

Investigation of an outbreak of vomiting in nurseries in South East England, May 2012

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SUMMARY

On 30 May 2012, Surrey and Sussex Health Protection Unit was called by five nurseries reporting children and staff with sudden onset vomiting approximately an hour after finishing their lunch that day. Over the following 24 h 50 further nurseries supplied by the same company reported cases of vomiting (182 children, 18 staff affected). Epidemiological investigations were undertaken in order to identify the cause of the outbreak and prevent further cases.

Investigations demonstrated a nursery-level attack rate of 55 out of 87 nurseries (63.2%, 95% confidence interval 52.2–73.3). Microbiological tests confirmed the presence of *Bacillus cereus* in food and environmental samples from the catering company and one nursery. This was considered microbiologically and epidemiologically consistent with toxin from this bacterium causing the outbreak. Laboratory investigations showed that the conditions used by the caterer for soaking of pearl haricot beans (known as navy bean in the USA) used in one of the foods supplied to the nurseries prior to cooking, was likely to have provided sufficient growth and toxin production of *B. cereus* to cause illness. This large outbreak demonstrates the need for careful temperature control in food preparation.

Key words: *Bacillus cereus*, bacterial infections, outbreaks.

INTRODUCTION

On 30 May 2012 Surrey and Sussex Health Protection Unit (SSHPU) received calls from five nursery schools

(for children aged 0–5 years) in rapid succession, reporting vomiting in children and staff within 1 h of eating their lunch. The nurseries were geographically

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dispersed across three counties in South East England. All had been supplied with the menu of shepherd's pie, cucumber and yoghurt, from the same catering company. This company prepared meals exclusively for nurseries at one central site and delivered ready-cooked food to 87 nurseries by 12 vans.

Investigations over the following 24 h uncovered 50 further affected nurseries and 200 individuals reported as having been unwell. In all cases the illness resolved within a few hours. No individuals were admitted to hospital. The catering company voluntarily ceased food production immediately. This paper describes the findings of environmental, microbiological and epidemiological investigations which identified that *Bacillus cereus* food poisoning was the likely underlying cause.

B. cereus is an endospore-forming bacterium which is a common contaminant of soil, dust, unprocessed plant material and foods such as rice, pasta and pastry. [1] This bacterium can cause an emetic foodborne illness with an incubation period of 0.5–6 h [2] due to the cereulide toxin which is preformed in food prior to consumption, [3]. The cereulide toxin is heat stable and retains the biological activity to cause food poisoning after normal food preparation processes including cooking [4].

METHODS

Epidemiological investigation

A descriptive epidemiological study was conducted for the nursery children and staff who had eaten food supplied by the catering company on 30 May 2012. Cases were defined as those who had eaten the food and then developed symptoms of vomiting and/or diarrhoea during the period 30 May 2012 to 1 June inclusive. A full list of nurseries supplied by the catering company was obtained and nursery staff were asked to complete a 'Select Survey' online questionnaire or hard copy version.

Data was collected and analysed mainly at the nursery level through questionnaires and where necessary a phone call for clarification. Information was collected on the attack rate by age group, timing of delivery and serving and symptoms experienced.

Nurseries' responses were handled and cleaned in Excel (Microsoft Corp., USA) and imported into Stata v. 12 (StataCorp., USA) for analysis. Attack rates (ARs) and binomial exact confidence intervals (CIs) were calculated by exposure status and other variables. Associations between ARs and continuous

variables such as time and temperature were explored with linear regression modelling. Fisher's exact test was used to test the association between exposure to menu items and outcome at nursery level. The χ^2 goodness-of-fit test was used to compare observed variation in ARs between individual nurseries or delivery runs with those that would be expected if ARs were homogeneous.

Environmental investigation

The catering company was contacted by the Environmental Health Officer (EHO) on 30 May and by the evening agreed to cease distribution of food. The EHO visited the company on 31 May when samples were taken of any remaining ingredients that had been used in the meal of 30 May, and environmental swab sampling was performed. On 1 June 2012 an EHO visited the dairy which produced yoghurt for the nursery meals, and took food and environmental samples. Food samples and washed food containers were also collected from one nursery. Food samples and environmental swabs were collected in accordance with the Food Standards Agency Food Law Code of Practice [5]. The samples were transported to the Health Protection Agency (HPA) Food Water and Environmental Microbiology Laboratory at Porton Down, Wiltshire for examination within 24 h of sampling.

