Lessons learnt from a birthday party: a Bacillus cereus outbreak,
Bari, Italy, January 2012

Domenico Martinelli(a), Francesca Fortunato(a), Silvio Tafuri(b), Vanessa Cozza(a),
Maria Chironna(b), Cinzia Germinario(b), Biagio Pedalino(a) and Rosa Prato(a)

(a) Dipartimento di Scienze Mediche e Chirurgiche, Università di Foggia, Foggia, Italy
(b) Dipartimento di Scienze Biomediche e Oncologia Umana,
Università degli Studi di Bari Aldo Moro, Bari, Italy

INTRODUCTION

Bacillus cereus is ubiquitously distributed in the environment [1] and can be isolated in various starchy food items (e.g., milk, rice, and pasta). Infection causes both emetic and diarrhoeal disease. The heat stable cereulide toxin causes the emetic syndrome. Typically, it is produced when the microorganism grows in food cooked and stored at room temperature for several hours [2, 3]. Emetic poisoning is characterized by a very short incubation period ranging from 0.5 to 6 hours, vomiting followed by diarrhoea after 8 to 16 hours in approximately a third of the cases [1, 4]. Usually the disease is self-limited within 24 hours. Severe and fatal outcomes are rare [5].

The real burden of B. cereus outbreaks is poorly known, especially because isolated strains are not always catalogued [6]. In Italy, in 2005, only 3 of 230 outbreaks reported to the computerised surveillance network for communicable diseases were associated to B. cereus [7]. In 2006 in Campania (South Italy), an outbreak of foodborne infection involved 149 participants at a wedding reception. The food item associated with illness was the ricotta cheese (RR 3.58; 95% CI 1.72-7.48); B. cereus was isolated in specimens of ricotta cheese collected at the dairy food supplier, but no laboratory confirmation was possible as no food leftovers were available. Main lessons learnt from this investigation included delay in notification, failure to use a reference laboratory with diagnostic protocols for the microbiological research in foodborne diseases, and deficiencies in hygienic measures of food handling, particularly refrigeration [8].

From 2002 to 2009, in Piedmont (North East of Italy), health authorities investigated 803 foodborne outbreaks with a total of 3699 ill individuals; B. cereus was responsible for 2.1% (no. = 76) of these cases [9].

We report a B. cereus foodborne outbreak that occurred during a birthday party in a pub in Bari (Puglia, South Italy). On 22 January 2012 at 4.30 pm, the owner of a pub reported a cluster of cases of emesis to the Local Health Authority. Cases were among a group of friends and relatives. We launched an investigation to estimate the magnitude of the outbreak, to identify the vehicle of contamination and to prevent further cases.

Key words
• Bacillus cereus
• emetic syndrome
• foodborne outbreak
MATERIALS AND METHODS

Field investigation

The epidemiological investigation was carried out between January 22nd and 24th, 2012.

We performed a retrospective cohort study among the guests of the party to identify risk factors associated with illness.

We defined cases as individuals presenting with vomiting, nausea, abdominal pain or diarrhoea within 24 hours after consuming lunch at the pub on 22 January. We administered a standardised questionnaire to collect information on: i) demographics; ii) if ill, type and symptoms onset; iii) food items consumed at lunch.

We calculated food-specific attack rates, relative risks (RRs) and 95% confidence intervals (95% CIs), along with p values using Fisher’s exact test using STATA-MP 10.1.

Environmental investigation

The list of food items served during the party was available (basmati rice, red rice, black rice, lasagne, ragù, sweet and sour vegetables, 2 apple cakes). We interviewed the food handler regarding food purchase, preparation process, storage, and cleaning procedures.

Microbiological investigation

Leftovers of 3 kinds of rice, lasagna, ragù, and 2 cakes were available for microbiological analysis. Food specimen were stored at 4 °C and processed for bacteriological analysis within 24 hours. Samples were tested for several enteric pathogens (B. cereus, Campylobacter, Clostridium perfringens, Enterobacteriaceae, E. coli, Listeria monocytogenes, Salmonella spp., Staphylococci) using routine methods [10]. Selective polymyxin-egg yolk-mannitol-bromothymol blue agar (PEMBA) medium was used to isolate B. cereus [1, 11].

Faecal specimens were collected from cases within 24 hours of onset. Part of the specimens was refrigerated and processed within 12 hours to detect Salmonella spp., Shigella, Campylobacter, Vibrios, and other enteropathogens using standard methods [10]. For isolation of B. cereus, dilutions of stool samples were seeded onto PEMBA [12].

In the routine laboratory investigations of leftovers and stool samples, no tests for staphylococcal enterotoxins or B. cereus emetic toxin were performed. Samples were not tested for enteric viruses.

Ethics statements

Ethical approval was not required for this study since the primary aim of the epidemiological investigation was to identify, characterize, and control disease in response to an immediate public health threat (Italian Ministry of Health, Circolare n. 4 of 13/03/1998, Prot. 400.3/26/1189, available at www.salute.gov.it/malattieInfettive/archivioNormativaMalattieInfettive.jsp?menu=normativa). All participants were explained the purpose of the investigation and participation was voluntary. Verbal informed consent was collected from participants or guardians of the minors involved in the study through the first question of the case report form. Laboratory tests were performed following routine diagnosis and treatment procedures.

