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.

Table 1

<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>RD: risk difference; RR: risk ratio; CI: confidence interval;</th>
</tr>
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<tbody>
<tr>
<td>Lettuce salad</td>
<td>0.197 1.31 0.74-2.32</td>
</tr>
<tr>
<td>Iced-cake</td>
<td>0.197 0.76 0.43-1.35</td>
</tr>
<tr>
<td>French-fries</td>
<td>0.06 0.92 0.47-1.79</td>
</tr>
<tr>
<td>Soup</td>
<td>0.385 1.63 1.06-2.50</td>
</tr>
<tr>
<td>Cheese</td>
<td>0.058 0.92 0.47-1.81</td>
</tr>
<tr>
<td>Bread</td>
<td>0.047 1.07 0.45-2.55</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 Monday 2 January 2009, six days after the dinner. This person had contact with one of the asymptomatic cases of the primary 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