Microbiological investigation

Patient specimens

Stool specimens were collected from nine symptomatic children or staff. The specimens were examined for the presence of *Salmonella*, norovirus, *Shigella*, *Campylobacter*, *Escherichia coli* O157, *Cryptosporidium*, rotavirus, adenovirus and *B. cereus* at the Brighton and Sussex University Hospital laboratory, according to standard procedures.

Food samples

A 10⁻¹ homogenate of each food sample was prepared in maximum recovery diluent (MRD), according to ISO 6887-1:1999 [6], and this was used to enumerate aerobic colony count (method based on ISO 4833:2003 but with the variation of using surface inoculation of the agar plate rather than a pour plate method; [7]), Enterobacteriaceae (according to ISO 21528-2:2004 [8]), *Escherichia coli* (using surface inoculation of TBX agar [9]),

coagulase-positive staphylococci (according to ISO 6888-1:1999 [10]), *Clostridium perfringens* (performed (based on ISO 7937:2004 but with inoculation of a single (rather than duplicate) Petri dish with 1 ml sample homogenate [11]) and *Bacillus* species (using surface inoculation of Polymyxin pyruvate egg-yolk mannitol Bromothymol Blue (PEMBA) agar [9]). Microbiological results for food samples were compared to the HPA Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market [12]. Spore stains were performed on homogenates of food samples according to the method described by Roberts & Greenwood [9].

Environmental swab samples

Sponge swabs were tested for Enterobacteriaceae, *E. coli*, *S. aureus*, *C. perfringens* and *Bacillus* species using standard microbiological methods [8–12].

Identification and typing of Bacillus isolates

Isolates of presumptive *B. cereus* were submitted to the Laboratory of Gastrointestinal Pathogens, Health Protection Agency, London, for identification and typing. Isolates were confirmed as *B. cereus* using a combination of 16S rDNA sequencing (performed by the Bioanalysis and Horizon Technologies Department, Health Protection Agency, Colindale), absence of parasporal crystals, fermentation of mannitol, lecithinase activity and resistance to both gamma phage and penicillin [4, 9, 13].

Genomic DNA extraction

DNA was extracted from single colonies after 18 h growth at 37 °C using Microlysis reagent according to the manufacturer's instructions (Microzone Ltd, UK). Cultures from the HPA National Collection of Type Cultures (NCTC), London and a non-template control containing only Microlysis solution were included with each batch of extractions. *B. cereus* NCTC 11143 (emetic), and NCTC 11145 (diarrhoeal) were used as positive and negative controls, respectively, for detection of the cereulide gene and *L. monocytogenes* NCTC 11994 was used as the control for ensuring reproducibility of fluorescent amplified fragment length polymorphism (fAFLP) profiling.

Detection of cereulide toxin genes

Cereulide gene was detected in DNA extracts using a real-time PCR assay adapted from Fricker *et al.* [14].

Forward and reverse primers (*CesF* and *CesR*) were as previously described and a modified *Ces* probe (5'-FAM-TGCATTTCTCGTTATTTTCCCACTAT-C-BHQ1-3') was used. Each reaction consisted of a total volume of 25 µl with: 1x TaqMan® *Fast* Universal PCR master mix (Life Technologies, USA), 0.5 µM each of *CesF* and *CesR* primers, 0.1 µM *Ces* probe, and 5 µl sample DNA. Reactions were performed using a 7500 FAST Sequence Detection System (Life Technologies), cycling conditions were 40 cycles at 95 °C for 15 s and 55 °C for 60 s.

fAFLP analysis of DNA samples

DNA profiles were generated by fAFLP analysis using a modification of the method described previously by Desai *et al.* [15] The modifications included a one-step double digestion with *HindIII* and *HhaI* restriction enzymes (Thermo Scientific, USA), and ligation of fragments to specific double-stranded adapters using 2 U of 400 U/µl T4 ligase (Thermo Scientific). Recommended buffers were used in combination with restriction and ligase enzymes in the same reaction and incubated for 1 h at 36 °C, 1 h at 22 °C then 65 °C for 10 min. The resulting fragments were amplified using a touchdown PCR procedure with the primers 5'-FAM-*HindIII* + A and *HhaI* + A. Fragment profiling was performed using a 3730 ABI sequencer (Life Technologies) by the Bioanalysis and Horizon Technologies Department, Health Protection Agency, Colindale.

