<tr>
<td>2) Duration of illness 12-60 hours</td>
<td>81% of cases had duration of illness between 12 - 60 hours*</td>
</tr>
<tr>
<td>3) Incubation period of 15-36 hours</td>
<td>94% of cases had incubation period of 15-36 hours</td>
</tr>
<tr>
<td>4) Bacterial pathogens not present</td>
<td>Stool samples found negative for bacteria</td>
</tr>
</tbody>
</table>

*This study questionnaire asked for the duration of illness in terms of days and not in hours. 81% of the cases presented duration of illness between 12 and 60 hours and 19% had duration of illness between 60 and 72 hours.
onset and nature of symptoms and duration of illness. They were also asked to report similar cases in their households and close environment during the same or the following week in order to obtain details about possible secondary cases caused by person-to-person transmission.

Primary case was defined as a person who ate at the restaurant on the night of 27 December 2008 and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within 72 hours after the restaurant meal. Secondary case was defined as a close contact (household member) of a primary case who did not participate in the dinner of 27 December and experienced diarrhoea (alone) or a vomiting episode plus one or more of the following symptoms: abdominal pain, nausea, and fever within a two week period after the meal.

The primary attack rate (AR) was calculated as the number of primary cases divided by the total number of people dining at the restaurant on 27 December and therefore possibly exposed to the causative agent.

To measure the association between eating specific food items served at the Christmas dinner and developing illness, Mantel-Haenszel estimates of the risk ratio (RR) with 95% confidence intervals for each food item were calculated.

Laboratory investigation

Two stool samples and one emesis sample were collected from the couple 36 hours after the Christmas dinner and tested for bacterial, parasitic and viral enteric pathogens. Routine bacterial culture for *Salmonella* and *Shigella* was performed according to standard procedures and microscopic methods were used to screen for protozoa and helminths. Stool specimens were examined for the presence of norovirus by reverse-transcription polymerase chain reaction (RT-PCR) using JV12y/JV13i oligonucleotide primers [7] followed by nucleotide sequencing of the RT-PCR products.

Results

Epidemiological and clinical characteristics of cases

Of the 22 dinner participants, 21 completed the questionnaire (response rate 96%) and 16 met the primary case definition yielding an overall attack rate of 73%. All cases (nine female and seven male) reported symptoms in compliance with Kaplan’s criteria [8,9] (Table 1).

Based on the answers to the questionnaires three further persons were identified who met the definition of secondary case, two of these were parents of two primary cases living in Porto, the third was identified in Lisbon and was a close contact of an asymptomatic person who had participated in the dinner (Figure 1).

The 16 primary cases reported the following clinical symptoms: diarrhoea (n=12, 75%), vomiting (n=15, 94%), abdominal pain (n=8, 50%), nausea (n=7, 44%), fever (n=5, 31%), fainting (n=1, 6%) and asthenia (n=7, 44%). Two persons (the 28-year-old couple) had to be hospitalised because of the severity of dehydration and received intravenous fluids. Among the five dinner participants who did not fully meet the case definition criteria, two had abdominal pain, two reported nausea and three reported asthenia.

<table>
<thead>
<tr>
<th>Food Item</th>
<th>Univariate analysis</th>
<th>RD</th>
<th>RR</th>
<th>95% CI (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce salad</td>
<td>0.197</td>
<td>1.31</td>
<td>0.74-2.32</td>
<td></td>
</tr>
<tr>
<td>Iced-cake</td>
<td>0.197</td>
<td>0.76</td>
<td>0.43-1.35</td>
<td></td>
</tr>
<tr>
<td>French-fries</td>
<td>0.06</td>
<td>0.92</td>
<td>0.47-1.79</td>
<td></td>
</tr>
<tr>
<td>Soup</td>
<td>0.385</td>
<td>1.63</td>
<td>1.06-2.50</td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>0.058</td>
<td>0.92</td>
<td>0.47-1.81</td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td>0.047</td>
<td>1.07</td>
<td>0.45-2.55</td>
<td></td>
</tr>
</tbody>
</table>