RESULTS

Description of the outbreak

We identified 12 cases (median age: 41, range: 15-70) among the 13 customers (attack rate: 92%) who had lunch at the pub on January 22nd, 2.00 p.m. The median incubation period was 2 hours (range: 1-5 hours). Cases started to occur on January 22nd, 3.00 p.m. and peaked at January 22nd, 4.00 p.m. with a
A Bacillus cereus outbreak in Bari, Italy

Brief notes

Table 1
Incidence of B. cereus infection according to selected characteristics, cohort study. Bari, Italy, 22 January 2012

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Among exposed</th>
<th>Among unexposed</th>
<th>Relative risk</th>
<th>95% Confidence interval</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; median age</td>
<td>5 6</td>
<td>7 7</td>
<td>0.17</td>
<td>0.86-1.58</td>
<td>1.00</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>6 6</td>
<td>6 7</td>
<td>0.83</td>
<td>0.58-1.19</td>
<td>0.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factors</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Basmati rice</td>
<td>12 12</td>
<td>0 1</td>
<td>Not calculable</td>
<td>Not calculable</td>
</tr>
<tr>
<td>Red rice</td>
<td>9 10</td>
<td>3 3</td>
<td>0.9</td>
<td>0.73-1.11</td>
</tr>
<tr>
<td>Black rice</td>
<td>11 12</td>
<td>1 1</td>
<td>91.67</td>
<td>0.77-1.09</td>
</tr>
<tr>
<td>Lasagne</td>
<td>5 6</td>
<td>7 7</td>
<td>0.83</td>
<td>0.58-1.19</td>
</tr>
<tr>
<td>Ragù</td>
<td>10 11</td>
<td>2 2</td>
<td>90.9</td>
<td>0.75-1.09</td>
</tr>
<tr>
<td>Sweet and sour vegetables</td>
<td>10 11</td>
<td>0 0</td>
<td>Not calculable</td>
<td>Not calculable</td>
</tr>
<tr>
<td>Apple cake 1</td>
<td>8 8</td>
<td>4 5</td>
<td>1.25</td>
<td>0.81-1.94</td>
</tr>
<tr>
<td>Apple cake 2</td>
<td>7 8</td>
<td>5 5</td>
<td>87.5</td>
<td>0.67-1.13</td>
</tr>
</tbody>
</table>

last case at January 22nd, 7.00 p.m. (Figure 1). The most commonly reported symptom was vomiting (83%), followed by nausea (75%), abdominal pain (50%) and diarrhoea (42%). No cases were hospitalised and no deaths were reported. Eleven cases recovered within one day; only in one case, diarrhoea persisted for 48 hours.

Cohort study

Food specific attack rates for basmati rice and one of the two apple cakes were 100%, and those who had consumed the apple cake were 1.25 times more likely to become ill (CI 95%: 0.81-1.94). All cases had consumed basmati rice and sweet and sour vegetables (aetiological fraction: 100%); the RR was thus not calculable for either of these food items. One individual, who had eaten sweet and sour vegetables but not basmati rice, did not develop symptoms (Table 1).

Environmental results

The food handler prepared food on three separate days using different storage methods. First, he cooked lasagne, ragù, sweet and sour vegetables 10 days before and froze them right after preparation; he defrosted them rapidly using microwave oven before the lunch. Second, he prepared bread, basmati rice, red rice, and black rice on 20 January 2012 at 5.00 pm and stored them at room temperature ≥ 22°C for 24 hours after cooking; then, he stored these food items in a refrigerator at temperature ≤ 4°C. He did not reheat rice dishes before consumption. Third, he cooked two apple cakes on 22 January, early in the morning.

Microbiological results

The culture of basmati rice and 2/5 faecal specimens grew B. cereus. Food and stool specimen were negative for all other tested pathogens.

DISCUSSION

Foodborne outbreaks linked to food consumption in restaurants and other public venues remain a relevant issue in terms of public health, economic impact and reputational damage to the commercial establishment. Improving the efficiency of outbreak investigations is critical to reduce illness and to formulate better prevention strategies [1].

The characteristics of this outbreak are consistent with available reports on foodborne outbreaks caused by B. cereus [5]. The short incubation period and the predominance of vomiting suggested an emetic toxin. The distribution of cases by time of onset suggested a common source of contamination by a bacteria or a toxin. We isolated B. cereus from basmati rice and faecal specimens.

Poor food handling and storage was most probably the cause of the outbreak. However, the source of contamination of the basmati rice remained unclear. Likely, B. cereus spores germinated after cooking and the emetic toxin was produced over the following 24 hours at temperature ≥ 22°C [2]. No unopened or opened pack of raw rice was available for testing as the rice had all been consumed specifically during the party.

We did not test samples for B. cereus emetic toxin. Together with the above mentioned inability to identify the source of contamination of the basmati rice this represents the main limitation of our investigation. The timely identification of B. cereus did not make the tests for enteric viruses necessary.

In this outbreak, the immediate alert by the pub owner contributed to prevent further cases. In the long term, measures to prevent foodborne outbreaks remain the control of storage procedures and temperatures, and to assure HACCP system [13]. Audits, including internal audits, are one of the most innovative but less known tools of official control among those covered by the European Community Regulation No...
The internal control system and the collaboration in the timely reporting of events to the competent authority by the food operators may prevent the spread of foodborne outbreaks and contribute to re-establish a good image of the food business operators. We recommended prompt closure of the pub and recovery of leftovers to avoid cross contamination of foods. Obtaining rapidly leftovers and stool specimens allowed us to perform timely microbiological investigations to isolate *B. cereus*. Two days after our investigation and after detailed inspection of the premises, the pub owner was given permission to re-open the pub. To our knowledge, this is one of the few reports on *B. cereus* outbreak in Italy [7-9].

**Acknowledgements**

The authors are very grateful to Salvatore Barbuti, who performed microbiological analysis, to Maria Filomena Gallone and Rocco Guerra for their contribution to the interviews, and, particularly, to Ivan Hutin for his precious advice on the description of the outbreak.

**Conflict of interest statement**

All authors have no financial interests related to the material in the manuscript.

Received on 10 May 2013.

Accepted on 7 August 2013.

**REFERENCES**