Fragments between 60 and 600 bp were analysed using Bionumerics v. 6.1 (Applied Maths, Belgium) and Peak scanner (Life Technologies). Fragment profiles were analysed using the Jaccard algorithm with 20% optimization, 15% minimum height, 1% tolerance using the UPMGA method and assigned to a type designated by Roman numerals.

RESULTS

Epidemiological investigations

Of the 87 nurseries contacted, 74 responded and were included in the analysis (participation rate 85.1%). Forty-six (83.6%) of 55 affected nurseries participated compared to 28 (87.5%) of 32 unaffected nurseries ($P = 0.63$) (Fig. 1).

Inspection of the questionnaire responses revealed some internal inconsistency in the data. Follow-up telephone calls for clarification reduced the overall proportion of responses with internal inconsistency

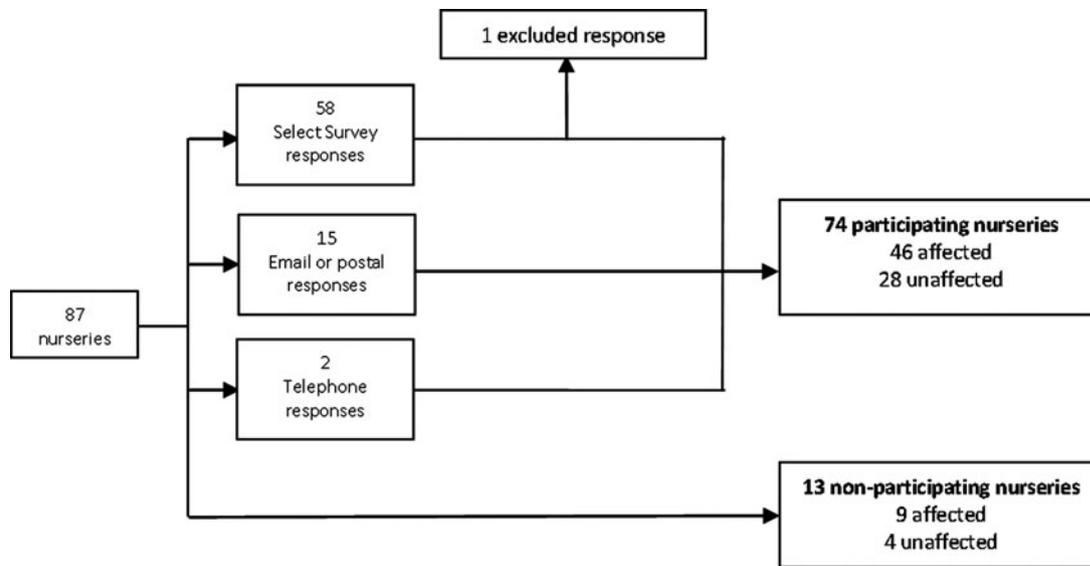


Fig. 1. Flowchart of questionnaire responses.

Table 1. Attack rate by age

| Age group | Affected | Exposed | AR (%) | 95% CI |
|------------------------------------|----------|---------|--------|-----------|
| Babies (about 6 weeks–1 year) | 8 | 111 | 7.2 | 3.2–13.7 |
| Younger children (about 1–2 years) | 66 | 543 | 12.2 | 9.5–15.2 |
| Older children (about 3–5 years) | 108 | 905 | 11.9 | 9.9–14.2 |
| Staff | 18 | 89 | 20.2 | 12.4–30.1 |

AR, Attack rate; CI, confidence interval.

from 25% to 4% in responses from nurseries reporting the presence of symptomatic individuals.

Of the 74 nurseries that participated in the questionnaire, information on exposure status was obtained for 2358 children and 718 staff, of whom 1648 (1559 children and 89 staff) had eaten food from the catering company.

Fifty-five of the 87 nurseries reported vomiting and/or diarrhoea in their children and staff (nursery level AR 63.2%, 95% CI 52.2–73.3). Of the 1648 individuals who were known to have eaten food from the catering company on 30 May, 200 (182 children and 18 staff) met the case definition for case-patient (individual level AR 12.1%, 95% CI 10.6–13.8). There was homogeneity in the menu items received by the nurseries and no associations were identified with individual items. Case-patient ARs increase with increasing age as shown in Table 1. The age groups were based on the room in which the children were cared for in the nursery and exact age cut-offs may vary by nursery.

