

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

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Abstract

Introduction. *Bacillus cereus*, a ubiquitous bacterium, can be isolated in various starchy food items, causing both emetic and diarrhoeal disease. The real burden of *B. cereus* outbreaks is actually poorly known in Italy.

We report a *B. cereus* foodborne outbreak that occurred in a pub in Bari (Italy) on January 22nd 2012 during a birthday party, promptly reported by the pub owner.

Materials and methods. 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. Leftovers of different meals were available for microbiological analysis. Faecal specimens were collected from cases.

Results. A total of 12 cases among the 13 customers (attack rate: 92%) were reported. All cases had consumed basmati rice and sweet and sour vegetables (aetiological fraction: 100%). *B. cereus* was isolated from both basmati rice served during the party and faecal specimens.

Discussion. The close collaboration between the pub owner and the public health officers and the possibility to test food leftovers and stool samples contributed to prevent further cases.

Key words

- *Bacillus cereus*
- emetic syndrome
- foodborne outbreak

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-limiting 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.

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 the 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 specimens 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

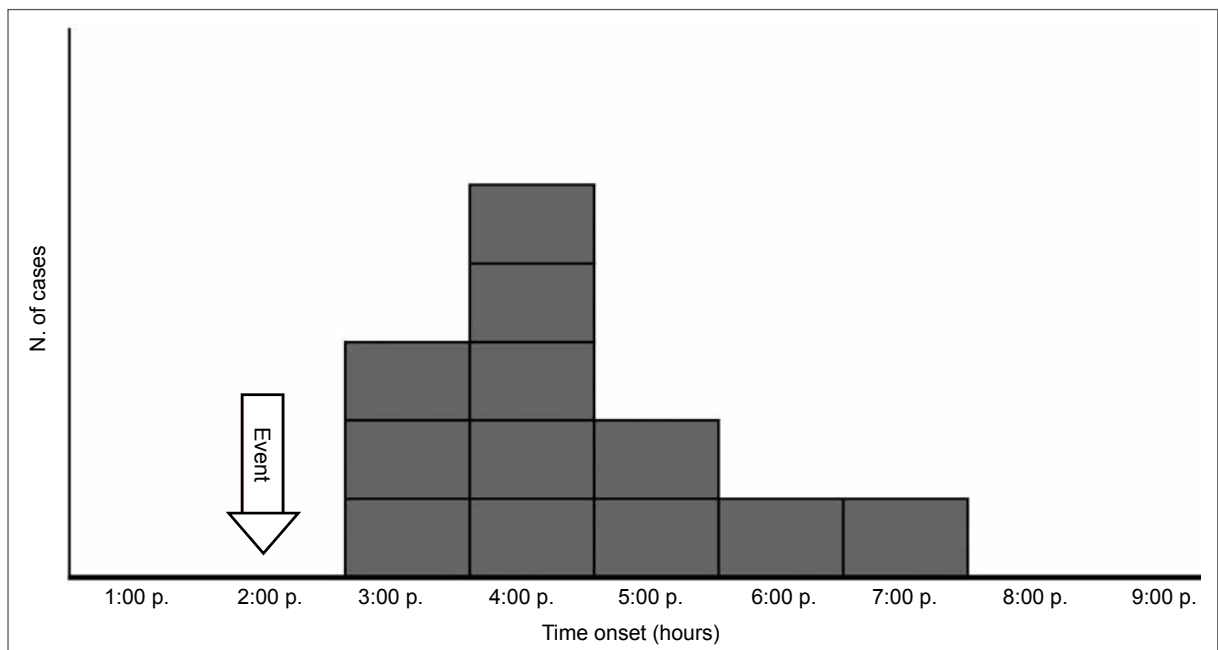


Figure 1

Number of cases by hour of symptom onset, *B. cereus* foodborne outbreak. Bari, Italy, 22 January 2012.

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

	Incidence of disease						Relative risk	95% Confidence interval	p
	Among exposed			Among unexposed					
	N	Total	%	N	Total	%			
Demographic characteristics									
Age > median age	5	6	0.83	7	7	100	1.17	0.86-1.58	1.00
Sex (male)	6	6	100	6	7	0.86	0.83	0.58-1.19	0.46
Risk factors									
Basmati rice	12	12	100	0	1	-	Not calculable	Not calculable	0.076
Red rice	9	10	90	3	3	100	0.9	0.73-1.11	1.00
Black rice	11	12	91.67	1	1	100	91.67	0.77-1.09	1.00
Lasagne	5	6	0.83	7	7	100	0.83	0.58-1.19	0.46
Ragù	10	11	90.9	2	2	100	90.9	0.75-1.09	1.00
Sweet and sour vegetables	10	11	90.9	0	0	-	Not calculable	Not calculable	Not calculable
Apple cake 1	8	8	100	4	5	1.25		0.81-1.94	0.38
Apple cake 2	7	8	87.5	5	5	100	87.5	0.67-1.13	1.00

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 $\geq 22^\circ\text{C}$ for 24 hours after cooking; then, he stored these food items in a refrigerator at temperature $\leq 4^\circ\text{C}$. 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 $\geq 22^\circ\text{C}$ [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

882/2004 of 29 April 2004 [13]. 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].

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Conflict of interest statement

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

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