RD: risk difference; RR: risk ratio; CI: confidence interval;
Clinical symptoms in the primary cases started abruptly 24-36 hours after the Christmas dinner, on Sunday and Monday, 28-29 December 2008. The mean incubation period was 28 hours (Figure 2). The duration of illness ranged from 12 to 76 hours (mean 45 hours). The last case associated with this outbreak was a secondary case in Lisbon who had onset of symptoms on Friday 2 January 2009, six days after the dinner. This person had contact with one of the asymptomatic guests of the dinner who traveled from Porto to Lisbon on 1 January.

**Food risk assessment**

From the data obtained through the questionnaires on food items consumed at the dinner soup was identified as the most likely source of the outbreak with a RR of 1.63 (95% CI: 1.06-2.50), followed by lettuce salad with a RR of 1.31 (95% CI: 0.74-2.32) (Table 2).

**Laboratory investigation**

Macroscopic analysis of one stool sample revealed live blood. This was confirmed by the presence of erythrocytes by optical microscopy. Both stool samples tested negative for Salmonella and Shigella and for rotavirus and adenovirus. The two stool samples and the emesis sample tested positive for norovirus. Nucleotide sequencing of the RT-PCR products demonstrated that all three isolates were identical and belonged to genotype GII.4 2006b.

**Discussion**

In the present study we describe a foodborne outbreak associated with a dinner in a restaurant in Porto, Portugal. Our combined epidemiological data and virological findings suggested that the causative pathogen was norovirus which was detected from the faecal and vomit specimens obtained from the couple who required hospitalisation. This strain was identified as a GII.4 2006b which has been predominant at a global scale for the past three years [10,11]. The involvement of other enteric pathogens in this outbreak cannot be ruled out with the exception of Salmonella, Shigella, enteric protozoa, helminths, rotavirus and adenovirus for which the faecal samples tested negative. The treatment of the hospitalised couple with loperamide is questionable since the use of antimotility agents in severe gastroenteritis may be harmful [12]. Normally, except the rehydration therapy, no further drugs are necessary in viral gastroenteritis treatment. The clinical and epidemiological characteristics of this outbreak including an attack rate of 73%, a mean incubation period of 28 hours, and a mean duration of illness of 45 hours as well as the occurrence of secondary cases are in accordance with a norovirus outbreak. Moreover, this cluster of cases met all four epidemiological criteria for a norovirus outbreak [8,9].

No definitive conclusion on the source of this outbreak could be reached, since food samples were not available for norovirus detection. However a foodborne origin was supported by the analysis performed with the web-based tool developed by the Foodborne Viruses in Europe (FBVE) network for the investigation of norovirus food-related outbreaks [13]. Risk associated with individual food items revealed, unexpectedly, that soup, despite being a warm food product, was the most likely source of the outbreak based on its highest RR (1.63, 95% CI: 1.06-2.50). Lettuce salad has been frequently associated with norovirus outbreaks [14] and in the present study was also associated with a high RR (1.31, 95% CI: 0.74-2.32). French fries, cheese and bread were not considered a risk factor given their RR (~1). Whether the food was contaminated before arriving at the restaurant or infection was due to poor food handling practices could not be determined since information on hygiene conditions, food handling practices and health status of the restaurant staff were not available.

Our data indicated that there were two routes of transmission in this outbreak. The origin was a foodborne transmission which caused infection in the primary cases who, subsequently, through person-to-person transmission, infected secondary cases among household and close contacts. The last case associated with this outbreak was detected six days after the dinner in a person resident in Lisbon who had contact with one of the participants of the Christmas dinner group. Although no laboratory confirmation was performed, the Lisbon case met in full the definition of secondary case, but the possibility that this patient was not associated with the outbreak cannot be ruled out.

To our knowledge this is the first study identifying norovirus as the causative agent of a foodborne outbreak in Portugal.

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**References**