Epidemic curve

The epidemic curve (Fig. 2) by nursery showed that 93% of the nurseries that reported symptomatic individuals had been affected within the 4 h from midday on 30 May 2012, and that the last nursery was affected during the evening of that same day. Data from 43 nurseries are included in the epidemic curve. It excludes nine nurseries that were affected but did not participate in the questionnaire, two that participated but did not provide a first time of onset, and one which provided a time of onset preceding the service of food.

Incubation

Forty-four of the nurseries that reported the presence of symptomatic individuals provided data on the times of serving of food and onset of symptoms. The time at which food from the catering company was served ranged from 11:00 to 12:25 hours. In the nurseries the interval from serving to first onset of symptoms ranged from 40 min to 10 h 5 min (median 1 h 48 min), excluding one nursery which reported symptoms prior to serving.

Symptom profile

Forty-five nurseries provided information about the symptoms experienced by affected individuals: 75% ($n = 162$) experienced vomiting, 10.6% ($n = 23$) nausea, 9.7% ($n = 21$) diarrhoea (none with blood), 12% ($n = 26$) abdominal pain or cramps and 3.2% ($n = 7$) fever.

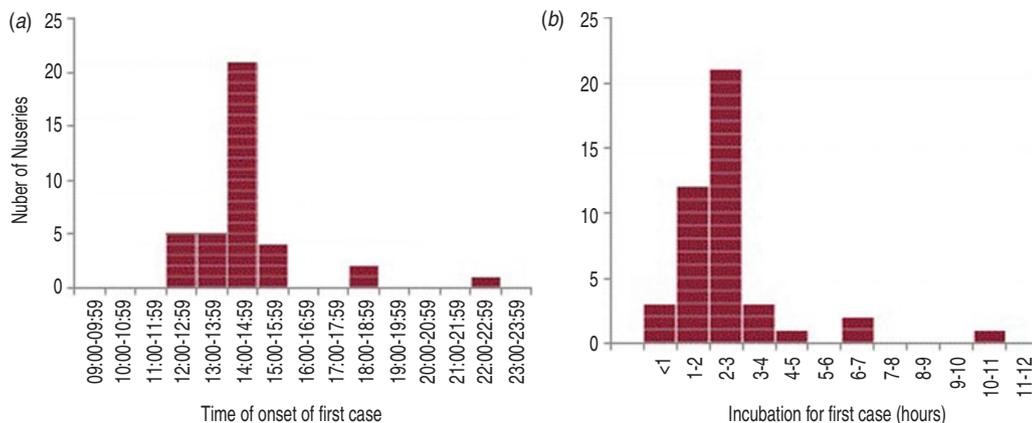


Fig. 2. (a) Epidemic curve by time of onset of symptoms and (b) incubation period for first case at 43 nurseries in South East England, 30 May 2012.

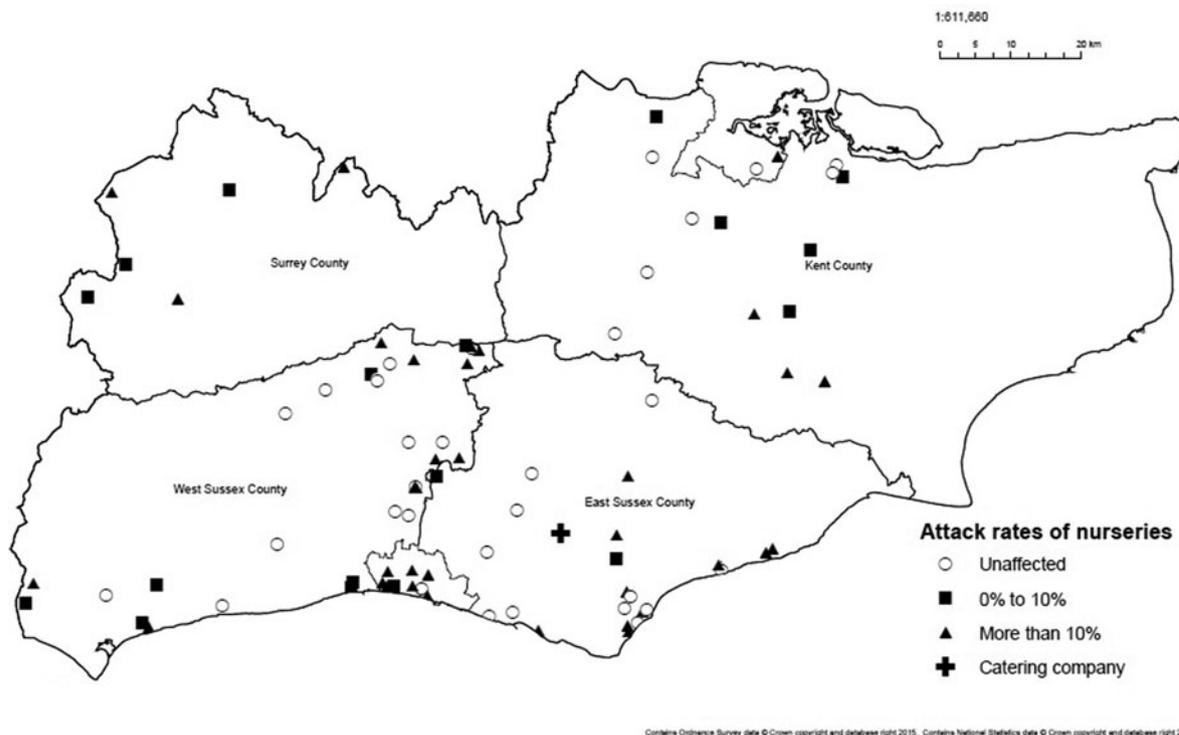


Fig. 3. Distribution of nurseries by attack rate ($n = 87$). (Source: Public Health England.)

Eighty percent of affected nurseries indicated that symptoms generally lasted <6 h, and none of the symptomatic individuals were reported as having been hospitalized.

Geographical distribution

AR by nursery ranged from 0% to 90% (Fig. 3). The distribution of ARs demonstrates positive skew, with a small number of nurseries having very high ARs. Nurseries were widely dispersed across four counties

(Kent, Surrey, East Sussex and West Sussex) in South East England. There may have been some clustering of nurseries with higher ARs in case-patients in the Brighton area and along the border between East and West Sussex, although this was not formally assessed.

There were no statistically significant associations between the time from delivery to serving of the food and AR. We also analysed the AR by delivery run in detail but no significant associations were found. There was a positive association between

temperature prior to serving and AR, and this was statistically significant ($P = 0.02$). However, this association was strongly influenced by the presence of a single positive outlying nursery, removal of which renders the association weak and non-significant ($P = 0.5$).

Environmental investigation

Inspection and review of food management systems at the catering company that supplied food to the affected nurseries led to the conclusion that the company's documented HACCP (hazard analysis critical control point) procedures were insufficiently robust and additional monitoring and recording was deemed necessary to ensure full confidence in the safety of the food.

A number of possible causes for the outbreak were considered including:

- Cooling processes for the minced beef and its separated cooking liquor could have been inadequate.
- The haricot beans were soaked at room temperature for 48 h potentially providing appropriate conditions for bacterial growth.
- Transportation of hot food may not have been at a consistently adequate temperature although it was reported to be at >63 °C and food was probed prior to serving.

Microbiological investigation

Stool samples

No potential pathogenic agent (including *B. cereus*) was detected in any of the nine stool specimens tested. Data on the time of collection in relation to onset of illness were not available.

Food and environmental microbiology

Thirty-nine isolates of *B. cereus* were recovered from nine separate food and environmental samples and their identities were confirmed. Between one and five separate *B. cereus* colonies were selected for further characterization from each sample and 13 distinct fAFLP profiles were detected. The cereulide gene (associated with the emetic toxin of *B. cereus* which was consistent with the clinical symptoms and incubation period) was only detected in isolates generating fAFLP profile I which were recovered from five food contact surfaces within the producer's premises, and from the shepherd's pie that had been supplied to one of the nurseries (Table 2).

S. aureus and *C. perfringens* were not detected in any sample. Enterobacteriaceae were detected at a level of 2.1×10^2 colony-forming units (c.f.u.) in a swab of a kettle strainer, and Enterobacteriaceae and *E. coli* were detected at 4.5×10^4 and 3.0×10^2 c.f.u., respectively in the swab of a storage tub at the producer's premises.

B. cereus was not isolated from a sample of shepherd's pie sampled at the catering company premises, but was detected at a level of 2.0×10^4 c.f.u./g in a sample of shepherd's pie eaten on the 30 May and obtained from one of the nurseries. Microscopic examination of suspensions of both samples of shepherd's pie indicated the presence of high numbers of bacterial spores. *B. cereus* was not recovered from a sample of dried haricot beans tested directly after collection from the catering company, but a level of 2.0×10^6 c.f.u./g was detected in the beans after a 25 g portion of dried beans was soaked in 50 ml sterile water for 48 h at 22 °C in the laboratory. When the beans were soaked in a similar manner for 48 h at 4 °C, no *B. cereus* were detected.

When testing all the *B. cereus* isolates recovered in this investigation, the cereulide gene was detected from all the isolates generating fAFLP profile I and was not detected in any of the other isolates recovered here. One out of three isolates recovered from the haricot beans which had been soaked for 48 h at 22 °C in the laboratory was identified as this same strain (fAFLP type I, cereulide gene positive).

DISCUSSION

This was a very large outbreak of vomiting, affecting 55 out of 87 nurseries supplied by a single catering company, and 200 of the 1648 individuals at those nurseries who were known to have eaten the food provided on that day. The predominant symptom was vomiting which was generally short-lived. The outbreak restricted in time, and occurred in the majority of nurseries that were affected within a 4-h period from mid-day on 30 May 2012. This suggests a single point source for the outbreak, with no person-to-person transmission.

The features of the outbreak are consistent with toxin-related foodborne poisoning. The absence of detection of any aetiological agents in specimens of faeces from any of the patients is possibly because either the specimens were collected too late, vomiting had occurred and expelled the agent after ingestion or that live bacteria had been sublethally injured by the

Table 2. *Bacillus cereus* counts and typing results for food samples and environmental swabs (for a selection of the samples taken)

| Location | Sample | <i>B. cereus</i> result | fAFLP Type I, cereulide gene detected |
|------------------------------|---|-------------------------------|---------------------------------------|
| Catering company | Swab storage tub | 3.5×10^3 c.f.u./swab | Present |
| | Swab kettle spout | 2.0×10^2 c.f.u./swab | Present |
| | Swab plastic scoop | 1.0×10^2 c.f.u./swab | n.d. |
| | Swab stainless-steel tin | 5.0×10^2 c.f.u./swab | Present |
| | Swab from transport container | 2.4×10^4 c.f.u./swab | n.d. |
| | Swab cutting plate | n.d. | |
| | Yoghurt | n.d. | |
| | Cucumber slices | n.d. | |
| | Shepherd's pie | n.d. | |
| | Haricot beans prior to soaking | n.d. | |
| | Haricot beans after 48 h at 4 °C | n.d. | |
| | Haricot beans after 48 h soaking at 22 °C | 2.0×10^6 c.f.u./g | Present |
| | Nursery | Shepherd's pie | 2.0×10^4 c.f.u./g |
| Cucumber slices | | n.d. | |
| Swab washed food container 1 | | 2.1×10^4 c.f.u./swab | n.d. |
| Swab washed food container 2 | | 3.5×10^4 c.f.u./swab | n.d. |

n.d., Not detected. The limit of detection was 100 c.f.u./swab and 200 c.f.u./g food.

cooking process and could not be recovered by conventional microbiological culture. Of the microbiological food-poisoning intoxications, those due to *S. aureus* enterotoxin and *B. cereus* emetic toxins are the most likely. Of these two aetiological agents, *S. aureus* may have a similar incubation period as the emetic *B. cereus* food poisoning (30 min to 6 h) although the former is generally 2–6 h and diarrhoea is more common [16–19]. Due to the symptoms shown by the patients in this outbreak together with the incubation period, the isolation of *B. cereus* from foods and the food production environment and the absence of microbiology evidence to suggest staphylococcal food poisoning, we suggest here that illness was most likely due to the *B. cereus* emetic toxin. Although it was not possible to test for this toxin in any food sample or clinical specimen, the incubation period of 0.5–6 h and sudden onset of nausea and vomiting [3] together with the results of food and environmental microbiological investigations are consistent with *B. cereus* emesis toxin as the cause of the outbreak. This is the largest outbreak caused by this organism recorded in England and Wales between 1992 and 2012 [20] although *B. cereus* outbreaks in nurseries served by external caterers, albeit affecting smaller numbers of patients, have been described previously [21]. In addition a large outbreak associated with a school catering organization has also been

described; however, in this outbreak an organism was not identified and the incubation period was much shorter than for this outbreak [22].

There was wide variation in the AR between nurseries: just over a third of nurseries that were supplied by the catering company reported no cases at all, whereas in one nursery, 90% of those that ate the food became unwell. The χ^2 goodness-of-fit test reveals that the variation between nurseries is highly statistically significant (χ^2 statistic = 292.5, 56 D.F., $P < 0.001$), suggesting that there is some factor other than chance that is responsible for the differences in AR between the nurseries. The outbreak was concentrated in time but dispersed in space. However, no specific aspect of the distribution or handling of the food following its dispatch from the catering company can be identified to explain this variation, and this may be due to methodological limitations of the study. There was variation in AR by age with higher AR in those of older age groups which contrasts with an outbreak of *B. cereus* documented in a similar age group [23]. The trend observed was likely to be a dose-response with older children and adults consuming more of the contaminated food and therefore experiencing more symptoms although it was not possible to test this hypothesis. We collected information about nursery staff and children attending nursery who did not eat food supplied by the catering company; of which there were 629 adults

and 799 children. Six reported gastrointestinal symptoms; giving an incidence of symptoms in those unexposed of 0.42% (95% CI 0.15–0.91). This reflects a low background incidence of diarrhoea and vomiting in this population.

There were aspects of this study which limit its ability to accurately describe the outbreak and to establish its cause. Case ascertainment was performed by nursery staff and consequently accurate reporting of numbers was dependent on them. There were significant inaccuracies in the responses which may have persisted despite clarification via telephone calls as children may have become unwell at home or staff may not have disclosed their illness. Although the online questionnaire was distributed promptly and enabled prompt investigation, recall bias may have influenced the information provided. Symptoms including diarrhoea were self-defined by the respondents this could have introduced misclassification of cases. Due to the nature of the outbreak it was necessary to collect data in aggregate form by nursery rather than at the individual level. As there was only a single menu delivered to each nursery this was not a major limitation of the study, but it may have skewed the epidemic curve, as this was based on time of onset of symptoms in only the first individual to become unwell in each nursery. However the detection of *B. cereus* (including strains that carried the cereulide gene) in seven environmental swabs and a sample of shepherd's pie, together with the observation of high numbers of spores within the two shepherd's pie samples, provide strong evidence that the shepherd's pie was the source of this outbreak. Furthermore, the demonstration that elevated levels of *B. cereus* were recovered from haricot beans used in the shepherd's pie following soaking at ambient temperature for 48 h suggests that this soaking process may have led to the hazardous levels of bacteria and the production of toxin in the final food product. Although the manufacturer's cooling processes for the minced beef and its separated cooking liquor may have been inadequate, the absence of geographical clustering did not support inadequate temperature maintenance during transport. Our conclusions are supported by Agata *et al.* [24] who reports that *B. cereus* toxin production depends on food type and is minimal in protein-rich food such as meat, and the toxin is heat stable, so although the bacteria may have been destroyed by heating, the toxin could have persisted and caused the cases to become symptomatic.

Environmental and microbiological investigations suggest that the cause of elevated levels of *B. cereus*

spores in food are likely to have been the soaking of haricot beans at room temperature for 48 h before they were used and this deficiency resulted in a large and widely distributed outbreak. Finlay and colleagues showed that *B. cereus* produces higher levels of toxin at 12–15 °C, but that toxin production is unlikely to occur below 12 °C or above 37 °C [25]. Given the low AR seen in this study the incident could have been missed in other settings, but the single menu and observation of cases on site for several hours after eating has enabled early identification in this incident. Other *B. cereus* food-poisoning outbreaks associated with young children have also been linked to poor food hygiene and temperature control [21, 23]. The demonstration that soaking at refrigeration temperature inhibited the growth of *B. cereus* suggests a mechanism for controlling this risk in future. The key recommendation of this study is that dried beans and pulses should be soaked at low temperature to prevent further outbreaks.

DECLARATION OF INTEREST

None.

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